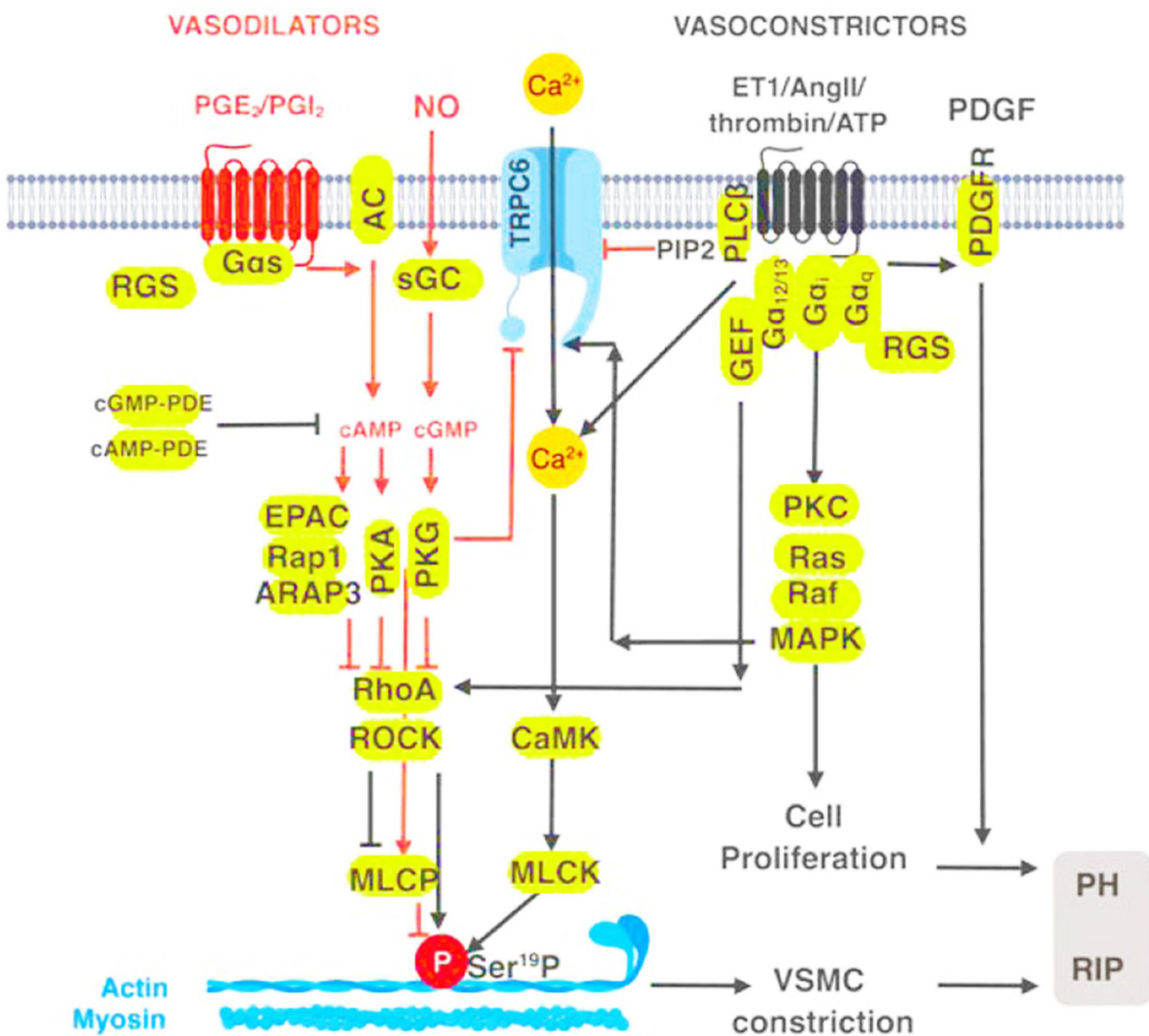


VP

Vessel Plus



EDITORIAL BOARD

Editors-in-Chief

Mario F. L. Gaudino (USA)
Alexander N. Orekhov (Russia)
Alexander D. Verin (USA)

Associate Editors

Evgenia V. Gerasimovskaya (USA)
Igor A. Sobenin (Russia)
Cristiano Spadaccio (Italy)
Robert M. Starke (USA)

Founding Editor

Aaron S. Dumont (USA)

Editorial Board Members

Mohamed Mohamed Rahouma
Ahmed (USA)

Manana Akhvediani (Georgia)
Wilbert S. Aronow (USA)
Juan A. Asensio (USA)
Sergio Berti (Italy)
Oleksandr Bilovol (Ukraine)
Peter Bolli (Canada)
Hong Chen (USA)
Lovely Chhabra (USA)
Yunzhou Dong (USA)
Ali H. Eid (Qatar)
Alberto Cordero Fort (Spain)
Mariann Harangi (Hungary)
Mohamed Kamel Kamel Hussein (USA)
Jahangir Iqbal (Saudi Arabia)
Simon Kennedy (UK)
Christopher Lau (USA)
Carl (Chip) Lavie (USA)
Veronika A. Myasoedova (Italy)

Paolo Nardi (Italy)
Narasimham L. Parinandi (USA)
Sampath Parthasarathy (USA)
Lisa Rong (USA)
Anna Shalimova (Ukraine)
Akira Sugawara (Japan)
Tamar Vakhtangadze (Georgia)
Ivar von Kügelgen (Germany)
Ya-Jing Wang (USA)
Keith A. Webster (USA)
Michael Yaroustovsky (Russia)
Li-Guo Zhao (UK)

Editorial Staffs

Mavis Wei (China)
Cai-Hong Wang (China)
Huan-Liang Wu (China)

GENERAL INFORMATION

About the Journal

Vessel Plus (VP), ISSN 2574-1209 (Online), is a peer-reviewed, open-access and continuously online published journal. The journal's full text is available online at www.vpjournal.net. The journal focuses on the latest clinical and basic research on the prevention, treatment, prognosis, and mechanisms of diseases of the blood vessels including original articles, case reports, commentaries, and expert reviews in molecular biology, physiology, and pathophysiology of the blood vessels. The journal is indexed by Chaoxing "Domain" Publishing Platform, CNKI, Google Scholar, J-Gate, ResearchBib, and Worldcat.

Information for Authors

Manuscripts should be prepared in accordance with Author Instructions. Please check www.vpjournal.net/pages/view/author_instructions for details.
All manuscripts should be submitted online at www.oaemesas.com/vp.

Copyright

The entire contents of the *VP* are protected under international copyrights. The journal, however, grants to all users a free, irrevocable, worldwide, perpetual right of access to, and a license to copy, use, distribute, perform and display the work publicly and to make and distribute derivative works in any digital medium for any reasonable purpose, subject to proper attribution of authorship and ownership of the rights. The journal also grants the right to make small numbers of printed copies for their personal use under the Creative Commons Attribution 4.0 License.
Copyright is reserved by © The Author(s) 2018.

Permissions

For information on how to request permissions to reproduce articles/information from this journal, please visit www.vpjournal.net.

Disclaimer

The information and opinions presented in the journal reflect the views of the authors and not of the journal or its Editorial Board or the Publisher. Publication does not constitute endorsement by the journal. Neither the *VP* nor its publishers nor anyone else involved in creating, producing or delivering the *VP* or the materials contained therein, assumes any liability or responsibility for the accuracy, completeness, or usefulness of any information provided in the *VP*, nor shall they be liable for any direct, indirect, incidental, special, consequential or punitive damages arising out of the use of the *VP*. The *VP*, nor its publishers, nor any other party involved in the preparation of material contained in the *VP* represents or warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such material. Readers are encouraged to confirm the information contained herein with other sources.

Publisher

OAE Publishing Inc.
245 E Main Street ste122, Alhambra, CA 91801, USA
Website: www.oaepublish.com

Contacts

E-mail: editorialoffice@vpjournal.net
Website: www.vpjournal.net

CONTENTS

- 1 Traumatic pulmonary artery injury: a review of the recent literature**
Youichi Yanagawa, Kouhei Ishikawa, Hiroki Nagasawa, Ikuto Takeuchi, Kei Jitsuiki, Hiromichi Ohsaka, Kazuhiko Omori
Vessel Plus 2018;2:1 <http://dx.doi.org/10.20517/2574-1209.2017.37>
- 2 Surgical revascularization for acute coronary syndromes: a narrative review**
Joseph H. Joo, Joshua M. Liao, Faisal G. Bakaeen, Danny Chu
Vessel Plus 2018;2:2 <http://dx.doi.org/10.20517/2574-1209.2017.36>
- 3 Temporizing amplatzer closure of an aorto-enteric fistula associated with a blind aortic stump via a translumbar approach**
S. Keisin Wang, Justin R. King, Ashley R. Gutwein, Raghu L. Motaganahalli, Andres Fajardo, Gary W. Lemmon
Vessel Plus 2018;2:3 <http://dx.doi.org/10.20517/2574-1209.2017.38>
- 4 Early treatment of functional tricuspid regurgitation at the time of mitral valve surgery: an increased risk or an additional benefit?**
Paolo Nardi, Calogera Pisano, Antonio Pellegrino, Fabio Bertoldo, Sabrina Ferrante, Monica Greci, Sara Rita Vacirca, Marco Russo, Giovanni Ruvolo
Vessel Plus 2018;2:4 <http://dx.doi.org/10.20517/2574-1209.2017.35>
- 5 Adherence to guidelines: primary prevention with aspirin in 1125 medical check-up participants**
Jin Hee Im, Sang Won Han, Seon Yeong Lee, Jong Sam Baik
Vessel Plus 2018;2:5 <http://dx.doi.org/10.20517/2574-1209.2018.07>
- 6 Metabolic risk in depression and treatment with selective serotonin reuptake inhibitors: are the metabolic syndrome and an increase in cardiovascular risk unavoidable?**
Mervin Chávez-Castillo, Ángel Ortega, Manuel Nava, Jorge Fuenmayor, Victor Lamed, Manuel Velasco, Valmore Bermúdez, Joselyn Rojas-Quintero
Vessel Plus 2018;2:6 <http://dx.doi.org/10.20517/2574-1209.2018.02>
- 7 Endovascular treatment of an iatrogenic superior mesenteric arteriovenous fistula after Nissen fundoplication**
S. Keisin Wang, Jie Xie, Raghu L. Motaganahalli
Vessel Plus 2018;2:7 <http://dx.doi.org/10.20517/2574-1209.2018.15>
- 8 Ruptured isolated descending thoracic aortic aneurysm: open or endovascular repair?**
Amer Harky, Nichola Manu, Rafal Al Nasiri, Dilan Sanli, Ciaran Grafton-Clarke, Jeffrey Shi Kai Chan, Chris Ho Ming Wong
Vessel Plus 2018;2:8 <http://dx.doi.org/10.20517/2574-1209.2018.12>

- 9 Repair of mitral subvalvular apparatus and a calcified left ventricle aneurysm**
Kasra Shaikhrezai, Sanjeet Singh Avtaar Singh, Karim Morcos, Steve Hunter
Vessel Plus 2018;2:9 <http://dx.doi.org/10.20517/2574-1209.2018.17>
- 10 The first INSPIRIS RESILIA Aortic Valve™ replacement (Edwards Lifesciences) in endocarditis**
Sanjeet Singh Avtaar Singh, Gwyn Beattie, David Reid, Philip Curry
Vessel Plus 2018;2:10 <http://dx.doi.org/10.20517/2574-1209.2018.18>
- 11 Bortezomib-induced posterior reversible encephalopathy syndrome: a case report**
Paolo Candelaresi, Maria Chiara Casorio
Vessel Plus 2018;2:11 <http://dx.doi.org/10.20517/2574-1209.2018.09>
- 12 Research into biodegradable polymeric stents: a review of experimental and modelling work**
Tianyang Qiu, Liguozhao
Vessel Plus 2018;2:12 <http://dx.doi.org/10.20517/2574-1209.2018.13>
- 13 Plasmatic biomarkers of inflammation correlate with ¹⁸FDG-PET-CT and microembolic signals in patients with carotid stenosis**
Hubertus Mueller, Loraine Fisch, Christophe Bonvin, Karl Lovblad, Osman Ratib, Patrice Lalive, Stephane Pagano, Nicolas Vuilleumier, Jean-Pierre Willi, Roman Sztajzel
Vessel Plus 2018;2:13 <http://dx.doi.org/10.20517/2574-1209.2018.19>
- 14 Percutaneous coronary intervention in the elderly: current updates and trends**
Mohammed J. Arisha, Dina A. Ibrahim, Ahmed A. Abouarab, Mohamed Rahouma, Mohamed K. Kamel, Massimo Baudo, Kritika Mehta, Mario F. L. Gaudino
Vessel Plus 2018;2:14 <http://dx.doi.org/10.20517/2574-1209.2018.29>
- 15 Histone deacetylases in vascular permeability and remodeling associated with acute lung injury**
Laszlo Kovacs, Anita Kovacs-Kasa, Alexander D. Verin, David Fulton, Rudolf Lucas, Yunchao Su
Vessel Plus 2018;2:15 <http://dx.doi.org/10.20517/2574-1209.2018.06>
- 16 Immunohistochemistry of the circadian clock in mouse and human vascular tissues**
Ciprian B. Anea, Ana M. Merloiu, David J. R. Fulton, Vijay Patel, R. Dan Rudic
Vessel Plus 2018;2:16 <http://dx.doi.org/10.20517/2574-1209.2018.46>
- 17 Who is the next for aortic valve implantation? Present and future indications**
Giuseppe Verolino, Alessia Delli Veneri, Myriam Carpenito, Francesco Piccirillo, Leonardo Aurino, Annunziata Nusca
Vessel Plus 2018;2:17 <http://dx.doi.org/10.20517/2574-1209.2018.32>
- 18 Iatrogenic injury to axillary artery: rescued by endovascular repair**
Chih-Chen Kao, Yao-Kuang Huang
Vessel Plus 2018;2:18 <http://dx.doi.org/10.20517/2574-1209.2018.42>
- 19 Genetic variants of renin on the prevalence of diabetic nephropathy**
Pulakes Purkait, Kalpataru Halder, Jammigumpula Masthanaiah Naidu, Biswanath Sarkar
Vessel Plus 2018;2:19 <http://dx.doi.org/10.20517/2574-1209.2018.16>

- 20 **Advantages and disadvantages of total arterial coronary artery bypass graft as compared to venous coronary artery bypass graft**
Dickson Dewantoro, Antonio Nenna, Umberto Satriano, Massimo Chello, Cristiano Spadaccio
Vessel Plus 2018;2:20 <http://dx.doi.org/10.20517/2574-1209.2018.50>
- 21 **Recent advances in cerebral cavernous malformation research**
Akhil Padarti, Jun Zhang
Vessel Plus 2018;2:21 <http://dx.doi.org/10.20517/2574-1209.2018.34>
- 22 **The endothelial progenitor cell dysfunction in hypertension: the diagnostic and predictive values**
Alexander E Berezin
Vessel Plus 2018;2:22 <http://dx.doi.org/10.20517/2574-1209.2018.23>
- 23 **Vascular approaches and its potential implications in transcatheter aortic valve implantation**
Alessandro Sticchi, Edoardo Bressi, Annunziata Nusca, Germano Di Sciascio
Vessel Plus 2018;2:23 <http://dx.doi.org/10.20517/2574-1209.2018.47>
- 24 **Is tacrolimus more likely to induce diabetes mellitus than ciclosporin in heart transplant patients?**
Anisha Jagpal, Sudeep Das De, Sanjeet Avtaar Singh, Alan Kirk
Vessel Plus 2018;2:24 <http://dx.doi.org/10.20517/2574-1209.2018.27>
- 25 **Fetal programming and its effects on vascular pulmonary circulation**
Carmela Rita Balistreri
Vessel Plus 2018;2:25 <http://dx.doi.org/10.20517/2574-1209.2018.35>
- 26 **Complications of transcatheter aortic valve replacement and rescue attempts**
Burak Can Depboylyu, Serkan Yazman, Bugra Harmandar
Vessel Plus 2018;2:26 <http://dx.doi.org/10.20517/2574-1209.2018.39>
- 27 **Age-associated features of oxidative stress as marker of vascular aging in comorbid course of hypertension and type 2 diabetes mellitus**
Valeriya Nemtsova, Olexander Bilovol, Irina Ilchenko, Anna Shalimova
Vessel Plus 2018;2:27 <http://dx.doi.org/10.20517/2574-1209.2018.48>
- 28 **Transcatheter aortic valve replacement: is anesthesiologic management linked to surgical outcomes?**
Chiara Candela, Annalaura Di Pumpo, Alessandro Centonze, Fabrizio Cucciniello, Domenico Sarubbi, Felice Eugenio Agrò
Vessel Plus 2018;2:28 <http://dx.doi.org/10.20517/2574-1209.2018.31>
- 29 **A current view of G protein-coupled receptor - mediated signaling in pulmonary hypertension: finding opportunities for therapeutic intervention**
Derek Strassheim, Vijaya Karoor, Kurt Stenmark, Alexander Verin, Evgenia Gerasimovskaya
Vessel Plus 2018;2:29 <http://dx.doi.org/10.20517/2574-1209.2018.44>

- 30 **Thrombospondins and remodeling of the tumor microenvironment**
Olga Stenina-Adognravi, Santoshi Muppala, Jasmine Gajeton
Vessel Plus 2018;2:30 <http://dx.doi.org/10.20517/2574-1209.2018.40>
- 31 **Selected meeting abstracts of 2018 healthcare and cardiology conference**
Ahmed Ahmed Fouad Abdelwahab Ahmed
Vessel Plus 2018;2:31 <http://dx.doi.org/10.20517/2574-1209.2018.58>
- 32 **Correction: Energetic metabolism in cardiomyocytes: molecular basis of heart ischemia and arrhythmogenesis**
María Sofía Martínez, Andrés García, Eliana Luzardo, Mervin Chávez-Castillo, Luis Carlos Olivar, Juan Salazar, Manuel Velasco, Joselyn Joanna Rojas Quintero, Valmore Bermúdez
Vessel Plus 2018;2:32 <http://dx.doi.org/10.20517/2574-1209.2018.68>
- 33 **Heart transplantation: a history lesson of Lazarus**
Sanjeet Singh Avtaar Singh, Nicholas Banner, Colin Berry, Nawwar Al-Attar
Vessel Plus 2018;2:33 <http://dx.doi.org/10.20517/2574-1209.2018.28>
- 34 **Risk factors for atherosclerosis and vascular calcification in patients with type 2 diabetes on long-term hemodialysis**
Tatyana Archakova, Liudmila Nedosugova
Vessel Plus 2018;2:34 <http://dx.doi.org/10.20517/2574-1209.2018.52>
- 35 **Revascularization method for patients with infrainguinal arterial disease**
Glushkov Nikolay, Ivanov Michael, Artemova Anastasia, Puzdriak Petr, Uryupina Anastasia, Bondarenko Pavel, Ivan Tigrov
Vessel Plus 2018;2:35 <http://dx.doi.org/10.20517/2574-1209.2018.45>
- 36 **Vascular smooth muscle cell contractile function and mechanotransduction**
Sultan Ahmed, Derek T. Warren
Vessel Plus 2018;2:36 <http://dx.doi.org/10.20517/2574-1209.2018.51>
- 37 **Blood pressure control and vascular protection with a fixed-dose combination of lisinopril + amlodipine + rosuvastatin in hypertensive patients**
Sergey V. Nedogoda, Elena V. Chumachek, Alla A. Ledyeva, Vera V. Tsoma, Alla S. Salasyuk, Victoria O. Smirnova, Victoria Yu. Hripaeva, Roman V. Palashkin, Ekaterina A. Popova
Vessel Plus 2018;2:37 <http://dx.doi.org/10.20517/2574-1209.2018.36>
- 38 **Endoscopic radial artery harvesting for coronary artery bypass grafting**
Ajita Naik, Mohamed Rahouma, Cristiano Spadaccio, Kritika Mehta, Massimo Baudo, Mohamed Kamel, Faiza Khan, Irbaz Hameed, Matthew Wingo, Yongle Ruan, Ahmed Abouarab, Mohamed Hossny, Leonard N. Girardi, Mario Gaudino
Vessel Plus 2018;2:38 <http://dx.doi.org/10.20517/2574-1209.2018.62>
- 39 **Evaluation of different surgical modalities for coronary reconstruction of diffusely diseased left anterior descending artery**
Mohamed H. Elsayed, Wael M. Hassanein, Samir A. Keshk, Mamdouh Zidan, Waheed G. Etman
Vessel Plus 2018;2:39 <http://dx.doi.org/10.20517/2574-1209.2018.65>

40 Ventricular septal defect and tricuspid and mitral valve insufficiency caused by penetrating trauma

Ana Lopez-Marco, Jennifer Williams, Christine Tan, Dheeraj Mehta

Vessel Plus 2018;2:40 <http://dx.doi.org/10.20517/2574-1209.2018.67>

41 Volatile anesthetics in cardiac surgery: the impalpable benefit

Annalaura Di Pumpo, Chiara Candela, Fabrizio Cucciniello, Domenico Sarubbi, Felice Eugenio Agrò

Vessel Plus 2018;2:41 <http://dx.doi.org/10.20517/2574-1209.2018.38>

Review

Open Access



Traumatic pulmonary artery injury: a review of the recent literature

Youichi Yanagawa, Kouhei Ishikawa, Hiroki Nagasawa, Ikuto Takeuchi, Kei Jitsuiki, Hiromichi Ohsaka, Kazuhiko Omori

Department of Acute Critical Care Medicine, Shizuoka Hospital, Juntendo University, Izunokuni 410-2295, Shizuoka, Japan.

Correspondence to: Dr. Youichi Yanagawa, Department of Acute Critical Care Medicine, Shizuoka Hospital, Juntendo University, 1129 Nagaoka, Izunokuni 410-2295, Shizuoka, Japan. E-mail: yyanaga@juntendo.ac.jp

How to cite this article: Yanagawa Y, Ishikawa K, Nagasawa H, Takeuchi I, Jitsuiki K, Ohsaka H, Omori K. Traumatic pulmonary artery injury: a review of the recent literature. *Vessel Plus* 2018;2:1. <http://dx.doi.org/10.20517/2574-1209.2017.37>

Received: 12 Dec 2017 **First Decision:** 8 Jan 2018 **Revised:** 19 Jan 2018 **Accepted:** 22 Jan 2018 **Published:** 26 Jan 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Pulmonary artery injury (PAI) is rare, lethal clinical entity. Traumatic PAI is anatomically classified into transection/rupture/laceration, pseudoaneurysm, dissection and fistula. In addition, traumatic PAI is clinically classified into two major categories: iatrogenic and non-iatrogenic, depending on the mechanism of the trauma. The frequency, clinical symptoms and treatment differ between the two clinical categories. If PAI can be managed appropriately and promptly in patients without cardiac arrest, the patient may be saved, as PAI can be easily controlled with appropriate procedures due to the low pressure in the PA circulation.

Keywords: Pulmonary artery, trauma, iatrogenic

INTRODUCTION

Pulmonary artery injury (PAI) is a rare, lethal clinical entity. Most vital emergencies involve proximal PAI. However, if PAI can be managed appropriately and promptly in patients without cardiac arrest, the patient may be saved, as PAI can be easily controlled with appropriate procedures due to the low pressure in the PA circulation, provided the injury site is small^[1]. In this review article, traumatic PAI is anatomically classified into four categories and clinically classified into two major categories: iatrogenic and non-iatrogenic, depending on the mechanism of the trauma. The frequency, clinical symptoms and treatment differ between the two clinical categories. The references are limited to reports in the English literature published since 1990.



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



ANATOMICAL CLASSIFICATION

Anatomically, traumatic PAI is classified into transection/rupture/laceration, pseudoaneurysm, dissection and fistula.

A transection, rupture, disruption, perforation, tear or laceration of the PA is thought to be a near-complete tear through all layers of the PA due to trauma; however, there is no consistent definitive terminology^[1]. Clinical symptoms due to such trauma include cardiac arrest or hemodynamic insufficiency due to massive hemorrhaging or cardiac tamponade, and dyspnea due to hemothorax or hemoptysis^[1,2]. Chest pain due to concomitant thoracic cage injury has also been reported. Rarely, this PAI, which involves hemostasis by clotting, is incidentally found on enhanced computed tomography (CT) without specific symptoms, as whole-body enhanced CT is routinely performed in patients following a high-energy accident^[3,4].

A pseudoaneurysm is an encapsulated hematoma in communication with the lumen of a ruptured vessel. This may form when re-epithelialization of the perforation does not occur, and a delayed diagnosis can occur even 60 years later^[5]. The pseudoaneurysm may stabilize and spontaneously resolve or expand and rupture, depending on the etiology, size and intravascular pressure^[1]. A pseudoaneurysm can be asymptomatic or characterized by symptoms of hemoptysis, shortness of breath and chest pain^[6,7]. An iatrogenic pseudoaneurysm of the PA is most common, followed by trauma-induced events. A pseudoaneurysm of the PA can also be congenital or have a non-traumatic cause, which includes infections and neoplasms^[6,7].

An arterial fistula is an abnormal connection between the artery and other lumen organs. If an abnormal connection between an artery and a vein occur, this is called as an arteriovenous (AV) fistula. In a trauma setting, arterial fistulas can be asymptomatic or characterized by right ventricular dysfunction, acute respiratory failure or transient ischemic attack (TIA)^[8-12]. Traumatism, fistulas occur between the PA and left atrium, internal mammary artery, aorta or pulmonary vein. Non-traumatic pulmonary AV fistulas can also be associated with hereditary hemorrhagic telangiectasia^[13]. The initial clinical manifestations include thrombotic or embolic stroke, brain abscess and TIA but can also be asymptomatic in non-traumatic cases^[13]. The clinical trial of cyanosis, exertional dyspnea and digital clubbing is common, but there have been no reports describing triads due to trauma^[13].

PA dissections (PADs) are created by the occurrence of a small tear in the tunica intima, which allows blood to enter and cause the intima layer to strip away from the media layer, in effect dividing the muscle layers of the vascular wall. The mechanism of blunt traumatic PAD is likely similar to that seen in the aorta as a result of shearing forces and differential deceleration of the mediastinum and the spine. However, unlike aortic dissection, PAD progresses rapidly and typically ruptures rather than developing a reentry site, which causes cardiogenic shock or sudden death, especially in non-traumatic cases with pulmonary hypertension^[14]. Five major etiological groups can be identified: congenital malformation, infection or inflammation, acquired cardiac diseases, iatrogenic causes and trauma^[15-17]. Traumatic PADs usually resolve or remain stable unless associated with pulmonary hypertension, in which case the risk of bleeding can be quite high^[1,16].

IATROGENIC PAI

The most common cause of PA ruptures and pseudoaneurysms is iatrogenic, with PA catheters being a particularly common culprit^[2,18-20]. Other iatrogenic causes include intraoperative surgical procedures^[21-24], indwelling chest tubes^[25,26], pacemaker implantation^[27], central venous catheterization^[28] and Kirschner wire migration^[29].

The incidence of PAI induced by catheters is not very high, averaging 0.01%-0.47%^[2]. The mortality rate of PAI induced by catheter averages 50% but can be as high as 75% in anticoagulated patients. If death occurs,

it is usually secondary to asphyxia rather than hypovolemia^[2]. The initial presentation may be as obvious as massive pulmonary hemorrhaging or as subtle as a cough associated with minimal hemoptysis, or it may even be totally asymptomatic^[30].

When catheter-induced PAI happens during insertion of a fluoroscope, it is relatively easy to retract the PA catheter a few centimeters and re-inflate the balloon under direct vision. It may therefore be possible to stop the bleeding^[2]. Additional diagnostic angiography and embolization also can be easily performed at that point.

In addition to treatments for PAI, the patient may need selective intubation to obtain lung isolation in accordance with clinical symptoms. Lung isolation can be performed with different techniques, including selective intubation with a standard endotracheal tube, bronchial blocker or double-lumen tube (DLT)^[2]. A bronchial blocker can be used for lung separation when a DLT is not immediately available or when it is difficult to insert the DLT. Bronchial blockers can be used to tamponade the bleeding side while waiting for diagnostic and therapeutic interventions. The most important aspects of treatment are lung isolation using selective intubation, bronchial blockers, or DLT as a temporary measure; rapid movement is important for more definitive therapy as it can avoid clotting of the entire lung on one side, which effectively causes pneumonectomy. Surgery, including pulmonary artery ligation, segmentectomy, lobectomy or pneumonectomy, is reserved for extreme cases, since these procedures are technically challenging and entail high morbidity^[2].

NON-IATROGENIC PAI

A majority of non-iatrogenic PAI cases occur due to chest trauma; however, most chest trauma cases do not involve PAI. PAI accounts for a small percentage of thoracic trauma cases. Epidemiologically, Kulshrestha *et al.*^[31] reported 102 patients sustaining cardiac injuries over a 4-year period. There were 45 blunt trauma, 36 stab injuries, and 21 gunshot injuries^[31]. The injury involved the ventricle in 85 patients, atrium in 7 and the PA in 5 (5%) and resulted in crush injury to the heart in the remaining 5 cases. Thirty-three patients (32.3%) died at the scene, and 58 (56.9%) died during transportation. Only 11 patients (10.8%) reached the hospital alive, and 10 of these survived following thoracotomy and repair of the cardiac injury. The patients with ventricular injuries had a greater prehospital mortality than those with atrial or PA injuries.

Deneuille^[32] reported 88 cases of penetrating chest trauma, focusing on non-iatrogenic PAIs. Of these 88 cases, 6 with PAI reached the hospital alive^[32]. All cases underwent urgent operation, and 4 survived. The mortality appears to be high in patients presenting with complex lesions involving vascular and pulmonary structures. As a result, they concluded that isolated injuries of the PA were amenable to surgical repair and had a good prognosis if the patients arrived at the hospital alive.

We summarized the cases of non-iatrogenic PAI in [Tables 1 and 2](#). Most cases were reported as case reports, except for the findings of Deneuille^[32]. Penetrating injuries were more frequent than blunt ones. Similar to Deneuille^[32], 46/50 (92%) cases survived. The diagnosis was made based on intraoperative findings, enhanced CT or pulmonary arteriography. The main treatment method was surgery or an interventional approach. These findings suggest that if hemorrhaging is not noted and the vital signs are stable, conservative treatment can be selected. There are no strict guidelines concerning the management of PAI, and the preferred approach depends on the lesion, patient and institution^[1].

CONCLUSION

PAI is a rare, lethal clinical entity; most vital emergencies involve proximal PAI. Anatomically, traumatic PAI is classified into transection/rupture/laceration, pseudoaneurysm, dissection and fistula. Iatrogenic

Table 1. Cases of non-iatrogenic injury of the pulmonary artery since 1990

No.	Reporter	Year	Age (year)	Gender	Type of injury	Cause of injury	Type of injury	Symptom	Treatment	Outcome	Arrest	Other
1	Demondion <i>et al.</i> ^[33]	2016	27	Male	Blunt	Snowmobile accident	Rupture	Mediastinal hematoma	Conservative	Survive	None	
2	Maury <i>et al.</i> ^[34]	2015	51	Male	Blunt	Traffic accident	Rupture	Hemothorax	Suture	Survive	None	
3	Lin <i>et al.</i> ^[35]	2014	23	Male	Blunt	Traffic accident	Rupture	Hemothorax	Ligation	Dead	None	MOF
4	Muthialu <i>et al.</i> ^[36]	2013	5	Female	Blunt	Traffic accident	Rupture	Hemothorax	Suture & lobectomy	Survive	None	
5	Vendrell and Gahide ^[3]	2010	42	Female	Blunt	?	Rupture	Hemothorax	Conservative	Survive	None	
6	Pereira and Narrod ^[37]	2009	55	Female	Blunt	Traffic accident	Rupture	Hemothorax	Suture	Survive	None	
7	Kanani <i>et al.</i> ^[38]	2002	31	Male	Blunt	Traffic accident	Rupture	Hemothorax	Suture	Survive	None	
8	Ambrose <i>et al.</i> ^[39]	2000	69	Male	Blunt	Traffic accident	Rupture	Hemothorax	Suture	Survive	None	
9	Weltman <i>et al.</i> ^[40]	1999	69	Male	Blunt	Traffic accident	Rupture	Hemothorax	Suture	Survive	None	
10	Clements <i>et al.</i> ^[41]	1997	42	Female	Blunt	Traffic accident	Rupture	Tamponade	Suture	Survive	None	
11	Daon and Gorton ^[42]	1997	44	Female	Blunt	Traffic accident	Rupture	Hemothorax	Suture	Survive	None	
12	Katz and Groskin ^[43]	1993	27	Female	Blunt	Traffic accident	Rupture	Hemothorax	Suture	Survive	None	
13	Ohsaka <i>et al.</i> ^[44]	2015	91	Male	Penetrating	Sword	Rupture	Hemothorax	Suture	Survive	PEA	
14	Greberski <i>et al.</i> ^[45]	2015	36	Male	Penetrating	Knife	Rupture	Hemothorax	Suture	Survive	None	
15	Senanayake <i>et al.</i> ^[46]	2012	54	Male	Penetrating	Stab	Rupture	Hemothorax	Suture	Survive	PEA	
16	Sanchez <i>et al.</i> ^[47]	2010	31	Male	Penetrating	Stab	Rupture	Hemothorax	Suture	Survive	None	
17	Atalay <i>et al.</i> ^[48]	2010	18	Male	Penetrating	Gun	Rupture	Hemothorax	Suture	Survive	None	
18	Deneville ^[32]	2000	32	Male	Penetrating	Knife	Rupture	Hemothorax	Suture	Survive	None	
19	Deneville ^[32]	2000	37	Male	Penetrating	Shotgun	Rupture	Hemothorax	Suture	Survive	None	
20	Deneville ^[32]	2000	24	Male	Penetrating	Knife	Rupture	Hemothorax	Suture	Survive	None	
21	Deneville ^[32]	2000	22	Male	Penetrating	Shotgun	Rupture	Hemothorax	Pneumonec-tomy	Dead	Yes	
22	Deneville ^[32]	2000	55	Male	Penetrating	Bull horn	Rupture	Hemothorax	Pneumonec-tomy	Dead	Yes	
23	Deneville ^[32]	2000	44	Male	Penetrating	Knife	Rupture	Hemothorax	Suture	Survive	None	
24	Babatasi <i>et al.</i> ^[49]	1999	69	Male	Penetrating	Gun	Rupture	Mediastinal hematoma	Suture	Survive	None	
25	Kiss <i>et al.</i> ^[50]	1999	34	Woman	Penetrating	Gun	Rupture	Tamponade	Suture	Survive	None	
26	Jain ^[51]	1998	7	Male	Penetrating	Air gun	Rupture	Tamponade	Suture	Survive	PEA	
27	Goel <i>et al.</i> ^[52]	2013	58	Female	Blunt	Traffic accident	Pseudoaneurysm	No specific	Conservative	Survive	None	
28	Sridhar <i>et al.</i> ^[53]	2010	32	Male	Blunt	?	Pseudoaneurysm	No specific	Embolization	Survive	None	
29	Reade <i>et al.</i> ^[54]	2006	57	Male	Blunt	Traffic accident	Pseudoaneurysm	No specific	Conservative	Survive	None	
30	Kasai and Kobayashi ^[55]	1992	17	Male	Blunt	Traffic accident	Pseudoaneurysm	No specific	Lobectomy	Survive	None	
31	Goel <i>et al.</i> ^[52]	2013	32	Male	Penetrating	Gun	Pseudoaneurysm	No specific	Conservative	Survive	None	
32	Quartey and Jessie ^[56]	2011	21	Male	Penetrating	Gun	Pseudoaneurysm	No specific	Coil embolization	Survive	None	
33	Blanié <i>et al.</i> ^[57]	2011	39	Male	Penetrating	Circular saw	Pseudoaneurysm	No specific	Pericardial patch	Survive	None	
34	Rai <i>et al.</i> ^[58]	2010	28	Woman	Penetrating	Gun	Pseudoaneurysm	No specific	Coil embolization	Survive	Yes	
35	Maddali <i>et al.</i> ^[59]	2007	35	Male	Penetrating	Knife	Pseudoaneurysm	No specific	Suture	Survive	None	
36	Khan <i>et al.</i> ^[60]	2005	50	Male	Penetrating	Gun	Pseudoaneurysm	No specific	Coil embolization	Survive	None	
37	Dimarakis <i>et al.</i> ^[61]	2005	29	Male	Penetrating	Knife	Pseudoaneurysm	No specific	Coil embolization	Survive	None	
38	Block <i>et al.</i> ^[7]	2004	40	Male	Penetrating	Gun	Pseudoaneurysm	No specific	Coil embolization	Survive	None	

39	de Jonge <i>et al.</i> ^[62]	2003	57	Male	Penetrating	Knife	Pseudoaneurysm	No specific	Coil embolization	Survive	None	
40	Donaldson and Ngo-Nonga ^[63]	2002	17	Male	Penetrating	Gun	Pseudoaneurysm	No specific	Lobectomy	Survive	Yes	CPC4
41	Savage <i>et al.</i> ^[64]	1999	49	Male	Penetrating	Gun	Pseudoaneurysm	No specific	Coil embolization	Survive	None	
42	Hubler <i>et al.</i> ^[65]	1997	20	Male	Penetrating	Knife	Pseudoaneurysm	No specific	Lobectomy	Survive	None	
43	Huet <i>et al.</i> ^[66]	1996	29	Male	Penetrating	Gun	Pseudoaneurysm	No specific	Stent	Survive	None	
44	Giglioli <i>et al.</i> ^[70]	2013	46	Female	Blunt	?	Fistula (aortopulmonary)	Right cardiac failure	Pericardial patch	Survive	None	
45	Rrapo <i>et al.</i> ^[71]	2013	20	Male	Penetrating	Gun	Fistula (pulmonary)	ARDS	Pericardial patch	Survive	None	
46	Roshanali <i>et al.</i> ^[72]	2012	48	Female	Penetrating	Missile debris	Fistula (pulmonary)	TIA	Plug occlusion	Survive	None	
47	Howell <i>et al.</i> ^[67]	2004	24	Male	Penetrating	Knife	Fistula (aortopulmonary)	No specific	Operation	Survive	None	
48	Kerr and Sauter ^[68]	1993	35	Male	Penetrating	Knife	Fistula (pulmonary)	Short of breath	Embolization	Survive	None	
49	Almdahl <i>et al.</i> ^[66]	2014	46	Female	Blunt	?	Dissection	No specific	Conservative	Survive	None	
50	Chung <i>et al.</i> ^[77]	2009	53	Male	Blunt	Boat accident	Dissection	No specific	Nitric oxide	Dead	None	

?: not described; ARDS: acute respiratory distress syndrome; TIA: transient ischemic attack; MOF: multiple organ failure; PEA: pulseless electrical activity; CPC: cerebral performance category; PAI: pulmonary artery injury

Table 2. Summary of non-iatrogenic injury of the pulmonary artery since 1990

Total		50 cases
Age, years	Range	5-91
	Average	38.4
Gender	Male	40 (80%)
	Female	10 (20%)
Type of injury	Blunt	19 (38%)
	Penetrating	31 (62%)
Cause of injury	Gun	15 (30%): shot gun, air gun include
	Traffic accident	13 (32%)
	Knife	10 (20%)
	Others	9 (18%)
Type of PAI	Rupture	26 (52%)
	Pseudoaneurysm	17 (34%)
	Fistula	5 (10%)
	Dissection	2 (4%)
Symptom	Hemothorax	21 (42%)
	No specific	20 (40%)
	Tamponade	3 (6%)
	Others	3 (6%)
Treatment	Surgical sutures	22 (44%)
	Endovascular	10 (20%): include coil, stent and other materials
	Conservative	6 (12%)
	Other surgical maneuver	10 (20%)
	Other treatment	2 (4%)
Survival	Number and rate	46 (92%)

PAI: pulmonary artery injury

procedures are the most common cause of iatrogenic PAI rupture and pseudoaneurysm, with PA catheters being a particularly common culprit. Non-iatrogenic PAIs occur due to chest trauma but most chest trauma does not involve PAI. Penetrating injuries were more frequent than blunt injuries. The diagnosis was made based on intraoperative findings, enhanced CT or pulmonary arteriography. The main treatment method was surgery or an interventional approach. If PAI can be managed appropriately and promptly in patients without cardiac arrest, the patient may be saved.

DECLARATIONS

Authors' contributions

Designed the study, gathered data and wrote the manuscript: Yanagawa Y

Gave technical support, conceptual advice and edited the manuscript: Ishikawa K, Nagasawa H, Takeuchi I, Jitsuiki K, Ohsaka H, Omori K

Financial support and sponsorship

This manuscript obtains financial support from the Ministry of Education, Culture, Sports, Science and Technology - Supported Program for the Strategic Research Foundation at Private Universities, 2015-2019 concerning (The constitution of total researching system for comprehensive disaster, medical management, corresponding to wide-scale disaster).

Conflicts of interest

The authors declare no conflicts of interest in association with this study.

Patient consent

Not applicable.

Ethics approval

This review article was approved by the review board of Juntendo Shizuoka Hospital, and all examinations were conducted according to the standards of good clinical practice and the Helsinki Declaration.

Copyright

© The Author(s) 2018.

REFERENCES

1. Abbas AE. Traumatic injury of the pulmonary artery: transection, rupture, pseudoaneurysm, or dissection? Sometimes semantics do matter. *J Thorac Cardiovasc Surg* 2016;152:1437-8.
2. Bussi eres JS. Iatrogenic pulmonary artery rupture. *Curr Opin Anaesthesiol* 2007;20:48-52.
3. Vendrell JF, Gahide G. Right pulmonary artery transection following blunt chest trauma. *Eur J Cardiothorac Surg* 2010;38:802.
4. Wurmb TE, Bernhard M. Total-body CT for initial diagnosis of severe trauma. *Lancet* 2016;388:636-8.
5. Surov A, Spielmann RP, Werdan K, Buerke M, Behrmann C. A late presentation of giant traumatic pulmonary artery aneurysm. *Circulation* 2010;122:2581-2.
6. Chen Y, Gilman MD, Humphrey KL, Salazar GM, Sharma A, Muniappan A, Shepard JO, Wu CC. Pulmonary artery pseudoaneurysms: clinical features and CT findings. *AJR Am J Roentgenol* 2017;208:84-91.
7. Block M, Lefkowitz T, Ravenel J, Leon S, Hannegan C. Endovascular coil embolization for acute management of traumatic pulmonary artery pseudoaneurysm. *J Thorac Cardiovasc Surg* 2004;128:784-5.
8. Abou Zahr R, Hellenbrand WE, Asnes JD. Iatrogenic left pulmonary artery to left atrium fistula. *Catheter Cardiovasc Interv* 2015;85:847-9.
9. Yan Y, Tweddle BA, Trerotola SO. Internal mammary artery-to-pulmonary artery and vein fistula acquired after video-assisted thoracoscopic surgery and pleurodesis. *J Vasc Interv Radiol* 2013;24:1759-61.
10. Giglioli C, Cecchi E, Angelotti P, Venditti F, Calabretta R, Scheggi V, Alterini B, Stefano P. Aortopulmonary fistula presenting with right ventricular dysfunction following blunt chest trauma. *J Card Surg* 2013;28:713.
11. Rrapo E, Lube MW, Smith CP. Right axillary artery bullet embolus and the formation of a pulmonary arteriovenous fistula after a gunshot wound to the back. *Am Surg* 2013;79:E172-4.
12. Roshanali F, Mandegar MH, Oraii S. Traumatic pulmonary arteriovenous fistula may be misdiagnosed with residual shunt after patent foramen ovale closure. *BMJ Case Rep* 2012;2012:bcr2012006802.
13. Yamakuchi M, Tanaka S, Tomosugi T, Moroki K, Yamada M, Toujou H, Uetsuhara K, Maruyama I. Pulmonary arteriovenous fistula manifesting as amaurosis fugax--case report. *Neurol Med Chir (Tokyo)* 2000;40:264-7.
14. Zhang C, Huang X, Li S, Yao H, Zhang B. Pulmonary artery dissection: a fatal complication of pulmonary hypertension. *Case Rep Med* 2016;2016:4739803.
15. Adodo DK, Kloeckner M, Bergoend E, Cou  til JP. Pulmonary artery dissection: a case treated by homograft replacement. *Ann Thorac Surg* 2017;103:e47-9.
16. Almdahl SM, Jakobsen  , Skatt r TH. Dissection of the right pulmonary artery after blunt trauma. *Eur J Cardiothorac Surg*

- 2014;46:141-2.
17. Chung JH, Mullins CD, Manchanda V, Gunn ML, Stern EJ. Pulmonary artery intimal injury associated with blunt trauma. *Emerg Radiol* 2009;16:497-9.
 18. Atreya AR, Arora S, Valania G. Pulmonary artery rupture with pseudoaneurysm formation secondary to Swan-Ganz catheter balloon inflation. *Acute Card Care* 2015;17:77-9.
 19. Inami T, Kataoka M, Shimura N, Ishiguro H, Yanagisawa R, Kawakami T, Fukuda K, Yoshino H, Satoh T. Incidence, avoidance, and management of pulmonary artery injuries in percutaneous transluminal pulmonary angioplasty. *Int J Cardiol* 2015;201:35-7.
 20. Ejiri K, Ogawa A, Matsubara H. Bail-out technique for pulmonary artery rupture with a covered stent in balloon pulmonary angioplasty for chronic thromboembolic pulmonary hypertension. *JACC Cardiovasc Interv* 2015;8:752-3.
 21. Berry MF. Pulmonary artery bleeding during video-assisted thoracoscopic surgery: intraoperative bleeding and control. *Thorac Surg Clin* 2015;25:239-47.
 22. Mei J, Pu Q, Liao H, Ma L, Zhu Y, Liu L. A novel method for troubleshooting vascular injury during anatomic thoracoscopic pulmonary resection without conversion to thoracotomy. *Surg Endosc* 2013;27:530-7.
 23. Cardillo G, Carleo F, DI Martino M, Ciamberlano B, Ialongo P, Cusumano G, Denitza Tinti M, Ricci A, Cafarotti S. Control of major pulmonary artery bleeds with a gelatin matrix-thrombin solution: a retrospective analysis. *J Cardiovasc Surg (Torino)* 2017;58:904-8.
 24. Péterffy A, Henze A. Haemorrhagic complications during pulmonary resection. A retrospective review of 1428 resections with 113 haemorrhagic episodes. *Scand J Thorac Cardiovasc Surg* 1983;17:283-7.
 25. Bozzani A, Arici V, Bellinzona G, Pirrelli S, Forni E, Otero A. Iatrogenic pulmonary artery rupture due to chest-tube insertion. *Tex Heart Inst J* 2010;37:732-3.
 26. Sundaramurthy SR, Moshinsky RA, Smith JA. Non-operative management of tube thoracostomy induced pulmonary artery injury. *Interact Cardiovasc Thorac Surg* 2009;9:759-60.
 27. Tokue H, Tokue A, Morita H, Tsushima Y. Successful interventional management for pulmonary arterial injury secondary to pacemaker implantation. *Case Rep Cardiol* 2016;2016:4340193.
 28. Hunt LB, Olshansky B, Hiratzka LF. Cardiac tamponade caused by pulmonary artery perforation after central venous catheterization. *JPEN J Parenter Enteral Nutr* 1984;8:711-3.
 29. Liu HP, Chang CH, Lin PJ, Chu JJ, Hsieh HC, Chang JP, Hsieh MC. Pulmonary artery perforation after Kirschner wire migration: case report and review of the literature. *J Trauma* 1993;34:154-6.
 30. Poplasky MR, Rozenblit G, Rundback JH, Crea G, Maddineni S, Leonardo R. Swan-Ganz catheter-induced pulmonary artery pseudoaneurysm formation: three case reports and a review of the literature. *Chest* 2001;120:2105-11.
 31. Kulshrestha P, Das B, Iyer KS, Sampath KA, Sharma ML, Rao IM, Venugopal P. Cardiac injuries--a clinical and autopsy profile. *J Trauma* 1990;30:203-7.
 32. Deneuville M. Injury of the pulmonary artery and its branches due to penetrating chest trauma. *Ann Vasc Surg* 2000;14:463-7.
 33. Demondion P, Bellemare P, El-Hamamsy I. Conservative management of an intrapericardial contained rupture of the right pulmonary artery in blunt trauma: a good idea? *J Thorac Cardiovasc Surg* 2016;152:1435-6.
 34. Maury JM, Deslandes L, Oheix S, David JS. Acute traumatic right pulmonary artery rupture in blunt trauma. *Intensive Care Med* 2015;41:134-5.
 35. Lin YY, Tiu CM, Chen JD, Chou YH, Hsueh HC, Tseng TK, Lee MH, Chang CY. Segmental pulmonary artery transection after blunt trauma. *J Chin Med Assoc* 2014;77:389-92.
 36. Muthialu N, Hoskote A, Deshpande R, Lister P. Right pulmonary hilar pedicle injury secondary to blunt chest trauma in a child. *Asian Cardiovasc Thorac Ann* 2013;21:235-8.
 37. Pereira SJ, Narrod JA. Repair of right pulmonary artery transection after blunt trauma. *Ann Thorac Surg* 2009;87:939-40.
 38. Kanani N, Ting P, Weber B, Gray RR, Maitland A. Blunt trauma resulting in systemic arterial and pulmonary artery injury: case report. *Can Assoc Radiol J* 2002;53:141-3.
 39. Ambrose G, Barrett LO, Angus GL, Absi T, Shaftan GW. Main pulmonary artery laceration after blunt trauma: accurate preoperative diagnosis. *Ann Thorac Surg* 2000;70:955-7.
 40. Weltman DI, Baykal A, Zhang D. CT diagnosis of laceration of the main pulmonary artery after blunt trauma. *AJR Am J Roentgenol* 1999;173:1361-2.
 41. Clements RH, Wagmeister LS, Carraway RP. Blunt intrapericardial rupture of the pulmonary artery in a surviving patient. *Ann Thorac Surg* 1997;64:258-60.
 42. Daon E, Gorton ME. Traumatic disruption of the innominate and right pulmonary arteries: case report. *J Trauma* 1997;43:701-2.
 43. Katz DS, Groskin SA. Pulmonary artery laceration and tension pneumothorax in blunt chest trauma. *J Thorac Imaging* 1993;8:156-8.
 44. Ohsaka H, Yanagawa Y, Miyasaka Y, Okamoto K. Successful treatment of a penetrating pulmonary artery injury caused by a Japanese sword in a patient transported by a physician-staffed helicopter. *J Emerg Trauma Shock* 2015;8:125-6.
 45. Greberski K, Bugajski P, Rzymiski S, Jarząbek R, Olczak B, Kalawski R. Penetrating thoracic injuries - treatment of two patients after suicide attempts. *Kardiochir Torakochirurgia Pol* 2015;12:62-4.
 46. Senanayake EL, Jeyatheesan J, Rogers V, Wilson IC, Graham TR. Stab to the chest causing severe great vessel injury. *Ann Thorac Surg* 2012;94:1716-8.
 47. Sanchez GP, Peng EW, Marks R, Sarkar PK. 'Scoop and run' strategy for a resuscitative sternotomy following unstable penetrating chest injury. *Interact Cardiovasc Thorac Surg* 2010;10:467-8.
 48. Atalay HH, Demirtürk OS, Kiliç D, Türköz R. Gunshot wound of the main pulmonary artery: a case report. *J Thorac Cardiovasc Surg*

- 2010;139:e17-8.
49. Babatasi G, Massetti M, Bhoyroo S, Le Page O, Khayat A. Pulmonary artery bullet injury following thoracic gunshot wound. *Eur J Cardiothorac Surg* 1999;15:87-90.
 50. Kiss SS, Tóth P, Kollár S, Nábrádi Z, Bóni J. Five-year study on the injury of the great thoracic vessels after penetrating chest injury. *Acta Chir Hung* 1999;38:75-8.
 51. Jain AK. Survival following cardiac tamponade and arrest in a paediatric patient with penetrating trauma to pulmonary artery. *Paediatr Anaesth* 1998;8:345-8.
 52. Goel S, Kumar A, Gamanagatti S, Gupta A. Spontaneous resolution of post-traumatic pulmonary artery pseudoaneurysm: report of two cases. *Lung India* 2013;30:203-5.
 53. Sridhar SK, Sadler D, McFadden SD, Ball CG, Kirkpatrick AW. Percutaneous embolization of an angiographically inaccessible pulmonary artery pseudoaneurysm after blunt chest trauma: a case report and review of the literature. *J Trauma* 2010;69:729.
 54. Reade CC, Jenkins NL, Bard MR, Kuszyk BS, Koutlas TC, Rotondo MF. Immediate diagnosis and nonoperative treatment of a pulmonary artery pseudoaneurysm after blunt traumatic injury. *J Trauma* 2006;60:894-6.
 55. Kasai T, Kobayashi K. Bilateral pseudoaneurysms of the pulmonary arteries caused by blunt chest injury. *Intensive Care Med* 1992;18:51-2.
 56. Quartey B, Jessie E. Pulmonary artery and vein pseudoaneurysm after gunshot wound to the chest. *J Emerg Trauma Shock* 2011;4:313-6.
 57. Blanié A, Fadel E, Duranteau J. Left pulmonary artery transection after penetrating thoracic trauma. *J Trauma* 2011;71:1479.
 58. Rai VK, Malireddy K, Dearmond D, Myers J, Dent DL. Traumatic pseudoaneurysm of the pulmonary artery. *J Trauma* 2010;69:730.
 59. Maddali MM, Zacharias S, Adhikari RK, Rajakumar MC, Ahmed AR. Traumatic pseudoaneurysm of pulmonary artery. *Asian Cardiovasc Thorac Ann* 2007;15:362-3.
 60. Khan AA, Bauer TL, Garcia MJ, Panasuk DB, Davies AL. Angiographic embolization of a traumatic pulmonary pseudoaneurysm. *Ann Thorac Surg* 2005;79:2136-8.
 61. Dimarakis I, Thorpe JA, Papagiannopoulos K. Successful treatment of a posttraumatic pulmonary artery pseudoaneurysm with coil embolization. *Ann Thorac Surg* 2005;79:2134-6.
 62. de Jonge I, Vahl A, van der Hulst V. Coil embolization of a left pulmonary artery pseudoaneurysm after penetrating injury. *J Endovasc Ther* 2003;10:681-3.
 63. Donaldson B, Ngo-Nonga B. Traumatic pseudoaneurysm of the pulmonary artery: case report and review of the literature. *Am Surg* 2002;68:414-6.
 64. Savage C, Zwischenberger JB, Ventura KC, Wittich GR. Hemoptysis secondary to pulmonary pseudoaneurysm 30 years after a gunshot wound. *Ann Thorac Surg* 2001;71:1021-3.
 65. Hubler B, Earls JP, Stevens K. Traumatic pulmonary arterial and venous pseudoaneurysms. *AJR Am J Roentgenol* 1997;169:1354.
 66. Huet N, Rodiere M, Badet M, Michoud M, Brichon PY, Ferretti G, Thony F. Covered stent and coils embolization of a pulmonary artery pseudoaneurysm after gunshot wound. *Cardiovasc Intervent Radiol* 2016;39:778-81.
 67. Howell A, Brown R, Ashley DW, Williams J 4th, Lane JE. Aortopulmonary fistula from penetrating thoracic trauma. *J Trauma* 2004;57:1374.
 68. Kerr A, Sauter D. Acquired traumatic pulmonary arteriovenous fistula: case report. *J Trauma* 1993;35:484-6.

Review

Open Access



Surgical revascularization for acute coronary syndromes: a narrative review

Joseph H. Joo¹, Joshua M. Liao², Faisal G. Bakaeen³, Danny Chu⁴

¹College of Medicine, Texas A&M University, Bryan, TX 77807, USA.

²Department of Medicine, University of Washington, Seattle, WA 98195, USA.

³Department of Thoracic and Cardiovascular Surgery, Cleveland Clinic, Cleveland, OH 44195, USA.

⁴Department of Cardiothoracic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA.

Correspondence to: Prof. Danny Chu, Division of Cardiac Surgery, Department of Cardiothoracic Surgery, University of Pittsburgh School of Medicine, 200 Lothrop Street, C-700 Pittsburgh, PA 15213, USA. E-mail: chud@upmc.edu

How to cite this article: Joo JH, Liao JM, Bakaeen FG, Chu D. Surgical revascularization for acute coronary syndromes: a narrative review. *Vessel Plus* 2018;2:2. <http://dx.doi.org/10.20517/2574-1209.2017.36>

Received: 30 Nov 2017 **First Decision:** 15 Jan 2018 **Revised:** 23 Jan 2018 **Accepted:** 24 Jan 2018 **Published:** 2 Feb 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Acute coronary syndrome (ACS) comprises a spectrum of disease that includes unstable angina, non-ST-elevation myocardial infarction, and ST-elevation myocardial infarction. Clinical management of patients with ACS has greatly evolved over the last two decades, but ACS remains an important cause of morbidity and mortality in patients with coronary artery disease. This narrative review describes the indication, timing, and approaches to surgical revascularization in the context of ACS. In particular, the review discusses and compares the utilization of off-pump coronary artery bypass grafting (CABG) vs. conventional on-pump CABG. Other surgical interventions, such as totally endoscopic coronary artery bypass and hybrid coronary revascularization, are also reviewed.

Keywords: Acute coronary syndromes, coronary artery bypass graft, off pump coronary artery bypass

INTRODUCTION

Acute coronary syndrome (ACS) comprises a spectrum of disease that includes unstable angina (UA), non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI), with distinctions based on symptom severity, electrocardiogram patterns, and degree of myocardial necrosis as reflected by cardiac biomarker levels^[1-3]. In-hospital and long-term mortality have improved largely because of improvements in ACS treatment modalities^[4-8]. However, ACS remains an important cause of morbidity and mortality in patients with coronary artery disease that is responsible for more than 1 million hospital admissions in the USA annually^[9]. Concurrently, indications for surgical revascularization in ACS patients



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



by coronary artery bypass grafting (CABG) as well as the overall management of ACS patients have evolved a great deal over the last 15 to 20 years^[4,10].

INDICATIONS FOR SURGICAL REVASCULARIZATION

For patients with UA or NSTEMI, treatment choices are based on the patient's level of risk as indicated by clinical symptoms, electrocardiogram changes, and cardiac biomarker levels^[5]. Based on joint guidelines from the American College of Cardiology and American Heart Association, CABG is recommended as primary treatment for patients with significant left main disease or left main equivalent (i.e. significant proximal left anterior descending and proximal left circumflex stenosis) and for patients unresponsive to maximal nonsurgical treatment (Class of Recommendation: I & Level of Evidence: A)^[10]. Surgery is also a reasonable consideration in patients with proximal left anterior descending (LAD) stenosis with 1- or 2-vessel disease, presence of complex coronary lesions, and for patients in whom percutaneous intervention is not feasible^[11-13].

For patients experiencing NSTEMI and UA, while indications for CABG vs. percutaneous coronary intervention (PCI) are similar to those for patients with stable angina, studies show that high-risk patients with left ventricular systolic dysfunction^[14,15], severe 3-vessel disease^[16-19], 2-vessel disease involving the proximal LAD, or diabetes mellitus^[20-22] should be considered for CABG. Existing guidelines affirm the indications for high-risk patients given the increased chances of long-term survival^[23,24]. In contrast, the survival benefits of CABG are much more modest in lower-risk patients. Thus, these patients should only be considered for early surgery if they are willing to accept the short-term risks associated with surgical revascularization in exchange for potentially improved functional status.

The accepted first-line treatment for STEMI is PCI or systemic thrombolysis. However, CABG is performed in up to 5% of STEMI cases^[25]. In particular, surgery is indicated among patients with good surgical targets but whose hemodynamic instability results in a complicated or failed angioplasty; after a failed fibrinolysis; who have persistent, refractory ischemia; who show evidence of mechanical or valvular disease; who are in cardiogenic shock; or who have life-threatening ventricular arrhythmias and either severe stenosis or multivessel disease^[10]. There is also class II evidence that CABG may be appropriate as primary intervention in patients for whom PCI failed, and it can also be considered in patients with evidence of severe left main or multivessel disease with poor left ventricular function or diabetes.

PROGNOSIS

Despite improvements over time, in-hospital mortality for patients with acute myocardial infarction (AMI) in the USA remains at 5% and is even higher among STEMI patients who undergo either PCI or emergency CABG^[26-30]. Additionally, NSTEMI patients undergoing surgical intervention have a poorer prognosis than their non-ACS counterparts^[31], and the hospital level 30-day risk-standardized mortality rates for patients discharged with AMI remains at approximately 16%^[32]. Outcomes for CABG are also worse in patients with ACS than in patients without ACS^[4,33]. The preoperative troponin I level has been promoted as the strongest independent predictor of short-term death^[1,31].

OFF-PUMP CABG

The advent of off-pump CABG (OPCABG) - which avoids cardiopulmonary bypass and its associated risks - brought the promise of reducing operative risk while producing long-term outcomes that were as good as or better than those of on-pump surgery^[34-36]. Several studies have since shown short-term outcomes comparable to those of on-pump CABG^[37,38], as well as lower rates of atrial fibrillation, less need for blood transfusions, less renal and neurocognitive dysfunction, and shorter hospital stays in mixed-risk patient

populations^[39]. Furthermore, OPCABG is associated with improved resource utilization and increased cost-effectiveness^[36,40].

However, the relative merits of OPCABG and on-pump CABG remain debatable and there has been national decline in the utilization of OPCABG^[41]. Randomized trials have demonstrated that short-term death or complications within a month of surgery occurred at similar frequency, but long-term mortality and complications occurred similarly if not at higher rates in patients undergoing OPCABG^[42,43]. Meta-analyses have also failed to demonstrate any significant benefit of OPCABG in mortality rates and showed comparable organ protection to conventional methods^[44,45].

Another aspect of understanding the comparative advantages of OPCABG^[46,47] is that patients who require intraoperative conversion from off-pump to on-pump surgery or abortion of the OPCABG procedure have poorer outcomes compared to patients undergoing successful OPCABG or on-pump operations^[48-51]. Additionally, patients who underwent OPCABG generally had fewer anastomoses than their on-pump counterparts, limiting the conclusions that can be drawn about OPCABG in patients with multiple targets and raising concerns about the completeness and effectiveness of revascularization in OPCABG^[39,52].

There are limited data regarding primary OPCABG for the treatment of ACS. In two studies, mortality was lower in off-pump vs. on-pump procedures (5% vs. 24%, $P = 0.015$ and 3.5% vs. 5.4%, $P = 0.690$)^[53,54]. Additionally, a European study with a cohort of 624 patients demonstrated that stratification and preselection of patients, as well as the timing of the intervention, are crucial considerations for ensuring that only appropriate candidates undergo and derive benefits from the procedure^[55]. An updated algorithm to stratify patients and better address the issue of conversion from off- to on-pump CABG has been put forth, which may help to reduce the frequency of off-pump to on-pump conversion in ACS patients^[54,56].

OTHER POTENTIAL SURGICAL INTERVENTIONS

It has been approximately two decades since several groups first described endoscopic techniques for less invasive, closed-chest totally endoscopic coronary artery bypass (TECAB) with the da Vinci robotic system^[57,58]. After a multicenter trial showed promising results, the US Food and Drug Administration approved robotically-assisted TECAB for non-emergent left internal mammary artery to LAD myocardial revascularization^[59]. Subsequently, there has been interest both in traditional arrested-heart TECAB with cardiopulmonary bypass and in beating-heart, off-pump TECAB with the use of endoscopic stabilizers. Although issues have been raised regarding technical challenges and conversion rates^[60-62], there are also data that suggest that with appropriate techniques and experience, excellent graft patency rates can be achieved^[63].

More recent advancement in this field is hybrid coronary revascularization (HCR), a procedure that combines PCI with OPCABG via minimally invasive entry through an anterolateral thoracotomy^[64]. In patients with multivessel and left main disease, HCR has been shown to be comparable to OPCABG performed via midline sternotomy with respect to short- and mid-term outcomes, without significant differences in repeat revascularization rates^[64,65].

Although no study has specifically examined the use of TECAB or HCR in the treatment of ACS, they may be alternative techniques to consider as the technology continues to advance and additional data are gathered regarding their outcomes and safety. Currently, only a few medical centers worldwide perform robotic TECAB due to the high complexity of operations, corresponding long learning curves and lack of an endoscopic surgical tradition^[66]. Therefore, more evidence is needed to quantify the benefits of HCR as an emerging procedure for ACS.

TIMING OF SURGICAL REVASCULARIZATION

In most patients with ACS who are to undergo CABG surgery, the procedure is postponed for several days to reduce procedure-related risk^[8]. The exceptions are patients with life-threatening conditions, such as severe disease or mechanical complications, who undergo early CABG. In another, AMI patients with persistent nonmechanical complications (persistent ischemia, shock), mortality rates when surgical revascularization was performed within 48 h of AMI were 7.7% for on-pump procedures performed because of persistent pain, but were negligible in those done more than 48 h later^[67]. Other work looking at patients undergoing CABG after AMI has produced similar numbers and has associated early operation with higher risk in both transmural and non-transmural AMI^[68]. There has been some suggestions, however, that even in higher-risk patients, early CABG is associated with very low in-hospital mortality and, therefore, could be considered in appropriate situations^[8]. For OPCABG, data suggest that patients taken to the operating room within 6 h from the onset of chest pain are more suitable for off-pump surgery and have a low incidence of conversion to on-pump CABG, which, as mentioned above, carries severe risks and consequences^[54].

CONCLUSION

Though the management of ACS has greatly evolved over the last two decades, the condition remains an important cause of morbidity and mortality in patients with coronary artery disease. Surgical revascularization is favored for more complex and high-risk patients. The merits of OPCABG remain debatable, and further study is needed to quantify the benefits of TECAB and HCR as emerging procedures for ACS.

DECLARATIONS

Acknowledgments

The authors would like to thank Stephen N. Palmer, PhD, ELS, for his contributions to the editing of earlier versions of the manuscript, for which he was not financially compensated.

Author's contributions

Concept/design: all authors

Definition of intellectual content: all authors

Literature search: all authors

Manuscript preparation, editing, and review: all authors

Financial support and sponsorship

None.

Conflicts of interest

Dr. Chu serves as an oral board review examiner for The Osler Institute, academic editor for Wolters Kluwer Health, and national proctor for Toray International America, Inc., and the Japanese Organization for Medical Device Development, Inc., none of which have relationship to this manuscript. Dr. Liao, Dr. Bakaeen, and Mr. Joo have no conflicts to disclose.

Patient consent

Not applicable.

Ethics approval

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Adams JE 3rd, Bodor GS, Dávila-Román VG, Delmez JA, Apple FS, Ladenson JH, Jaffe AS. Cardiac troponin I: a marker with high specificity for cardiac injury. *Circulation* 1993;88:101-6.
2. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined - a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000;36:959-69.
3. Antman EM, Tanasijevic MJ, Thompson B, Schactman M, McCabe CH, Cannon CP, Fischer GA, Fung AY, Thompson C, Wybenga D, Braunwald E. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. *N Engl J Med* 1996;335:1342-9.
4. Bertrand ME, Simoons ML, Fox KA, Wallentin LC, Hamm CW, McFadden E, De Feyter PJ, Specchia G, Ruzyllo W; Task Force on the Management of Acute Coronary Syndromes of the European Society of Cardiology. Management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J* 2002;23:1809-40.
5. Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, Jones RH, Kereiakes D, Kupersmith J, Levin TN, Pepine CJ, Schaeffer JW, Smith EE 3rd, Steward DE, Theroux P, Gibbons RJ, Alpert JS, Faxon DP, Fuster V, Gregoratos G, Hiratzka LF, Jacobs AK, Smith SC Jr; American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina). ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina). *Circulation* 2002;106:1893-900.
6. Van de Werf F, Ardissino D, Betriu A, Cokkinos DV, Falk E, Fox KA, Julian D, Lengyel M, Neumann FJ, Ruzyllo W, Thygesen C, Underwood SR, Vahanian A, Verheugt FW, Wijns W; Task Force on the Management of Acute Myocardial Infarction of the European Society of Cardiology. Management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J* 2003;24:28-66.
7. Antman EM, Anbe DT, Armstrong PW, Bates ER, Green LA, Hand M, Hochman JS, Krumholz HM, Kushner FG, Lamas GA, Mullany CJ, Ornato JP, Pearle DL, Sloan MA, Smith SC Jr, Alpert JS, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Gregoratos G, Halperin JL, Hiratzka LF, Hunt SA, Jacobs AK; American College of Cardiology; American Heart Association Task Force on Practice Guidelines; Canadian Cardiovascular Society. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of Patients With Acute Myocardial Infarction). *Circulation* 2004;110:e82-292.
8. Monteiro P; Portuguese Registry on Acute Coronary Syndromes. Impact of early coronary artery bypass graft in an unselected acute coronary syndrome patient population. *Circulation* 2006;114:I467-72.
9. Eisen A, Giugliano RP, Braunwald E. Updates on acute coronary syndrome: a review. *JAMA Cardiol* 2016;1:718-30.
10. Eagle KA, Guyton RA, Davidoff R, Edwards FH, Ewy GA, Gardner TJ, Hart JC, Herrmann HC, Hillis LD, Hutter AM Jr, Lytle BW, Marlow RA, Nugent WC, Orszulak TA, Antman EM, Smith SC Jr, Alpert JS, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Gregoratos G, Halperin JL, Hiratzka LF, Hunt SA, Jacobs AK, Ornato JP; American College of Cardiology; American Heart Association Task Force on Practice Guidelines; American Society for Thoracic Surgery and the Society of Thoracic Surgeons. ACC/AHA 2004 guideline update for coronary artery bypass graft surgery: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1999 Guidelines for Coronary Artery Bypass Graft Surgery). *Circulation* 2004;110:1168-76.
11. Aldea GS, Bakaeen FG, Pal J, Fremes S, Head SJ, Sabik J, Rosengart T, Kappetein AP, Thourani VH, Firestone S, Mitchell JD; Society of Thoracic Surgeons. The Society of Thoracic Surgeons Clinical Practice Guidelines on Arterial Conduits for Coronary Artery Bypass Grafting. *Ann Thorac Surg* 2016;101:801-9.
12. Farooq V, van Klaveren D, Steyerberg EW, Meliga E, Vergouwe Y, Chieffo A, Kappetein AP, Colombo A, Holmes DR Jr, Mack M, Feldman T, Morice MC, Stähle E, Onuma Y, Morel MA, Garcia-Garcia HM, van Es GA, Dawkins KD, Mohr FW, Serruys PW. Anatomical and clinical characteristics to guide decision making between coronary artery bypass surgery and percutaneous coronary intervention for individual patients: development and validation of SYNTAX score II. *Lancet* 2013;381:639-50.
13. Tarakji KG, Sabik JF 3rd, Bhudia SK, Batizy LH, Blackstone EH. Temporal onset, risk factors, and outcomes associated with stroke after coronary artery bypass grafting. *JAMA* 2011;305:381-90.
14. Yusuf S, Zucker D, Passamani E, Peduzzi P, Fisher LD, Takaro T, Kennedy JW, Davis K, Killip T, Norris R, Morris C, Mathur V, Varnauskas E, Chalmers TC. Effect of coronary artery bypass graft surgery on survival: overview of 10-year results from randomised trials by the Coronary Artery Bypass Graft Surgery Trialists Collaboration. *Lancet* 1994;344:563-70.
15. Mock MB, Fisher LD, Holmes DR Jr, Gersh BJ, Schaff HV, McConney M, Rogers WJ, Kaiser GC, Ryan TJ, Myers WO, Killip T 3rd; Participants in the Coronary Artery Surgery Study. Comparison of effects of medical and surgical therapy on survival in severe angina pectoris and two-vessel coronary artery disease with and without left ventricular dysfunction: a Coronary Artery Surgery Study Registry Study. *Am J Cardiol* 1988;61:1198-203.
16. Yusuf S, Wittes J, Friedman L. Overview of results of randomized clinical trials in heart disease. II. Unstable angina, heart failure, primary prevention with aspirin, and risk factor modification. *JAMA* 1988;260:2259-63.
17. The BARI Investigators. Seven-year outcome in the Bypass Angioplasty Revascularization Investigation (BARI) by treatment and diabetic status. *J Am Coll Cardiol* 2000;35:1122-9.
18. Mohr FW, Morice MC, Kappetein AP, Feldman TE, Stähle E, Colombo A, Mack MJ, Holmes DR Jr, Morel MA, Van Dyck N, Houle

- VM, Dawkins KD, Serruys PW. Coronary artery bypass graft surgery versus percutaneous coronary intervention in patients with three-vessel disease and left main coronary disease: 5-year follow-up of the randomised, clinical SYNTAX trial. *Lancet* 2013;381:629-38.
19. Serruys PW, Morice MC, Kappetein AP, Colombo A, Holmes DR, Mack MJ, Ståhle E, Feldman TE, van den Brand M, Bass EJ, Van Dyck N, Leadley K, Dawkins KD, Mohr FW; SYNTAX Investigators. Percutaneous coronary intervention versus coronary-artery bypass grafting for severe coronary artery disease. *N Engl J Med* 2009;360:961-72.
 20. Jones RH, Kesler K, Phillips HR 3rd, Mark DB, Smith PK, Nelson CL, Newman MF, Reves JG, Anderson RW, Califf RM. Long-term survival benefits of coronary artery bypass grafting and percutaneous transluminal angioplasty in patients with coronary artery disease. *J Thorac Cardiovasc Surg* 1996;111:1013-25.
 21. First-year results of CABRI (Coronary Angioplasty versus Bypass Revascularisation Investigation). CABRI Trial Participants. *Lancet* 1995;346:1179-84.
 22. Weintraub WS, Stein B, Kosinski A, Douglas JS Jr, Ghazzal ZM, Jones EL, Morris DC, Guyton RA, Craver JM, King SB 3rd. Outcome of coronary bypass surgery versus coronary angioplasty in diabetic patients with multivessel coronary artery disease. *J Am Coll Cardiol* 1998;31:10-9.
 23. Gu YL, van der Horst IC, Douglas YL, Svilaas T, Mariani MA, Zijlstra F. Role of coronary artery bypass grafting during the acute and subacute phase of ST-elevation myocardial infarction. *Neth Heart J* 2010;18:348-54.
 24. Wright RS, Anderson JL, Adams CD, Bridges CR, Casey DE Jr, Ettinger SM, Fesmire FM, Ganiats TG, Jneid H, Lincoff AM, Peterson ED, Philippides GJ, Theroux P, Wenger NK, Zidar JP, Anderson JL, Adams CD, Antman EM, Bridges CR, Califf RM, Casey DE Jr, Chavey WE 2nd, Fesmire FM, Hochman JS, Levin TN, Lincoff AM, Peterson ED, Theroux P, Wenger NK, Wright RS. 2011 ACCF/AHA Focused Update of the Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction (Updating the 2007 Guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the American College of Emergency Physicians, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Am Coll Cardiol* 2011;57:1920-59.
 25. Anderson JL, Adams CD, Antman EM, Bridges CR, Califf RM, Casey DE Jr, Chavey WE 2nd, Fesmire FM, Hochman JS, Levin TN, Lincoff AM, Peterson ED, Theroux P, Wenger NK, Wright RS, Smith SC Jr, Jacobs AK, Halperin JL, Hunt SA, Krumholz HM, Kushner FG, Lytle BW, Nishimura R, Ornato JP, Page RL, Riegel B; American College of Cardiology; American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non ST-Elevation Myocardial Infarction); American College of Emergency Physicians; Society for Cardiovascular Angiography and Interventions; Society of Thoracic Surgeons; American Association of Cardiovascular and Pulmonary Rehabilitation; Society for Academic Emergency Medicine. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non ST-Elevation Myocardial Infarction): developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons: endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. *Circulation* 2007;116:e148-304.
 26. McNamara RL, Kennedy KF, Cohen DJ, Diercks DB, Moscucci M, Ramee S, Wang TY, Connolly T, Spertus JA. Predicting in-hospital mortality in patients with acute myocardial infarction. *J Am Coll Cardiol* 2016;68:626-35.
 27. Matetzky S, Sharir T, Domingo M, Noc M, Chyu KY, Kaul S, Eigler N, Shah PK, Cercek B. Elevated troponin I level on admission is associated with adverse outcome of primary angioplasty in acute myocardial infarction. *Circulation* 2000;102:1611-6.
 28. Giannitsis E, Muller-Bardorff M, Lehrke S, Wiegand U, Tolg R, Weidtmann B, Hartmann F, Richardt G, Katus HA. Admission troponin T level predicts clinical outcomes, TIMI flow, and myocardial tissue perfusion after primary percutaneous intervention for acute ST-segment elevation myocardial infarction. *Circulation* 2001;104:630-5.
 29. Albes JM, Gross M, Franke U, Wippermann J, Cohnert TU, Vollandt R, Wahlers T. Revascularization during acute myocardial infarction: risks and benefits revisited. *Ann Thorac Surg* 2002;74:102-8.
 30. Lee DC, Oz MC, Weinberg AD, Ting W. Appropriate timing of surgical intervention after transmural acute myocardial infarction. *J Thorac Cardiovasc Surg* 2003;125:115-20.
 31. Thielmann M, Jakob H. Surgical revascularization and perioperative management in patients with non-ST-elevation acute coronary syndromes. *Rocz Akad Med Białymst* 2005;50:37-44.
 32. Krumholz HM, Wang Y, Chen J, Drye EE, Spertus JA, Ross JS, Curtis JP, Nallamothu BK, Lichtman JH, Havranek EP, Masoudi FA, Radford MJ, Han LF, Rapp MT, Straube BM, Normand SL. Reduction in acute myocardial infarction mortality in the United States: risk-standardized mortality rates from 1995-2006. *JAMA* 2009;302:767-73.
 33. Louagie YAG, Jamart J, Buche M, Eucher PM, Schoevaerdts D, Collard E, Gonzalez M, Marchandise B, Schoevaerdts JC. Operation for unstable angina pectoris: factors influencing adverse in-hospital outcome. *Ann Thorac Surg* 1995;59:1141-9.
 34. Cleveland JC Jr, Shroyer AL, Chen AY, Peterson E, Grover FL. Off-pump coronary artery bypass grafting decreases risk-adjusted mortality and morbidity. *Ann Thorac Surg* 2001;72:1282-9.
 35. Aub-Omar Y, Taggart DP. Off-pump coronary artery bypass grafting. *Lancet* 2002;360:327-9.
 36. Van Dijk D, Nierich AP, Jansen EWL, Nathoe HM, Suyker WJL, Diephuis JC, van Boven WJ, Borst C, Buskens E, Grobbee DE, de Medina EOR, de Jaegere PPT. Early outcome after off-pump versus on-pump coronary bypass surgery: results from a randomized study. *Circulation* 2001;104:1761-6.
 37. Nathoe HM, Van Dijk D, Jansen E, Suyker W, Diephuis JC, van Boven WJ, de la Rivière AB, Borst C, Kalkman CJ, Grobbee DE, Buskens E, de Jaegere PP; Octopus Study Group. A comparison of on-pump and off-pump coronary bypass surgery in low-risk patients.

- N Engl J Med* 2003;348:394-402.
38. Reston JT, Tregear SJ, Turkelson CM. Outcomes following off-pump coronary artery bypass grafting. *Ann Thorac Surg* 2003;76:1510-5.
39. Cheng DC, Bainbridge D, Martin JE, Novick RJ. Does off-pump coronary artery bypass reduce mortality, morbidity, and resource utilization when compared with conventional coronary artery bypass? A meta-analysis of randomized trials. *Anesthesiology* 2005;102:188-203.
40. Puskas J, Cheng D, Knight J, Angelini G, Decannier D, Diegeler A, Dullum M, Martin J, Ochi M, Patel N, Sim E, Trehan N, Zamvar V. Off-pump versus conventional coronary artery bypass grafting: a meta-analysis and consensus statement from the 2004 ISMICS Consensus Conference. *Innovations (Phila)* 2005;1:3-27.
41. Bakaeen FG, Shroyer AL, Gammie JS, Sabik JF, Cornwell LD, Coselli JS, Rosengart TK, O'Brien SM, Wallace A, Shahian DM, Grover FL, Puskas JD. Trends in use of off-pump coronary artery bypass grafting: results from the Society of Thoracic Surgeons Adult Cardiac Surgery Database. *J Thorac Cardiovasc Surg* 2014;148:856-63, 864.e1; discussion 863-4.
42. Shroyer AL, Grover FL, Hattler B, Collins JF, McDonald GO, Kozora E, Lucke JC, Baltz JH, Novitzky D; Veterans Affairs Randomized On/Off Bypass (ROOBY) Study Group. On-pump versus off-pump coronary-artery bypass surgery. *N Engl J Med* 2009;361:1827-37.
43. Lamy A, Devereaux PJ, Prabhakaran D, Taggart DP, Hu S, Straka Z, Piegas LS, Avezum A, Akar AR, Lanus Zanetti F, Jain AR, Noiseux N, Padmanabhan C, Bahamondes JC, Novick RJ, Tao L, Olavegogeascoechea PA, Airan B, Sulling TA, Whitlock RP, Ou Y, Gao P, Pettit S, Yusuf S; CORONARY Investigators. Five-year outcomes after off-pump or on-pump coronary-artery bypass grafting. *N Engl J Med* 2016;375:2359-68.
44. Möller CH, Penninga L, Wetterslev J, Steinbrüchel DA, Gluud C. Off-pump versus on-pump coronary artery bypass grafting for ischaemic heart disease. *Cochrane Database Syst Rev* 2012;(3):CD007224.
45. Raja S. Off-pump versus on-pump coronary artery bypass grafting: comparative effectiveness. *Comp Effect Res* 2015;5:73-9.
46. Al-Ruzzeh S, Ambler G, Asimakopoulos G, Omar RZ, Hasan R, Fabri B, El-Gamel A, DeSouza A, Zamvar V, Griffin S, Keenan D, Trivedi U, Pullan M, Cale A, Cowen M, Taylor K, Amrani M. Off-pump coronary artery bypass (OPCAB) surgery reduces risk-stratified morbidity and mortality: a United Kingdom multi-center comparative analysis of early clinical outcome. *Circulation* 2003;108:1-8.
47. Mack MJ, Pfister A, Bachand D, Emery R, Magee MJ, Connolly M, Subramanian V. Comparison of coronary bypass surgery with and without cardiopulmonary bypass in patients with multivessel disease. *J Thorac Cardiovasc Surg* 2004;127:167-73.
48. Patel NC, Patel NU, Loulmet DF, McCabe JC, Subramanian VA. Emergency conversion to cardiopulmonary bypass during attempted off-pump revascularization results in increased morbidity and mortality. *J Thorac Cardiovasc Surg* 2004;128:655-61.
49. Edgerton JR, Dewey TM, Magee MJ, Herbert MA, Prince SL, Jones KK, Mack MJ. Conversion in off-pump coronary artery bypass grafting: an analysis of predictors and outcomes. *Ann Thorac Surg* 2003;76:1138-43.
50. Iaco AL, Contini M, Teodori G, Di Mauro M, Di Giammarco G, Vitolla G, Iovino T, Calafiore AM. Off or on bypass: what is the safety threshold? *Ann Thorac Surg* 1999;68:148614-89.
51. Jin R, Hiratzka LF, Grunkemeier GL, Krause A, Page US. Aborted off-pump coronary artery bypass patients have much worse outcomes than on-pump or successful off-pump patients. *Circulation* 2005;112:1332-7.
52. Hattler B, Messenger JC, Shroyer AL, Collins JF, Haugen SJ, Garcia JA, Baltz JH, Cleveland JC Jr, Novitzky D, Grover FL; Veterans Affairs Randomized On/Off Bypass (ROOBY) Study Group. Off-pump coronary artery bypass surgery is associated with worse arterial and saphenous vein graft patency and less effective revascularization: results from the Veterans Affairs Randomized On/Off Bypass (ROOBY) Trial. *Circulation* 2012;125:2827-35.
53. Locker C, Shapira I, Paz Y, Kramer A, Gurevitch J, Matsa M, Pevni D, Mohr R. Emergency myocardial revascularization for acute myocardial infarction: survival benefits of avoiding cardiopulmonary bypass. *Eur J Cardiothorac Surg* 2000;17:234-8.
54. Kaya K, Cavalli R, Telli A, Soyul MF, Aslan A, Gokaslan G, Mursel S, Tasoz R. Off-pump versus on-pump coronary artery bypass grafting in acute coronary syndrome: a clinical analysis. *J Cardiothorac Surg* 2010;5:31.
55. Jasinski MJ, Wos S, Olszowska P, Bachowski R, Ceglarek W, Widenka K, Gemel M, Domaradzki W, Deja M, Szafranek A, Golba K, Szurlej D. Primary OPCAB as a strategy for acute coronary syndrome and acute myocardial infarction. *Heart Surg Forum* 2003;6:31-5.
56. Borde D, Asegaonkar B, Apsingekar P, Khade S, Futane S, Khodve B, Annachatre A, Puranik M, Borgaonkar V, Belapurkar Y, Joshi S. Risk stratification in off-pump coronary artery bypass (OPCAB) surgery - role of EuroSCORE II. *J Cardiothorac Vasc Anesth* 2015;29:1167-71.
57. Loulmet D, Carpentier A, d'Attellis N, Berrebi A, Cardon C, Ponzio O, Aupécle B, Relland JY. Endoscopic coronary artery bypass grafting with the aid of robotic assisted instruments. *J Thorac Cardiovasc Surg* 1999;118:4-10.
58. Falk V, Diegeler A, Walther T, Banusch J, Brucerius J, Raumans J, Autschbach R, Mohr FW. Total endoscopic computer enhanced coronary artery bypass grafting. *Eur J Cardiothorac Surg* 2000;17:38-45.
59. Argenziano M, Katz M, Bonatti J, Srivastava S, Murphy D, Poirier R, Loulmet D, Siwek L, Kreaden U, Ligon D; TECAB Trial Investigators. Results of the prospective multicenter trial of robotically assisted totally endoscopic coronary artery bypass grafting. *Ann Thorac Surg* 2006;81:1666-75.
60. de Cannière D, Wimmer-Greinecker G, Cichon R, Guliemos V, Van Praet F, Seshadri-Kreaden U, Falk V. Feasibility, safety, and efficacy of totally endoscopic coronary artery bypass grafting: multicenter European experience. *J Thorac Cardiovasc Surg* 2007;134:710-6.
61. Bonatti J, Schachner T, Bonaros N, Ohlinger A, Danzmayr M, Jonetzko P, Friedrich G, Kolbitsch C, Mair P, Laufer G. Technical challenges in totally endoscopic robotic coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 2006;131:146-53.
62. Bolton JW, Connolly J. Results of a phase one study on robotically assisted myocardial revascularization on the beating heart. *Ann Thorac Surg* 2004;78:154-8.

63. Srivastava S, Gadasalli S, Agusala M, Kolluru R, Barrera R, Quismundo S, Kreaden U, Jeevanandam V. Beating heart totally endoscopic coronary artery bypass. *Ann Thorac Surg* 2010;89:1873-80.
64. Halkos ME, Vassiliades TA, Douglas JS, Morris DC, Rab ST, Liberman HA, Samady H, Kilgo PD, Guyton RA, Puskas JD. Hybrid coronary revascularization versus off-pump coronary artery bypass grafting for the treatment of multivessel coronary artery disease. *Ann Thorac Surg* 2011;92:1695-702.
65. Halkos ME, Rab T, Vassiliades TA, Morris DC, Douglas JS, Kilgo PD, Liberman HA, Guyton RA, Thourani VH, Puskas JD. Hybrid coronary revascularization versus off-pump coronary artery bypass for the treatment of left main coronary stenosis. *Ann Thorac Surg* 2011;92:2155-60.
66. Canale LS, Mick S, Mihaljevic T, Nair R, Bonatti J. Robotically assisted totally endoscopic coronary artery bypass surgery. *J Thorac Dis* 2013;5 Suppl 6:S641-9.
67. Nunley DL, Grunkemeier GL, Teply JF, Abbruzzese PA, Davis JS, Khonsari S, Starr A. Coronary bypass operation following acute complicated myocardial infarction. *J Thorac Cardiovasc Surg* 1983;85:485-91.
68. Lee DC, Oz MC, Weiberg AD, Lin SX, Ting W. Optimal timing of revascularization: Transmural versus nontransmural acute myocardial infarction. *Ann Thorac Surg* 2001;71:1198-204.

Case Report

Open Access



Temporizing amplatzer closure of an aorto-enteric fistula associated with a blind aortic stump via a translumbar approach

S. Keisin Wang, Justin R. King, Ashley R. Gutwein, Raghu L. Motaganahalli, Andres Fajardo, Gary W. Lemmon

Division of Vascular Surgery, Department of Surgery, Indiana University School of Medicine, Indianapolis, IN 46202, USA.

Correspondence to: Dr. S. Keisin Wang, Division of Vascular Surgery, Department of Surgery, Indiana University School of Medicine, 1801 Senate Blvd MPC# 2-3500, Indianapolis, IN 46202, USA. E-mail: wangkei@iupui.edu

How to cite this article: Wang SK, King JR, Gutwein AR, Motaganahalli RL, Fajardo A, Lemmon GW. Temporizing amplatzer closure of an aorto-enteric fistula associated with a blind aortic stump via a translumbar approach. *Vessel Plus* 2018;2:3. <http://dx.doi.org/10.20517/2574-1209.2017.38>

Received: 13 Dec 2017 **First Decision:** 17 Jan 2018 **Revised:** 18 Jan 2018 **Accepted:** 27 Jan 2018 **Published:** 7 Feb 2018

Science Editor: Aaron S. Dumont **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

We present a case of an aorto-enteric fistula (AEF) with chronic, persistent bleeding from a blind aortic stump managed by endovascular means. This novel approach may have extended the life of a patient who would otherwise have been subject to a high perioperative morbidity or persistent bleeding and death. While our patient ultimately expired, we believe this technique can be considered for temporization in highly-selected patients.

Keywords: Aorto-enteric fistula, amplatzer vascular plug, endovascular treatment

INTRODUCTION

Aorto-enteric fistula (AEF) is a highly-morbid complication following aortic reconstruction. Although the traditionally accepted method of treatment is open surgical repair, alternative methods need to be considered in high operative-risk patients. We present a case of endovascular plug placement within a blind aortic stump for treatment of an AEF 16 years after an index aorto-bifemoral bypass (ABF). While the patient initially experienced resolution of his symptoms, this ultimately proved to be a temporizing measure as the patient eventually died of hemorrhagic shock related to AEF progression.



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





Figure 1. Double-balloon upper endoscopy demonstrated a mucosal defect in the 3rd portion of the duodenum suggestive of a sinus tract. This area was clipped to mark the anatomic location. Computed tomography angiogram following endoscopy demonstrated a diverticulum immediately adjacent to the clip suggestive of a small aorto-enteric fistula

CASE REPORT

A 75-year-old male was transferred to our facility for evaluation of intermittent gastrointestinal (GI) bleeding requiring over 20 units of packed red blood cells over the 6 months previous to admission. His past surgical history was significant for ABF bypass to treat and infrarenal abdominal aortic aneurysm in 2001. Three years following his initial operation, the patient developed a perigraft infection complicated by AEF treated by partial colectomy, end ileostomy, aortic graft explantation, ligation of the infrarenal aorta, and axillo-bifemoral bypass. The blind aortic stump was created two centimeters distal to the renal ostia.

The GI bleeding was initially evaluated at an outside facility with multiple computed tomography angiograms (CTAs), capsule endoscopies, and direct upper/lower GI endoscopies. Following transfer to our facility, double-balloon endoscopy demonstrated a small mucosal defect suspicious for a sinus tract in the 3rd portion of the duodenum [Figure 1]. An endoclip was placed for as a reference point. Repeat CTA visualized the endoclip near an aortic diverticulum at the inferior border of the blind stump [Figure 2]. Because of his medical comorbidities (chronic kidney disease III, hypertension, anemia, frailty, multiple abdominal operations creating a hostile abdomen with left-sided ostomy) and patient preference, open surgery was ruled out as a treatment option. At this point, we elected to proceed with embolization of the distal aortic stump.

Under moderate sedation, the left radial artery was accessed. After heparin infusion, a long 5-F catheter was positioned in the aorta and a diagnostic aortogram was performed. Unfortunately, the brachial artery was of unsuitable size for the sheath required for our planned embolization. Using a “down the barrel” technique, an 18-G trocar needle was advanced into the terminal aspect of the abdominal aortic stump via a translumbar approach. An 8-F sheath was inserted via the translumbar access, positioned in the abdominal aorta, and confirmed via angiography. A 22-mm Amplatzer II Vascular Plug (St. Jude Medical) was then deployed such that the plug spanned from the luminal aspect of the terminal abdominal aorta to the extraluminal portion of the aortic stump to prevent migration. Through the radial artery, multiple Ruby (Penumbra) coils were deployed past the vascular plug to thrombose the residual stump [Figure 3].

The patient had an uneventful postoperative course and was discharged 2 days later on lifelong oral antibiotics. At a 5-week postoperative visit, imaging demonstrated continued thrombosis of the aortic stump, decreased periaortic soft tissue swelling, and no extravasation of contrast [Figure 4]. The patient stated that his GI bleeding had completely resolved.



Figure 2. After placement of a metallic clip in the duodenum at the visualized lesion, a computed tomography angiogram demonstrated a diverticulum of the aortic stump (black arrow) near the duodenal clip (white arrow) concerning for another aorto-enteric fistula

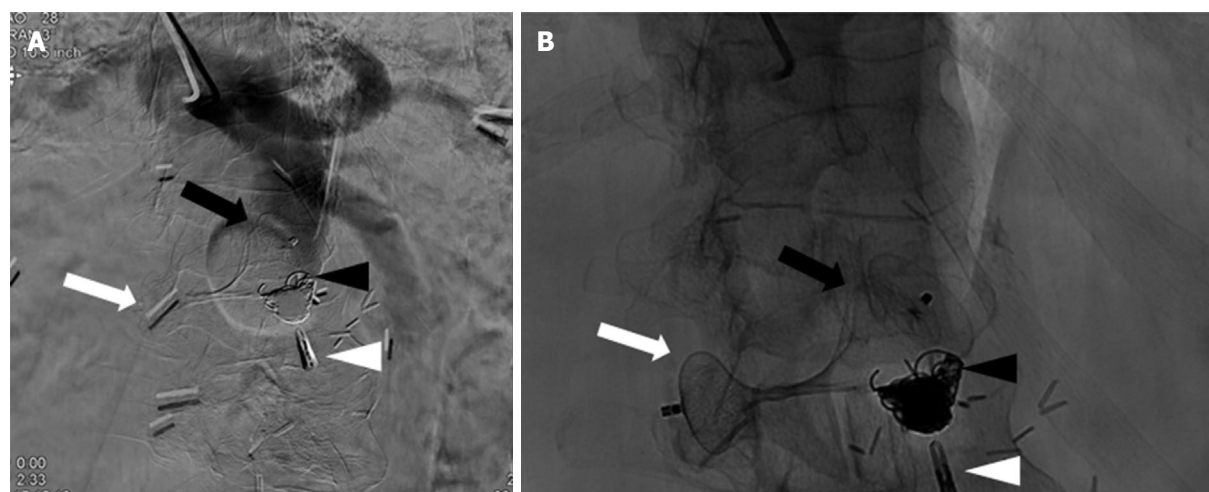


Figure 3. Digital subtraction angiography (A) and fluoroscopy (B) demonstrated placement of the Amplatzer II Vascular Plug so that 2/3 of the plug remained intraluminally (black arrow) in the aortic stump while 1/3 remained in the periaortic space (white arrow). Microcoils were deployed distal to the intraluminal plug to further assist in stump thrombosis (black arrowhead). The close association between the aortic stump and the duodenum is demonstrated by the endoclip placed at the time of double-balloon endoscopy (white arrowhead)

Approximately 14 weeks after our procedure, the patient presented with recurrent GI bleeding. The AEF was again identified by endoclip on upper GI endoscopy. The patient underwent further coil placement within the aortic stump via the radial artery. Following this reintervention, he was discharged home without repeat bleeding. Unfortunately, he proceeded to present in similar fashions 2 additional times; the first was managed with endoscopic clipping. However, on his final presentation 5 months postoperatively, the decision was made to proceed with definitive surgical ligation of the aortic stump given multiple failed endovascular and endoscopic interventions. On the day prior to his scheduled surgery, the patient developed acute hemorrhagic shock identified via his nasogastric tube. Unfortunately, he became hemodynamically unstable, was emergently intubated, and initiated on a massive transfusion protocol. After discussion with the patient's family, code status was changed to "do not resuscitate", comfort care was initiated, and he ultimately expired.

DISCUSSION

AEF is a rare and highly morbid complication following aortic intervention with an incidence of approximately 1%^[1]. In the aortic reconstruction patient, it is thought that pathogenesis is related to

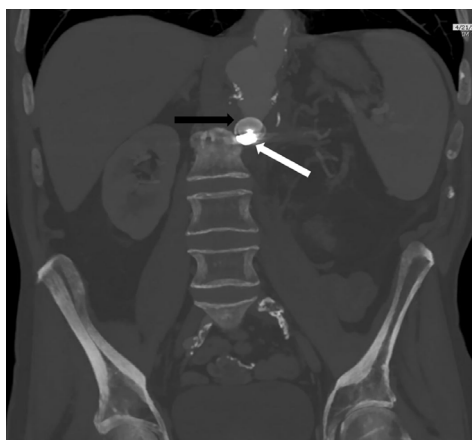


Figure 4. Five weeks post-operatively, the patient remained bleed-free without leukocytosis or fever. Computed tomography angiogram demonstrated improvement in peri-aortic inflammation and stable positioning of the intraluminal vascular plug (black arrow) and the microcoils (white arrow)

instrumentation, enteric contact with aortic wall or graft, and persistent inflammation^[2-4]. Spontaneous rupture of the aorta through the fistulous connection can present as subclinical, persistent bleeding, or life threatening exsanguination and carries a mortality rate approaching 100% if left untreated^[5,6].

Patients developing AEF may prove to be difficult to diagnose as they tend to present with episodic bleeding. Therefore, a high index of suspicion must be maintained. Other clinical manifestations may include abdominal pain, palpable abdominal mass, and nausea^[2]. However, these symptoms are either vague or often absent. Our patient presented with episodic bleeding without other accompanying symptoms for 6 months and underwent multiple CTAs and endoscopies before he was diagnosed. This illustrates well the difficulty in making the diagnosis and locating the site of pathology.

High-quality evidence regarding outcomes after treatment of AEFs are severely lacking^[5]. One of the larger experiences was published by Armstrong *et al.*^[7] in 2005. In this study, the authors described their experience with secondary AEFs in 29 patients. The most common procedures performed were excision with extra-anatomic bypass including axillo-femoral and cross-femoral bypass grafting ($n = 25$), aorto-femoral grafting with additional lower-limb bypasses ($n = 2$), and *in situ* reconstruction with rifampin-soaked Dacron ($n = 2$). Perioperative mortality in those who received an operation at 30-day was 21%^[7].

A European meta-analysis of both endovascular and open intervention for secondary AEFs was published in 2016 by Kakkos *et al.*^[8]. The authors included 98 patients who received endovascular intervention along with 725 open repairs from 1999 to 2015. In-hospital mortality was 7.1% in the endovascular group compared to the 33.9% in the open repair cohort, though this would be expected given the intent of each treatment modality, specifically palliation versus definitive repair. Interestingly, there was no difference in recurrence between the two treatment modalities. While the early survival benefit dampened during follow-up, it continued to remain significant. Not surprisingly, late sepsis was twice as high in the endovascular group compared to open repair at two years postoperatively (42% vs. 19%). The authors conclude that endovascular repair is associated with early benefit which is lost over time; therefore, they argue for staged repair with eventual conversion to *in situ* vein grafting in selected patients^[8].

Based on our previous experience and the established literature, we did not believe our patient would have survived an open operation and therefore reserved open repair initially. He had multiple abdominal procedures stemming from his original aortic reconstruction which resulted in takebacks, ostomies, and

intraabdominal abscesses. Additionally, he suffered from frailty, malnutrition, and chronic kidney disease. After discussion regarding his treatment options, he was adamant on intervention to stop the GI bleeding. Therefore, an endovascular intervention was attempted as a palliative procedure. Although our patient initially demonstrated cessation of his GI bleeding following endovascular plug deployment, this success was short-lived and the patient experienced recurrence of his symptoms with ultimate exsanguination and death.

DECLARATIONS

Authors' contributions

Concept: Wang SK

Design, literature search, manuscript preparation, manuscript editing, review: Wang SK, King JR, Gutwein AR, Motaganahalli RL, Fajardo A, Lemmon GW

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Written consent was obtained from the family of the deceased and is available upon request.

Ethics approval

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Song Y, Liu Q, Shen H, Jia X, Zhang H, Qiao L. Diagnosis and management of primary aortoenteric fistulas--experience learned from eighteen patients. *Surgery* 2008;143:43-50.
2. Duncan JR, Renwick AA, Mackenzie I, Gilmour DG. Primary aortoenteric fistula: pitfalls in the diagnosis of a rare condition. *Ann Vasc Surg* 2002;16:242-5.
3. Sweeney MS, Gadacz TR. Primary aortoduodenal fistula: manifestation, diagnosis, and treatment. *Surgery* 1984;96:492-7.
4. van Olffen TB, Knippenberg LH, van der Vliet JA, Lastdrager WB. Primary aortoenteric fistula: report of six new cases. *Cardiovasc Surg* 2002;10:551-4.
5. Saers SJ, Scheltinga MR. Primary aortoenteric fistula. *Br J Surg* 2005;92:143-52.
6. Voorhoeve R, Moll FL, de Letter JA, Bast TJ, Wester JP, Slee PH. Primary aortoenteric fistula: report of eight new cases and review of the literature. *Ann Vasc Surg* 1996;10:40-8.
7. Armstrong PA, Back MR, Wilson JS, Shames ML, Johnson BL, Bandyk DF. Improved outcomes in the recent management of secondary aortoenteric fistula. *J Vasc Surg* 2005;42:660-6.
8. Kakkos SK, Bicknell CD, Tsolakis IA, Bergqvist D; Hellenic Co-operative Group on Aortic Surgery. Management of secondary aortoenteric and other abdominal arterio-enteric fistulas: a review and pooled data analysis. *Eur J Vasc Endovasc Surg* 2016;52:770-86.

Editorial

Open Access



Early treatment of functional tricuspid regurgitation at the time of mitral valve surgery: an increased risk or an additional benefit?

Paolo Nardi, Calogera Pisano, Antonio Pellegrino, Fabio Bertoldo, Sabrina Ferrante, Monica Greci, Sara Rita Vacirca, Marco Russo, Giovanni Ruvo

Department of Cardiac Surgery, Tor Vergata University Policlinic, Rome 00133, Italy.

Correspondence to: Dr. Paolo Nardi, Department of Cardiac Surgery, Tor Vergata University Policlinic, Viale Oxford 81, Rome 00133, Italy. E-mail: pa.nardi4@libero.it

How to cite this article: Nardi P, Pisano C, Pellegrino A, Bertoldo F, Ferrante S, Greci M, Vacirca SR, Russo M, Ruvo G. Early treatment of functional tricuspid regurgitation at the time of mitral valve surgery: an increased risk or an additional benefit? *Vessel Plus* 2018;2:4. <http://dx.doi.org/10.20517/2574-1209.2017.35>

Received: 9 Nov 2017 **First Decision:** 9 Feb 2018 **Revised:** 24 Feb 2018 **Accepted:** 7 Mar 2018 **Published:** 13 Mar 2018

Science Editor: Alexander D. Verin **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

INTRODUCTION

Functional tricuspid regurgitation (FTR) refers to tricuspid insufficiency occurring secondarily to left-sided heart valve disease, especially mitral stenosis or regurgitation, in the absence of organic lesions of the tricuspid valve. In the late 1960s, the observation that mitral valve surgery sometimes led to an improvement in FTR suggested a conservative approach^[1]. On the contrary, in the 1970s, Carpentier *et al.*^[2] reported excellent results with tricuspid valve repair, arguing for systematic repair of FTR.

With the increasing population of long-term survivors of prosthetic mitral valve replacement, it has been observed that many patients developed late heart failure as the result of onset or progression of FTR in a severe form. Severe FTR is associated with substantially poorer functional outcomes and survival if untreated^[3,4]. Moreover, data showing late development of severe FTR in patients with mild or mild-to-moderate regurgitation at the time of mitral valve surgery have more recently pushed towards early aggressive intervention on the tricuspid valve in concomitance with the treatment of mitral valve disease. This raised the question if FTR in the presence of a lesser degree of regurgitation should be treated during the first operation, supporting Carpentier's assertion that "surgical abstention" may be somewhat a dangerous policy. Moreover, when patients require reoperation for tricuspid valve dysfunction, a high operative mortality has been observed, mainly due to the irreversible right ventricular systolic or liver dysfunction^[5-7]. Increasing data now support an early surgical treatment of FTR^[8-11].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



We have reviewed the current guidelines and several reports for recommending tricuspid valve repair, also in the light of our recent experience in the treatment of FTR. It is our opinion that a dysfunction of the tricuspid valve even if in the early stages of manifestation, should be corrected at the time of concomitant left-sided heart surgery, i.e., mitral valve surgery.

AMERICAN AND EUROPEAN GUIDELINES

The 2014 American Heart Association/American College of Cardiology guidelines indicate that surgery for the treatment of FTR is required for patients affected by a severe degree of regurgitation (stages C and D of the tricuspid valve disease) undergoing left-sided valve surgery^[12]. This type of indication is in Class I, with Level C of evidence. Risks and benefits of tricuspid valve surgery should be carefully evaluated in the presence of severe right ventricle systolic dysfunction or irreversible pulmonary hypertension, potentially causing a right ventricle failure after operation. In Class IIa with Level B of evidence, it is recommended the repair of FTR in the presence of mild or moderate tricuspid regurgitation (stage B of the tricuspid valve disease) at the time of left-sided valve surgery either in the presence of tricuspid annular dilation or with prior evidence of right heart failure. In Class IIb with Level C of evidence tricuspid valve repair may be recommended in the presence of moderate FTR (stage B) and pulmonary artery hypertension at the time of left-sided valve surgery. The 2017 European Society of Cardiology/European Association for Cardio-Thoracic Surgery guidelines focus on the timing of surgical intervention based on the concept that surgery of the tricuspid valve should be carried out sufficiently early to avoid late irreversible right ventricular dysfunction or progression of FTR^[13].

In presence of FTR, adding tricuspid valve repair, if indicated during left-sided surgery, not only does not increase the operative risk, but also has been demonstrated to provide reverse remodeling of the right ventricle and to improve the functional status. The indication with Level C of evidence in Class I, IIa, IIb are similar to those reported by the American guidelines, with the exception of Class IIb, where it is stated that surgery may be considered in patients with mild or moderate FTR, even in the absence of annular dilatation, when previous right heart failure has been documented. In both American and European Guidelines the annulus dilatation of the tricuspid valve defined as greater than 40 mm or 21 mm/m² by 2D echocardiography represents a surgical indication for the treatment. In fact, a diastolic diameter greater than 40 mm or 21 mm/m² increases the risk of persistent or progressive FTR after isolated mitral valve surgery.

TRICUSPID VALVE REPAIR TECHNIQUES

Repair techniques for the treatment of FTR have been introduced by Kay *et al.*^[14] in 1965 and De Vega^[15] in 1972. Kay's technique provides the obliteration of the posterior tricuspid leaflet by placement of several sutures across the posterior segment of the tricuspid valve annulus making the valve as bicuspid. De Vega's technique provides the annuloplasty by placement of two semicircular sutures around the annulus anchored with two pledgets (2-0 Ti-cron), starting from the anterior-septal commissure and ending in front of the origin of the coronary sinus.

Ring annuloplasty, first introduced by Carpentier *et al.*^[2] in 1974, is thought to offer the best long-term outcomes for severe FTR, by means of a more complete annular stabilization. However, this procedure leads to prolongation of the operation and cardiopulmonary bypass time. Therefore intervening on moderate functional TR in the context of another cardiac procedure may become a decision-making dilemma.

CURRENT EARLY AND LATE RESULTS OF THE REPAIR TECHNIQUES

Marquis-Gravel *et al.*^[16] examined the outcomes of 926 cases of tricuspid valve surgery performed over a 30-year period. Of them, 792 patients underwent tricuspid valve repair (85%) more frequently in concomitance

with mitral valve surgery (85%). Tricuspid valve repair was done by the use of De Vega or ring annuloplasty. Operative mortality was 14%, 15-year survival 34%. Risk factors for late mortality included the preoperative severity $> 3+$ of the FTR, whereas tricuspid valve surgery concomitantly performed with mitral and/or aortic surgery was not a predictive factor for increased mortality^[16]. Chan *et al.*^[17] studied 624 mitral valve replacement patients. They performed in 125 out of 231 patients having preoperatively a FTR $> 2+$ tricuspid repair using De Vega or ring annuloplasty techniques. During a mean follow-up of 6.8 years among patients who had preoperative FTR $> 2+$, the regurgitation worsened in 10 (8%) patients who received repair compared with 85 (17%) who did not. Moreover, the progression of FTR was less developed in the repair group ($P = 0.008$)^[17]. Navia *et al.*^[18] have compared the effectiveness of several tricuspid valve repair techniques in 2277 patients who had undergone left-sided valve surgery. At 10 years of follow-up, the use of a rigid prosthetic ring provided the most sustained reduction of FTR^[18].

On the other hand, Yilmaz *et al.*^[19] in a series of 699 patients undergoing mitral valve repair, showed that at 3 years of follow-up, a clinically silent non-severe FTR was unlikely to progress. Huang *et al.*^[20] in a series of 448 patients undergoing tricuspid annuloplasty with concomitant procedures, evaluated the results of the De Vega (216 patients) or ring (232 patients) annuloplasty. The indication to FTR treatment was done on the symptomatic tricuspid regurgitation grade (4+) (91.3%) or in presence of moderate FTR ($< 4+$) or marked tricuspid annular dilatation (diameter > 4.0 cm) (8.7%). With both types of tricuspid valve repair techniques postoperative echocardiography showed significant improvement of the FTR grade (from 3.4 preoperatively to 0.6, $P < 0.05$); 5-year freedom from reoperation (81% *vs.* 75%, $P = 0.124$) was similar. They concluded that the De Vega annuloplasty is an acceptable strategy, improving both clinical and echocardiographic status of the patients during long-term follow-up, although the event-free survival appeared to be lower in comparison with that observed for the ring annuloplasty (74.5% *vs.* 78.8%, $P = \text{NS}$)^[20].

Finally, Takano *et al.*^[21] in a smaller series of 71 patients undergoing mitral valve replacement and tricuspid valve repair, but with a follow-up period of 20 years, identified the preoperative moderate grade of FTR as a significant risk factor for the development of late severe tricuspid regurgitation. They claimed that an aggressive early treatment of FTR at the time of mitral valve surgery may prevent the late progression of the FTR.

In our recent experience, from January 2015 to October 2017, on a series of 156 patients treated for left-sided heart valve disease (mitral, mitral and aortic valve disease), 57 patients (36.5%) underwent suture annuloplasty techniques (De Vega, 49 patients; Kay, 8 patients). In the mitral surgery group of patients ($n = 114$), FTR was treated in 35 cases (30.7%). Indication for the surgical treatment was given in the presence of symptomatic severe or moderate FTR, or when the diameter of the tricuspid valve annulus reached 40 mm, regardless of symptoms^[22,23]. We have adopted those tricuspid valve repair techniques because they require less surgical time in comparison with the use of a ring implant. The increased incidence of the surgical treatment of FTR observed in our series is in accordance with that reported in the database of the Society of Thoracic Surgeons. The trend of the tricuspid valve surgery increased with the time: 11,405 patients treated in the first period of analysis (2000-2003), 21,804 and 21,166 in the last periods (2004-2007 and 2008-2010). In this report operative mortality declined from 10.6% in 2000 to 8.2% in 2010 ($P < 0.001$)^[24].

In our series the operative mortality for mitral valve repair (29 patients) and mitral plus tricuspid valve repair (9 patients) was similar (0% *vs.* 0%), as well as that observed for mitral valve replacement (50 patients) and mitral valve replacement plus tricuspid valve repair (26 patients) (2% *vs.* 3.6%, $P = \text{NS}$).

As compared to preoperative period, clinical status of patients surgically treated for FTR during the short-term follow-up showed a significant improvement in NYHA class (3.0 ± 0.7 preoperatively *vs.* 1.4 ± 0.6 at follow-up), pulmonary artery pressure mean value (60 ± 22 *vs.* 32 ± 10 mmHg), mean value of FTR ($2.8 \pm 1.0/4+$ *vs.* $0.7 \pm 0.6/4+$) ($P < 0.001$, for all comparisons). None of the patients required permanent pacemaker

Table 1. Early and late results of the FTR surgical repair

Authors	Repair techniques	Operative mortality	Late survival	Freedom from recurrence of significant FTR
Marquis-Gravel <i>et al.</i> ^[16]	De Vega, ring implant	14%	55% at 10 years	46% at 10 years
Chan <i>et al.</i> ^[17]	De Vega, ring implant	3%	80% at 10 years	75% at 10 years
Navia <i>et al.</i> ^[18]	De Vega, Kay, ring implant		44% at 10 years	98% at 5 years
Huang <i>et al.</i> ^[20]	De Vega, ring implant	1.1%	84%, 97% (De Vega <i>vs.</i> ring) at 5 years	75%, 79% (De Vega <i>vs.</i> ring) at 5 years
Takano <i>et al.</i> ^[21]	Ring implant	0	59% at 15 years	93% at 15 years
Filsoufi <i>et al.</i> ^[25]	Ring implant	5.3%	85% at 2 years	100% at 2 years
Fukuda <i>et al.</i> ^[26]	Ring implant	0	100% at 1 year	70 at 1 year
Ghanta <i>et al.</i> ^[27]	Kay, ring implant	6.4%	75%, 61% (Kay <i>vs.</i> ring) at 3 years	75%, 69% (Kay <i>vs.</i> ring) at 3 years
Chang <i>et al.</i> ^[28]	De Vega, Kay	3.4%	96% at 8 years	72% at 8 years
Tang <i>et al.</i> ^[29]	De Vega, ring implant	7%, 4% (De Vega <i>vs.</i> ring)	36%, 49% (De Vega <i>vs.</i> ring) at 15 years	39%, 83% (De Vega <i>vs.</i> ring) at 15 years
McCarthy <i>et al.</i> ^[30]	De Vega, ring implant	8%	50% at 8 years	67%, 83% (De Vega <i>vs.</i> ring) at 8 years
Our recent experience, 2015-2017	De Vega, Kay	0	100% at 1 year	100% at 1 year

FTR: functional tricuspid regurgitation

implantation at discharge, or during follow-up. Early and late results of surgical treatment of FTR are summarized in Table 1, reporting either data above mentioned than other surgical series.

CONCLUSIONS

Current data suggest that tricuspid valve repair together with early elective surgical intervention for mitral valve disease should be done in order to improve late outcomes and avoid the risk of a late redo operation due to progression of FTR. In the presence of severe FTR, surgery continues to be recommended in Class I. Annular dilatation and history of congestive heart failure symptoms are important to take the decision to early repair of FTR, although more recent guidelines continue to indicate surgical intervention in these specific subgroup of patients in Class II. We agree that a dysfunction of the tricuspid valve, even if not associated with a severe insufficiency, should be corrected at the time of a surgical operation on the mitral valve, especially if the technique used to repair the tricuspid valve requires a short time of execution.

DECLARATIONS

Authors' contributions

Study design: Nardi P

Development of methodology: Nardi P

Collection of data: Ferrante S, Greci M, Vacirca SR, Russo M

Analysis and/or interpretation of data: Pisano C, Pellegrino A, Bertoldo F

Writing of the manuscript: Nardi P

Supervision: Ruvolo G

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Braunwald NS, Ross J Jr, Morrow AG. Conservative management of tricuspid regurgitation in patients undergoing mitral valve replacement. *Circulation* 1967;35:163-9.
2. Carpentier A, Deloche A, Hanania G, Forman J, Sellier P, Piwnica A, Dubost C, McGoon DC. Surgical management of acquired tricuspid valve disease. *J Thorac Cardiovasc Surg* 1974;67:53-65.
3. King RM, Shaff HV, Danielson GK, Gersh BJ, Orszulak TA, Piehler JM, Puga FJ, Pluth JR. Surgery for tricuspid regurgitation late after mitral valve replacement. *Circulation* 1984;70:1193-7.
4. Cohen SR, Sell JE, McIntosh CL, Clark RE. Tricuspid regurgitation in patients with acquired, chronic, pure mitral regurgitation. I. Prevalence, diagnosis, and comparison of preoperative clinical and hemodynamic features in patients with and without tricuspid regurgitation. *J Thorac Cardiovasc Surg* 1987;94:481-7.
5. Mangoni AA, DiSalvo TG, Vlahakes GJ, Polanczyk CA, Fifer MA. Outcome following isolated tricuspid valve replacement. *Eur J Cardiothorac Surg* 2001;19:68-73.
6. Kwon DA, Park JS, Chang HJ, Kim YJ, Sohn DW, Kim KB, Ahn H, Oh BH, Park YB, Choi YS. Prediction of outcome in patients undergoing surgery for severe tricuspid regurgitation following mitral valve surgery and role of tricuspid annular systolic velocity. *Am J Cardiol* 2006;98:659-61.
7. Kim YJ, Kwon DA, Kim HK, Park JS, Hahn S, Kim KH, Kim KB, Sohn DW, Ahn H, Oh BH, Park YB. Determinants of surgical outcome in patients with isolated tricuspid regurgitation. *Circulation* 2009;120:1672-8.
8. Benedetto U, Melina G, Angeloni E, Refice S, Roscitano A, Comito C, Sinatra R. Prophylactic tricuspid annuloplasty in patients with dilated tricuspid annulus undergoing mitral valve surgery. *J Thorac Cardiovasc Surg* 2012;143:632-8.
9. Calafiore AM, Gallina S, Iacò AL, Contini M, Bivona A, Gagliardi M, Bosco P, Di Mauro M. Mitral valve surgery for functional mitral regurgitation: should moderate-or-more tricuspid regurgitation be treated? A propensity score analysis. *Ann Thorac Surg* 2009;87:698-703.
10. Van de Veire NR, Braun J, Delgado V, Versteegh MI, Dion RA, Klautz RJ, Bax JJ. Tricuspid annuloplasty prevents right ventricular dilatation and progression of tricuspid regurgitation in patients with tricuspid annular dilatation undergoing mitral valve repair. *J Thorac Cardiovasc Surg* 2011;141:1431-9.
11. Chikwe J, Itagaki S, Anyanwu A, Adams DH. Impact of concomitant tricuspid annuloplasty on tricuspid regurgitation, right ventricular function, and pulmonary artery hypertension after repair of mitral valve prolapse. *J Am Coll Cardiol* 2015;65:1931-8.
12. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP 3rd, Guyton RA, O'Gara PT, Ruiz CE, Skubas NJ, Sorajja P, Sundt TM 3rd, Thomas JD; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. AHA/ACC guideline for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014;63:57-185.
13. Baumgartner H, Falk V, Bax JJ, De Bonis M, Hamm C, Holm PJ, Jung B, Lancellotti P, Lansac E, Muñoz DR, Rosenhek R, Sjögren J, Tornos Mas P, Vahanian A, Walther T, Wendler O, Windecker S, Zamorano JL; ESC Scientific Document Group. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. *Eur Heart J* 2017;38:2739-91.
14. Kay JH, Maselli-Campagna G, Tsuji HK. Surgical treatment of tricuspid insufficiency. *Ann Surg* 1965;162:53-8.
15. De Vega NF. La anuloplastiasselectiva, regulable y permanente. *Rev Esp Cardiol* 1972;25:555-6.
16. Marquis-Gravel G, Bouchard D, Perrault LP, Pagé P, Jeanmart H, Demers P, Carrier M, Cartier R, Poirier NC, Hébert Y, Pellerin M. Retrospective cohort analysis of 926 tricuspid valve surgeries: clinical and hemodynamic outcomes with propensity score analysis. *Am Heart J* 2012;163:851-8.
17. Chan V, Burwash IG, Lam BK, Auyeung T, Tran A, Mesana TG, Ruel M. Clinical and echocardiographic impact of functional tricuspid regurgitation repair at the time of mitral valve replacement. *Ann Thorac Surg* 2009;88:1209-15.
18. Navia JL, Nowicki ER, Blackstone EH, Brozzi NA, Nento DE, Atik FA, Rajeswaran J, Gillinov AM, Svensson LG, Lytle BW. Surgical management of secondary tricuspid valve regurgitation: annulus, commissure, or leaflet procedure? *J Thorac Cardiovasc Surg* 2010;139:1473-82.
19. Yilmaz O, Suri RM, Dearani JA, Sundt TM 3rd, Daly RC, Burkhart HM, Li Z, Enriquez-Sarano M, Schaff HV. Functional tricuspid regurgitation at the time of mitral valve repair for degenerative leaflet prolapse: the case for a selective approach. *J Thorac Cardiovasc Surg* 2011;142:608-13.
20. Huang X, Gu C, Men X, Zhang J, You B, Zhang H, Wei H, Li J. Repair of functional tricuspid regurgitation: comparison between suture annuloplasty and rings annuloplasty. *Ann Thorac Surg* 2014;97:1286-92.
21. Takano H, Hiramatsu M, Kida H, Uenoyama M, Horiguchi K, Yamauchi T, Kin K, Shirakawa Y, Kaneko M, Daimon T. Severe tricuspid regurgitation after mitral valve surgery: the risk factors and results of the aggressive application of prophylactic tricuspid valve repair. *Surg Today* 2017;47:445-56.

22. Dreyfus GD, Corbi PJ, Chan KM, Bahrami T. Secondary tricuspid regurgitation or dilatation: which should be the criteria for surgical repair? *Ann Thorac Surg* 2005;79:127-32.
23. Anyanwu AC, Chikwe J, Adams DH. Tricuspid valve repair for treatment and prevention of secondary tricuspid regurgitation in patients undergoing mitral valve surgery. *Curr Cardiol Rep* 2008;10:110-7.
24. Kilic A, Saha-Chaudhuri P, Rankin JS, Conte JV. Trends and outcomes of tricuspid valve surgery in North America: an analysis of more than 50,000 patients from the Society of Thoracic Surgeons database. *Ann Thorac Surg* 2013;96:1546-52; discussion 1552.
25. Filsoufi F, Salzborg SP, Coutu M, Adams DH. A three-dimensional ring annuloplasty for the treatment of tricuspid regurgitation. *Ann Thorac Surg* 2006;81:2273-7.
26. Fukuda S, Gillinov AM, McCarthy PM, Stewart WJ, Song JM, Kihara T, Daimon M, Shin MS, Thomas JD, Shiota T. Determinants of recurrent or residual functional tricuspid regurgitation after tricuspid annuloplasty. *Circulation* 2006;114:1582-7.
27. Ghanta RK, Chen R, Narayanasamy N, McGurk S, Lipsitz S, Chen FY, Cohn LH. Suture bicuspidization of the tricuspid valve versus ring annuloplasty for repair of functional tricuspid regurgitation: midterm results of 237 consecutive patients. *J Thorac Cardiovasc Surg* 2007;133:117-26.
28. Chang BC, Song SW, Lee S, Yoo KJ, Kang MS, Chung N. Eight-year outcomes of tricuspid annuloplasty using autologous pericardial strip for functional tricuspid regurgitation. *Ann Thorac Surg* 2008;86:1485-92; discussion 1493.
29. Tang GH, David TE, Singh SK, Maganti MD, Armstrong S, Borger MA. Tricuspid valve repair with an annuloplasty ring results in improved long-term outcomes. *Circulation* 2006;114:1577-81.
30. McCarthy PM, Bhudia SK, Rajeswaran J, Hoercher KJ, Lytle BW, Cosgrove DM, Blackstone EH. Tricuspid valve repair: durability and risk factors for failure. *J Thorac Cardiovasc Surg* 2004;127:674-85.

Original Article

Open Access



Adherence to guidelines: primary prevention with aspirin in 1125 medical check-up participants

Jin Hee Im¹, Sang Won Han¹, Seon Yeong Lee², Jong Sam Baik¹

¹Department of Neurology, Sanggye Paik Hospital, Inje University College of Medicine, Seoul 01757, South Korea.

²Department of Family Medicine, Sanggye Paik Hospital, Inje University College of Medicine, Seoul 01757, South Korea.

Correspondence to: Dr. Jong Sam Baik, Department of Neurology, Sanggye Paik Hospital, Inje University College of Medicine, 761-1 Sanggye 7-dong, Nowon-gu, Seoul 01757, South Korea. E-mail: jsbaik@paik.ac.kr

How to cite this article: Im JH, Han SW, Lee SY, Baik JS. Adherence to guidelines: primary prevention with aspirin in 1125 medical check-up participants. *Vessel Plus* 2018;2:5. <http://dx.doi.org/10.20517/2574-1209.2018.07>

Received: 5 Mar 2018 **First Decision:** 22 Mar 2018 **Revised:** 4 Apr 2018 **Accepted:** 11 Apr 2018 **Published:** 18 Apr 2018

Science Editor: Aaron S. Dumont **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Aim: The aim of the present study was to assess the 10-year cardiovascular disease (CVD) risk and to apply the current recommendations on aspirin use for primary prevention in Korean participants undergoing a medical check-up.

Methods: Adults aged 50 to 69 years were eligible for the study if they did not have a history of atherosclerotic CVD (ASCVD) or stroke. The 10-year CVD risk was calculated using the ASCVD risk estimator (<http://tools.acc.org/ASCVD-Risk-Estimator>).

Results: A total of 1125 participants were enrolled in this study. The mean age was 57 years, and 32% of the participants were women. Based on the 2016 US Preventive Services Task Force recommendations, aspirin was indicated in 266 (23.6%) participants but only 44 (3.9%) participants were taking aspirin regularly. Among these participants, aspirin was prescribed appropriately in 36% of the participants, suggesting that only 6% of the participants were taking aspirin appropriately and 3.3% of the participants were taking aspirin inappropriately. Logistic regression analysis showed that treatment for hypertension was significantly associated with taking aspirin (odds ratio 7.49; 95% confidence interval 3.62-15.49).

Conclusion: Our study suggested that there may be an opportunity for decreasing the rate of CVD as well as the risk for major bleeds through tailored education on aspirin use.

Keywords: Aspirin, cardiovascular disease, guideline, primary prevention, stroke



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



INTRODUCTION

Aspirin provides the benefit of primary prevention of vascular events in men or women whose risk for myocardial infarction (MI) or ischemic stroke, respectively, is high enough to outweigh the risk for bleeding^[1]. In patients with atherosclerotic vascular disease, long-term antiplatelet therapy reduces the risk of vascular events. The benefits of long-term antiplatelet therapy substantially exceed the bleeding risk. For primary prevention of vascular events, aspirin is frequently taken regularly with or without a doctor's prescription. In such cases, the balance is less clear because the risks without aspirin and the benefits of aspirin are generally an order of magnitude lower than in secondary prevention^[2-4]. In a collaborative meta-analysis, the use of aspirin provided a 12% proportional reduction in serious vascular events, due mainly to a reduction in non-fatal MI, and the net effect on stroke was not significant^[5]. In 2016, the US Preventive Services Task Force (USPSTF) updated the recommendations on low-dose aspirin use for primary prevention of cardiovascular disease (CVD) and colorectal cancer (CRC), based on the American College of Cardiology/American Heart Association (ACC/AHA) risk calculator^[4]. Prevention of CVD events is important, and understanding the physician's recommendations for aspirin use is essential for the management of quality of health care. The aim of the present study was to assess the 10-year CVD risk and to apply the current recommendations on aspirin use for primary prevention in Korean participants undergoing a medical check-up.

METHODS

Participants

Between January 2014 and December 2016, the participants who underwent a medical check-up at Sanggye Paik Hospital Health Promotion Center were enrolled into this study. Adults aged 50 to 69 years were eligible for the study if they did not have a history of atherosclerotic cardiovascular disease (ASCVD) or stroke with a low density lipoprotein (LDL)-cholesterol < 190 mg/dL and if they agreed to provide informed consent. They were classified as having an ASCVD history when they reported a history of MI, coronary bypass, or angioplasty. The participants' demographics, vascular risk factors, and medication history were collected at baseline. The medications taken regularly during the month preceding their visit were recorded. Physical examination, including blood pressure measurements, electrocardiogram, and blood testing were also performed.

The 2016 USPSTF recommendations on low-dose aspirin use for primary prevention of CVD and CRC are as follows: adults aged 50 to 69 years who have $\geq 10\%$ 10-year CVD risk, are willing to take low-dose aspirin daily and have a life expectancy of at least 10 years, and are not at an increased risk for bleeding. With respect to adults aged 60 to 69 years, the decision to initiate aspirin use should be an individual one. The evidence of aspirin use in adults younger than 50 years and aged 70 years or older is insufficient^[4]. The 10-year CVD risk was calculated using the ASCVD risk estimator (<http://tools.acc.org/ASCVD-Risk-Estimator>). The risk factors for the ACC/AHA ASCVD risk calculation were gender, age, race, total cholesterol, high density lipoprotein (HDL)-cholesterol, diabetes, treatment for hypertension, systolic blood pressure (SBP), and cigarette smoking. In this study, hypertension was defined as a SBP of at least 140 mmHg or a diastolic blood pressure (DBP) of at least 90 mmHg. Diabetes mellitus was defined as present if the participant was receiving hypoglycemic agents or the fasting serum glucose level was 126 mg/dL or higher^[6]. Participants who smoked regularly during the previous year were classified as current smokers.

High-resolution B-mode ultrasound measurements were performed in some participants according to the guidelines of the Mannheim intima-media thickness (IMT) Consensus^[7]. The methods of IMT measurement have been published previously^[8]. In brief, a single longitudinal lateral image of bilateral common carotid arteries (CCA) was obtained proximal to the carotid bulb, with the patient in the supine position. Automatic measurements of the CCA-IMT were performed approximately at 10 mm proximal to the carotid bulb. In this study, mean IMT of both carotid arteries was used for analysis.

Table 1. Baseline demographic and clinical characteristics of participants

Characteristics	Total (n = 1125)	Men (n = 764)	Women (n = 361)	P value
Age (years)	57.1 (4.86)	56.7 (4.66)	57.7 (5.18)	0.001
SBP (mmHg)	121.6 (14.33)	123.0 (13.84)	118.8 (14.96)	< 0.0001
DBP (mmHg)	77.7 (10.78)	79.3 (10.53)	74.3 (10.50)	< 0.0001
Hypertension	316 (28.1)	228 (29.8)	88 (24.4)	0.057
Diabetes mellitus	111 (9.9)	85 (11.1)	26 (7.2)	0.039
Dyslipidemia	256 (22.8)	169 (22.1)	87 (24.1)	0.460
Current smoking	230 (20.4)	222 (29.1)	8 (2.2)	< 0.0001
Total cholesterol (mg/dL)	192.8 (33.21)	189.8 (32.93)	199.1 (32.97)	< 0.0001
LDL cholesterol (mg/dL)	119.9 (24.02)	118.8 (24.04)	122.3 (23.84)	0.021
HDL cholesterol (mg/dL)	50.4 (11.79)	47.9 (10.35)	55.6 (12.87)	< 0.0001
Regular alcohol drinking	669 (59.5)	559 (73.2)	110 (30.5)	< 0.0001
Regular physical activity	642 (57.1)	454 (59.4)	188 (52.1)	< 0.0001
Regular antihypertensive therapy	282 (25.1)	202 (26.4)	80 (22.2)	0.122
Current aspirin use	44 (3.9)	34 (4.5)	10 (2.8)	0.175

Data are means (SD) or numbers (%). SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein

All study participants provided informed consent, and the study design was approved by the appropriate ethics review board and conducted in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki.

Statistical analysis

Data are expressed as mean (standard deviation) or number (percent). The baseline characteristics of the two groups were compared by a Student *t* test for continuous variables and by a χ^2 test for categorical variables. Logistic regression models were used to analyze the relationship between ASCVD risk calculation factors and taking aspirin. Due to this trial enrolled single ethnicity, gender, age, total cholesterol, HDL-cholesterol, diabetes, treatment for hypertension, SBP, and cigarette smoking were used as variables for the study. Two-sided null hypotheses of no difference were rejected at $P < 0.05$. SAS version 4.2 software (SAS Institute Inc, Cary, NC, USA) was used for statistical analysis.

RESULTS

During the study period, a total of 1125 participants were enrolled in this study. Table 1 shows the baseline characteristics of the enrolled participants. The mean age was 57 years, and 32% of the participants were women. With respect to clinical history and vascular risk factors, 28% of the participants had a history of hypertension, 10% of the participants had diabetes, 23% of the participants had dyslipidemia, and 20% of the participants were current tobacco smokers. The SBP and DBP were significantly higher in men. The frequency of diabetes, smoking, and regular alcohol drinking/physical activity was also higher in men, whereas age, total, LDL and HDL-cholesterol levels were higher in women.

Based on the 2016 USPSTF recommendations, aspirin was indicated in 266 (23.6%) participants but only 44 (3.9%) participants were taking aspirin regularly [Table 2]. Among these participants, aspirin was prescribed appropriately in 36% of the participants, suggesting that only 6% of the participants were taking aspirin appropriately and 3.3% of the participants were taking aspirin inappropriately. Table 3 demonstrates the 10-year CVD risk and the aspirin use according to gender and age. Among men, 253 (33.1%) participants had $\geq 10\%$ 10-year CVD risk [Figure 1]. Aspirin was recommended in 20.1% of the male participants aged 50-59 years and in 75.1% of the male participants aged 60-69 years. Among these male participants, however, only 15 (5.9%) were taking aspirin appropriately and 3.7% were taking aspirin inappropriately. Among women, 13 (3.6%) participants had $\geq 10\%$ 10-year CVD risk and none of the participants were taking aspirin appropriately. Aspirin was recommended only in 9.8% of the female participants aged 60-69 years [Table 3].

Table 2. The 10-year CVD risk and aspirin use

	Total (n = 1125)	Men (n = 764)	Women (n = 361)
10-year CVD risk (%)	0.5-46.2	1.4-46.2	0.5-20.2
≥ 10% 10-year CVD risk	266 (23.6)	253 (33.1)	13 (3.6)
Current aspirin use	44 (3.9)	34 (4.5)	10 (2.8)
Appropriate	16 (36)	15 (44)	1 (10)
Inappropriate	28 (64)	19 (56)	9 (90)

Data are numbers (%). CVD: cardiovascular disease

Table 3. The 10-year CVD risk and aspirin use according to gender and age

	Men			Women		
	Total (n = 764)	50-59 years (n = 583)	60-69 years (n = 181)	Total (n = 361)	50-59 years (n = 229)	60-69 years (n = 132)
10-year CVD risk (%)	1.4-46.2	1.4-25.8	4.3-46.2	0.5-20.2	0.5-8	1.8-20.2
≥ 10% 10-year CVD risk	253 (33.1)	117 (20.1)	136 (75.1)	13 (3.6)	0 (0)	13 (9.8)
Current aspirin use	34 (4.5)	20 (3.4)	14 (7.7)	10 (2.8)	4 (1.7)	6 (4.5)
Appropriate	15 (44)	5 (25)	10 (71)	1 (10)	0 (0)	0 (0)
Inappropriate	19 (56)	15 (75)	4 (29)	9 (90)	4 (100)	6 (100)

Data are numbers (%). CVD: cardiovascular disease

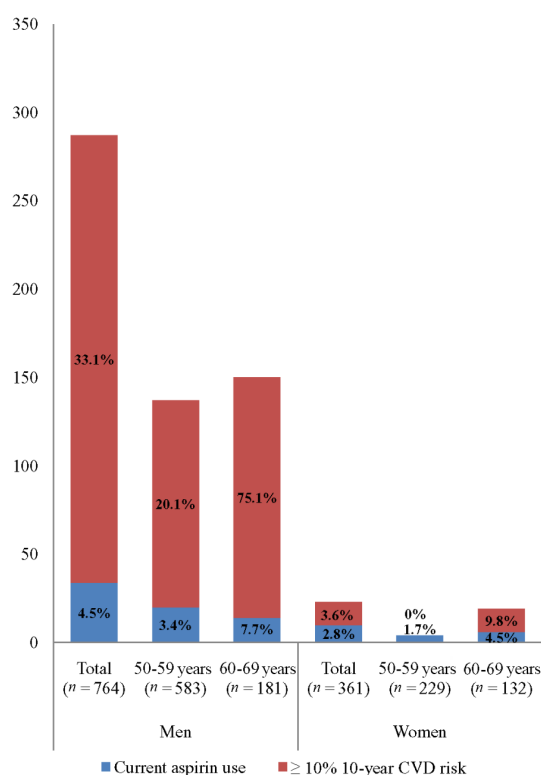
**Figure 1.** The 10-year cardiovascular disease (CVD) risk and the aspirin use according to gender and age

Table 4 shows the relationship between the factors of ASCVD risk calculation and taking aspirin. Treatment for hypertension was significantly associated with taking aspirin [odd ratio (OR) 7.49; 95% confidence interval (CI) 3.62-15.49]. Though there were no significant differences, a trend toward taking aspirin was related with age (OR 1.02; 95% CI 0.99-1.12) and men (OR 1.64; 95% CI 0.79-3.35). Smoking was inversely related with aspirin use (OR 0.59; 95% CI 0.24-1.42). Of the 1125 participants, 265 (23.6%) underwent IMT measurements. The mean IMT was significantly greater in the ≥ 10% 10-year CVD risk group ($n = 70$, 0.83 ± 0.13 mm) than in the < 10% 10-year CVD risk group ($n = 195$, 0.78 ± 0.12 mm; $P = 0.003$).

Table 4. Logistic regression analysis for the factors of ASCVD risk calculation and taking aspirin

ASCVD risk calculation factors	OR (95% CI)	P value
Men	1.64 (0.79-3.35)	0.179
Age	1.05 (0.99-1.12)	0.106
HDL-cholesterol	0.98 (0.94-1.01)	0.124
Total cholesterol	0.99 (0.98-1.01)	0.346
Treatment for hypertension	7.49 (3.62-15.49)	< 0.0001*
Systolic blood pressure	0.98 (0.96-1.01)	0.179
Cigarette smoker	0.59 (0.24-1.42)	0.236
Diabetes	1.27 (0.56-2.86)	0.565

ASCVD: atherosclerotic cardiovascular disease; OR: odds ratio; CI: confidence interval; HDL: high density lipoprotein. Significant *P* is marked with *

DISCUSSION

Based on the ACC/AHA ASCVD risk estimator and the 2016 USPSTF recommendations, this study demonstrated that aspirin was indicated in 23.6% of the participants undergoing medical check-up but only 6% of the participants were taking it appropriately. These results are similar to previous findings that showed the frequency of aspirin use^[9-11]. The role of aspirin in primary prevention among individuals without known CVD is currently unclear^[2,4,12]. However, high-risk patients who are not receiving aspirin are at an increased risk of CVD events. Low-risk patients are also exposed to the adverse bleeding risk with unnecessary use of aspirin. For primary prevention of CVD, decisions regarding aspirin use should be highly individualized^[13]. An alternative approach that may be helpful in determining the risk and benefit from aspirin therapy is using a risk assessment tool. It is helpful that healthcare providers will be able to estimate the CVD risk for an individual patient.

In this study, logistic regression analysis revealed that though SBP did not have any effect on the aspirin use (OR 0.98; 95% CI 0.96-1.01), treatment for hypertension was strongly associated with taking aspirin. It might be related with doctor's coprescription of aspirin with antihypertensive drugs in outpatient clinic, suggesting that there may be an opportunity for decreasing the rate of CVD as well as the risk for major bleeds through tailored education for physicians on aspirin use. Our study also showed that there was a trend toward taking aspirin with men and aging. Advancing age is a well-known non-modifiable risk factor for CVD. The cumulative effects of aging substantially increase the CVD risk, but the burden of CVD risk can be reduced in part by the modification of traditional risk factors^[14]. A higher frequency of diabetes and smoking in men may be associated with these results as well. Potentially modifiable risk factors, such as hypertension, diabetes, dyslipidemia, tobacco use, and physical inactivity, account for most of the risk of CVD^[15,16]. Medications to control blood pressure and lipids, smoking cessation, diet, and exercises are the interventions broadly applicable to the general population. There is another chance for decreasing the rate of CVD through personalized education for individuals on modifiable risk factors. The optimization of CVD prevention for individuals can identify and achieve the control of risk factors safely, expeditiously, and cost-effectively.

In our study, the mean IMT was significantly greater in the $\geq 10\%$ 10-year CVD risk group than in the $< 10\%$ 10-year CVD risk group. Several longitudinal studies have demonstrated that an increased carotid IMT can have an independent, synergistic risk prediction power for stroke and MI^[17]. While a carotid ultrasonography screening policy is not warranted in the general population, it might be considered in subjects with a higher 10-year CVD risk to better stratify their actual risk^[18,19]. Further studies are required to address the role of carotid ultrasonography in primary prevention of vascular events in high-risk subjects.

There were several limitations in this study. The most important limitation was that aspirin use was determined based on a self-report and this might have led to an underestimation of the actual use. The socioeconomic status was not determined and this could have resulted in overestimation of the number of individuals in

the population taking aspirin. Due to the small sample size, we could not evaluate the factors regarding to the inappropriate use of aspirin. Only 3.9% of participants were taken aspirin regularly in this study. Finally, this trial enrolled only Korean participants, limiting the generalizability of our findings to other geographic regions. Concerns have been raised that this guideline is the only US-based, externally validated equations that report risk as a combination of CVD, stroke, and CRC events. There may be limitations in general applications of the risk functions to other ethnic populations due to the differences in diet pattern, life style, social environment, or genetic predisposition^[20,21]. These limitations should be considered during the interpretation of our data.

In conclusion, the decision to take aspirin is still an individual one, which should be made after careful evaluation of the trade-off between the benefits and risks, particularly the risk of major bleeding. This study showed that aspirin would be indicated in nearly one-quarter of the adults aged 50 to 69 years who undergo a medical check-up but only 6% were taking aspirin appropriately based on the 2016 USPSTF recommendations. These results suggested that there may be an opportunity for decreasing the rate of CVD as well as the risk for major bleeds through tailored education on aspirin use.

DECLARATIONS

Authors' contributions

Conceived of the study: Baik JS

Recruitment and clinical assessment: Im JH, Han SW, Lee SY

Statistical analysis: Han SW

Drafted the initial version of the report: Im JH

Revision and editing of the report: all authors

Data source and availability

The dataset used and analysed during the current study are available from the corresponding author on reasonable request.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

All study participants provided informed consent.

Ethics approval

The study design was approved by the appropriate ethics review board and conducted in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki.

Copyright

© The Author(s) 2018.

REFERENCES

1. US Preventive Services Task Force. Aspirin for the prevention of cardiovascular disease: US Preventive Services Task Force recommendation statement. *Ann Intern Med* 2009;150:396-404.
2. Ittaman SV, VanWormer JJ, Rezkalla SH. The role of aspirin in the prevention of cardiovascular disease. *Clin Med Res* 2014;12:147-54.

3. Xie M, Shan Z, Zhang Y, Chen S, Yang W, Bao W, Rong Y, Yu X, Hu FB, Liu L. Aspirin for primary prevention of cardiovascular events: meta-analysis of randomized controlled trials and subgroup analysis by sex and diabetes status. *PLoS One* 2014;9:e90286.
4. Bibbins-Domingo K; US Preventive Services Task Force. Aspirin use for the primary prevention of cardiovascular disease and colorectal cancer: US Preventive Services Task Force recommendation statement. *Ann Intern Med* 2016;164:836-45.
5. Antithrombotic Trialists' (ATT) Collaboration; Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, Buring J, Hennekens C, Kearney P, Meade T, Patrono C, Roncaglioni MC, Zanchetti A. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet* 2009;373:1849-60.
6. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003;26 Suppl 1:S5-20.
7. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Hernandez Hernandez R, Jaff M, Kownator S, Naqvi T, Prati P, Rundek T, Sitzer M, Schminke U, Tardif JC, Taylor A, Vicaute E, Woo KS. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. *Cerebrovasc Dis* 2012;34:290-6.
8. Kim SN, Lee HS, Nam HS, Lee HR, Kim JM, Han SW, Park JH, Baik JS, Kim JY. Carotid intima-media thickness is inversely related to bone density in female but not in male patients with acute stroke. *J Neuroimaging* 2016;26:83-8.
9. Hira RS, Kennedy K, Nambi V, Jneid H, Alam M, Basra SS, Ho PM, Deswal A, Ballantyne CM, Pettersen LA, Virani SS. Frequency and practice-level variation in inappropriate aspirin use for the primary prevention of cardiovascular disease: insights from the National Cardiovascular Disease Registry's Practice Innovation and Clinical Excellence registry. *J Am Coll Cardiol* 2015;65:111-21.
10. Mainous AG, Tanner RJ, Shorr RI, Limacher MC. Use of aspirin for primary and secondary cardiovascular disease prevention in the United States, 2011-2012. *J Am Heart Assoc* 2014;3:e000989.
11. Manes C, Giacci L, Sciarilli A, D'Alleva A, De Caterina R. Aspirin overprescription in primary cardiovascular prevention. *Thromb Res* 2006;118:471-7.
12. Miedema MD, Huguelet J, Virani SS. Aspirin for the primary prevention of cardiovascular disease: in need of clarity. *Curr Atheroscler Rep* 2016;18:4.
13. Mora S, Ames JM, Manson JE. Low-dose aspirin in the primary prevention of cardiovascular disease: shared decision making in clinical practice. *JAMA* 2016;316:709-10.
14. Dhingra R, Vasan RS. Age as a risk factor. *Med Clin North Am* 2012;96:87-91.
15. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004;364:937-52.
16. Cannon CP. Cardiovascular disease and modifiable cardiometabolic risk factors. *Clin Cornerstone* 2007;8:11-28.
17. Touboul PJ, Labreuche J, Vicaute E, Amarenco P. Carotid intima-media thickness, plaques, and Framingham risk score as independent determinants of stroke risk. *Stroke* 2005;36:1741-5.
18. Prati P, Tosoletto A, Vanuzzo D, Bader G, Casaroli M, Canciani L, Castellani S, Touboul PJ. Carotid intima media thickness and plaques can predict the occurrence of ischemic cerebrovascular events. *Stroke* 2008;39:2470-6.
19. Jonas DE, Feltner C, Amick HR, Sheridan S, Zheng ZJ, Watford DJ, Carter JL, Rowe CJ, Harris R. Screening for asymptomatic carotid artery stenosis: a systematic review and meta-analysis for the US Preventive Services Task Force. *Ann Intern Med* 2014;161:336-46.
20. Bineau S, Dufouil C, Helmer C, Ritchie K, Empana JP, Ducimetiere P, Alperovitch A, Bousser MG, Tzourio C. Framingham stroke risk function in a large population-based cohort of elderly people: the 3C study. *Stroke* 2009;40:1564-70.
21. Jee SH, Park JW, Lee SY, Nam BH, Ryu HG, Kim SY, Kim YN, Lee JK, Choi SM, Yun JE. Stroke risk prediction model: a risk profile from the Korean study. *Atherosclerosis* 2008;197:318-25.

Review

Open Access



Metabolic risk in depression and treatment with selective serotonin reuptake inhibitors: are the metabolic syndrome and an increase in cardiovascular risk unavoidable?

Mervin Chávez-Castillo^{1,2}, Ángel Ortega¹, Manuel Nava¹, Jorge Fuenmayor¹, Victor Lameda¹, Manuel Velasco³, Valmore Bermúdez^{1,4}, Joselyn Rojas-Quintero^{1,5}

¹Endocrine and Metabolic Diseases Research Center, School of Medicine, The University of Zulia, Maracaibo 4001, Venezuela.

²Psychiatric Hospital of Maracaibo, Maracaibo 4001, Venezuela.

³Department of Pharmacology, "JM Vargas" Medical School, Central University of Venezuela, Caracas 1050, Venezuela.

⁴Advanced Frontier Studies Research Group (ALEF), Simón Bolívar University, Cúcuta 540006, Colombia.

⁵Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA.

Correspondence to: Dr. Mervin Chávez-Castillo, Endocrine and Metabolic Diseases Research Center, School of Medicine, The University of Zulia, Maracaibo 4001, Venezuela. E-mail: mervinch12@gmail.com

How to cite this article: Chávez-Castillo M, Ortega Á, Nava M, Fuenmayor J, Lameda V, Velasco M, Bermúdez V, Rojas-Quintero J. Metabolic risk in depression and treatment with selective serotonin reuptake inhibitors: are the metabolic syndrome and an increase in cardiovascular risk unavoidable? *Vessel Plus* 2018;2:6. <http://dx.doi.org/10.20517/2574-1209.2018.02>

Received: 2 Feb 2018 **First Decision:** 2 Apr 2018 **Revised:** 3 Apr 2018 **Accepted:** 9 Apr 2018 **Published:** 18 Apr 2018

Science Editor: Alexander D. Verin **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Depression is one of the most common psychiatric disorders, and has become an epidemic in modern medical practice; notorious for frequently co-occurring with multiple comorbidities, especially cardiovascular disease (CVD), type 2 diabetes mellitus (DM2), and its various risk factors comprised in the metabolic syndrome (MS). Selective serotonin reuptake inhibitors (SSRIs) are the most widely used class of psychotropic drugs in this and many other clinical scenarios; yet their impact on cardiometabolic health has not been elucidated. The objective of this review was to summarize current views on the pharmacology of SSRIs and cardiometabolic risk, as well as available epidemiological evidence regarding its clinical significance. SSRIs appear to intervene in cardiometabolic physiology fundamentally by modulating chronic inflammation, a key pathophysiologic phenomenon in MS, DM2 and CVD. However, the dosing necessary to achieve a beneficial impact in this regard, as well as their clinical correlations, remain controversial. Each SSRI displays a particular profile regarding each of the components of the MS: weight gain seems to be the most common effect of SSRIs, more frequent with paroxetine, followed by citalopram and escitalopram. As a drug class, SSRIs also appear to promote hypercholesterolemia rather uniformly, while fluoxetine and citalopram appear to particularly increase triacylglyceride levels. In contrast, fluvoxamine and paroxetine seem to have the greatest impact on dysglycemia. Lastly, most SSRIs appear to be innocuous or even beneficial regarding blood pressure and



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



high-density lipoprotein cholesterol. Nevertheless, many of these effects may vary significantly upon specific clinical circumstances, especially timing. This topic remains rather unexplored in clinical psychopharmacology, and further, larger-scale epidemiological studies are needed in order to offer improved care in this field.

Keywords: Depression, selective serotonin reuptake inhibitors, metabolic syndrome, cardiovascular risk, cardiovascular disease, type 2 diabetes mellitus, chronic inflammation

INTRODUCTION

Depression has become an emerging epidemic in recent years, with prevalence rates of 10%-15% across the globe^[1]. This trend has resulted in ever-increasing financial costs, along with a significant decay in the life quality of patients^[2]. A substantial portion of this burden may stem from the multiple medical comorbidities associated with depression, in particular, cardiovascular disease (CVD)^[3], with these conditions coexisting in up to 15% of cases^[4].

CVD remains the leading cause of morbidity and mortality worldwide^[5], significantly driven by a myriad of modifiable risk factors consequent upon a predominantly Westernized lifestyle^[6]. The metabolic syndrome (MS), conceptualized as a cluster of cardiovascular risk factors - obesity, hypertension, hyperglycemia and atherogenic dyslipidemia - which in co-occurrence substantially increase the risk of CVD and type 2 diabetes mellitus (DM2), is widely regarded as a useful clinical tool in the prevention of these conditions^[7]. These factors also appear to be involved in the pathophysiology of depression, and may account for the higher cardiovascular risk observed in this disorder^[8,9].

In this context, the pharmacological management of depression presents a clinical conundrum: depression is accompanied by increased risk of MS - and by extension, CVD and DM2 - yet many antidepressant drugs appear to exacerbate these risks as well^[10,11]. However, in contrast with antipsychotic drugs, whose clinical relevance in regards to deleterious cardiometabolic effects has been well-characterized^[12,13], the impact of antidepressant drugs in clinical outcomes remains less clear. This is an especially pressing matter in the field of neuropsychopharmacology, as antidepressant drugs, and selective serotonin reuptake inhibitors (SSRIs) in particular, have become one of the most prescribed drug classes in contemporary medical practice^[14,15]. This review aims to summarize current views on the pharmacology of SSRIs and cardiometabolic risk, as well as available epidemiological evidence regarding its clinical significance.

SSRI-ASSOCIATED CARDIOMETABOLIC RISK: MOLECULAR PATHWAYS

SSRIs have become very popular in clinical use owing to various beneficial characteristics, including their ease of administration, increased pharmacodynamic specificity, and enhanced tolerability with relatively minor side effects; in contrast to the “dirty”, less specific and tolerable older antidepressant drugs, such as tricyclics and monoamine oxidase inhibitors^[16]. Although this distinction is notorious regarding cardiovascular safety, the underlying molecular differences in their pharmacologic profiles remain largely elusive^[17].

Chronic systemic inflammation may be an especially important target for SSRIs in this context, given the comprehensive involvement of this phenomenon in the pathophysiology of MS, CVD and DM2^[18]. Furthermore, this kind of low-grade inflammation is also present in depression, as patients with disorder tend to show increased levels of proinflammatory biomarkers such as tumor necrosis factor alpha (TNFα), C-reactive protein, interleukin (IL)-6 and IL-1β^[19]. This is compounded by the frequent accompaniment of depression with unhealthy dietary habits and physical inactivity, which themselves also promote chronic inflammation^[20], and are prominent in the development of depressive symptoms such as loss of energy, sleep disturbances and irritability^[21].

Interestingly, the onset of the antidepressant effect of SSRIs has been reported to coincide with a reduction in the circulating levels of proinflammatory biomarkers^[22]. A novel hypothesis posits these changes to be due to a T helper (Th1)-like response, triggering inflammatory activity via interferon γ (IFN γ)-related pathways^[23]. SSRIs appear to decrease the production of IFN γ and stimulate the release of IL-10, by modulating the corresponding mRNA in immune cells^[24]. Consistent with this, SSRIs also appear to upregulate the expression of genes involved in apoptotic pathways in T cells^[25]. In addition, blockade of serotonin reuptake results in increased circulating serotonin levels, which have been reported to be able to suppress cytokine synthesis by T cells, B cells, natural killer cells and monocytes/macrophages^[26-29], resembling what occurs in sepsis after massive platelet degranulation^[30].

Macrophages may be particularly relevant regarding the immunomodulatory effects of SSRIs due to their high expression of serotonin receptors^[31,32]. By acting as ambient serotonin level sensors, macrophages could modulate genotype expression patterns in macrophages: activation of 5HT₇ receptors in macrophages has been noted to induce polarization towards the antiinflammatory M2 phenotype^[33,34]. Inhibition of TNF α and IL-6 release, as well as promotion of IL-10 synthesis, are prominent among the antiinflammatory features of M2 macrophages^[33,35,36].

Nevertheless, these antiinflammatory effects have been speculated to occur only at doses greater than the usual therapeutic range^[37], and SSRIs may rather be proinflammatory at lower doses, especially with prolonged use^[38]. This is consistent with evidence from Kubera *et al.*^[27], who found physiological levels of intracellular serotonin tend to promote TNF α and IL-6 synthesis in macrophages, whereas supraphysiological levels of extracellular serotonin were linked with downregulation of serotonin receptors and with decreased release of proinflammatory cytokines. Further research is required to elucidate the clinical correlates and significance of this molecular framework for SSRI-mediated immunomodulation.

Chronic inflammation is also closely linked to insulin resistance and obesity, two fundamental elements of the MS. Thus, by intervening through immunomodulation, SSRIs could have a pivotal role in the pathophysiology of this cluster of manifestations^[39]. Paroxetine may be a particularly powerful inductor of insulin resistance by interfering with IRS-1 signaling^[40]. Indeed, each SSRI seems to exert distinct effects on insulin resistance, body weight composition, and serum lipids, independently of their impact on chronic inflammation. For example, paroxetine has been linked with higher low-density lipoprotein cholesterol (LDL-C) levels, possibly due to increased appetite^[41]; whereas fluoxetine, by inhibiting PON1 activity, may favor lower high-density lipoprotein cholesterol (HDL-C) levels^[42]. In addition, certain pharmacokinetic interactions, such as that of fluoxetine with statins - including inhibition of CYP3A and modulation of glucuronidation, P-glycoprotein (Pgp) and organic anion transport peptide 1B1 (OATP), may result in potentiated reduction of cholesterol^[43]. This complexity warrants further insight and an individual assessment of each specific SSRI in this context. In stark contrast, SSRIs seem to be relatively innocuous regarding blood pressure, unlike other antidepressant drug classes such as serotonin-norepinephrine reuptake inhibitors, monoamine oxidase inhibitors, and tricyclic antidepressants^[44-46].

Lastly, the prominent role of serotonin in platelet physiology has ignited speculation regarding SSRIs as antiplatelet agents^[47,48]. The higher concentrations of circulating serotonin induced by SSRIs could reduce platelet aggregation^[49,50] and impair reactivity to vasoconstriction^[51]. However, SSRIs do not appear to intervene in the functionality of vitronectin - a fundamental component of glycoprotein IIb/IIIa^[48] - or fibrinogen^[52]; but are able to regulate the expression of vascular adhesion molecules such as VCAM-1, ICAM-1, P-selectin and E-selectin^[53,54]. Nevertheless, the relative relevance of these effects remains unknown in the context of the chronic inflammatory milieu which SSRIs could promote simultaneously, as does the clinical significance of this antiplatelet activity.

Table 1. Comparison of the clinical effects of SSRIs in key metabolic variables

SSRIs	Body weight	Glycemia	Serum Lipids				Blood pressure	References
			TAG	HDL-C	LDL-C	TC		
Paroxetine	Short-term: N Long-term: ↑↑↑	Short-term: ↓ Long-term: ↑↑↑	↑	N	↑	↑	N	[59,67,68,75,80,81,85,88-90,94,96,99,101-104]
Fluoxetine	Short-term: ↓ Long-term: N	↓	↑↑	N	↑	↑	N	[58,59,64,65,67-70,78,85,90-92,94,96,99,105-107]
Fluvoxamine	N	↑↑↑	SE	SE	SE	↑	SE	[58,75,84,108-110]
Sertraline	Short-term: ↓ Long-term: N	↓	↑	N	↑	↑	N	[62,63,67,76,85,89,90,93,94,96,99,111]
Citalopram	Short-term: ↑ Long-term: ↑	N	↑↑	N	↑	↑	N	[58,68,71,79,84,85,90,92,94,96,99,112]
Escitalopram	Short-term: ↑ Long-term: ↑	↓	↑	↑	↑	↑	N	[66,71,77,85,96,99,113,114]

↑: increase; ↑↑: high increase; ↑↑↑: very high increase; ↓: decrease; N: neutral; SE: scarce evidence; TAG: triacylglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; SSRI: selective serotonin reuptake inhibitor

SSRI-ASSOCIATED CARDIOMETABOLIC RISK: CLINICAL EVIDENCE

Management of metabolic risk is a frequent clinical challenge when prescribing psychiatric medications^[55]. However, in contrast to other psychotropic drug classes - which tend to behave more or less uniformly in regards to metabolic risk^[56], SSRIs appear to have more heterogeneous patterns^[57,58]. The following sections summarize key clinical evidence regarding the impact of SSRIs on each of the components of the MS [Tables 1 and 2].

Obesity

Historically, weight changes have been a hallmark in the side effect profile of most antidepressant classes^[59-61]. In the case of SSRIs, the specific agent used and the length of therapy may account for the very variable effects these drugs appear to have on body weight^[59]. For example, in two multicenter, double-blind, randomized, placebo-controlled clinical trials assessing sertraline therapy in 376 pediatric patients with major depressive disorder (MDD) over 10 weeks, Wagner *et al.*^[62] found a mean weight loss of 0.38 kg in subjects treated with sertraline. Croft *et al.*^[63] obtained similar outcomes in an 8-week case-control study, with an average weight loss of 0.79 kg in patients treated with sertraline. Likewise, use of fluoxetine has been linked to short-term weight loss, as reported by de Jonghe *et al.*^[64] in a randomized, double-blind 6-week study, where patients treated with fluoxetine showed an average loss of 0.84 kg; as well as by Michelson *et al.*^[65] in a prospective, placebo-controlled trial, where subjects on fluoxetine showed an average weight loss of 0.35 kg after 12 weeks. However, escitalopram has been linked with a significant increase in waist circumference in the short term^[66].

Nevertheless, the clinical value of these findings may be limited due to the short length of these studies, especially when considering treatment guidelines for MDD suggest use of antidepressants for at least 6 months. Therefore, in recent years, more studies have attempted to evaluate the long-term effect of SSRIs on weight^[67-71]. In a similar 32-week study by Fava *et al.*^[67] with fluoxetine, sertraline and paroxetine, weight gain was significantly higher with the latter. Mansoor *et al.*^[68] found a similar outcome, where citalopram and paroxetine were associated with long-term weight gain, whereas fluoxetine and venlafaxine were not. Fluoxetine in particular is consistently reported not to be linked with any major long-term weight changes^[69,70]. In contrast, Calarge *et al.*^[71] found citalopram and escitalopram are associated with significant changes in all body composition parameters, including visceral fat mass, after 2 years in treatment for MDD and generalized anxiety disorder.

Notwithstanding this outline, some research has suggested patients with MDD are intrinsically more susceptible to changes in body weight, and these shifts may occur independently of antidepressant use^[72]. At any rate, at present, there is sufficient evidence to highlight long-term weight gain as a clinically relevant effect for certain SSRIs, underlining the importance of patient-centric prescribing in psychopharmacology.

Table 2. Summary of key clinical evidence regarding SSRIs and the components of the metabolic syndrome

Authors	SSRIs studied	Methodology	Relevant results
Andersohn <i>et al.</i> ^[75]	Paroxetine, sertraline, fluoxetine, fluvoxamine and citalopram	Case-control study with data from the UK General Practice Research Database, including 165,968 patients with depression who received at least one prescription for antidepressants between January 1990 and June 2005	Fluvoxamine and paroxetine were associated with increased risk of type 2 diabetes mellitus: OR 9.0 (95% CI 1.08-75.58) and OR 1.75 (95% CI 1.13-2.72), respectively
Raeder <i>et al.</i> ^[58]	Paroxetine, sertraline, fluoxetine, fluvoxamine and citalopram	Cross-sectional study with data from the Hordaland Health Study, including 25,315 subjects with ages between 40-49 years and 70-74 years	As a group, SSRIs were significantly associated with type 2 diabetes mellitus, hypercholesterolemia and abdominal obesity. Paroxetine showed the strongest association with general and abdominal obesity. Conversely, citalopram was not linked with any of the aforementioned metabolic variables
Wagner <i>et al.</i> ^[62]	Sertraline	Two multicenter, double-blind, randomized, placebo-controlled clinical trials which assessed the safety and efficacy of sertraline during 10 weeks in 376 pediatric patients diagnosed with MDD	Use of sertraline was associated with a mean weight loss of 0.38 kg at 10 weeks
Fava <i>et al.</i> ^[67]	Fluoxetine, sertraline and paroxetine	Double-blind, randomized trial which assessed body weight changes during treatment with SSRIs over 26-32 weeks	Patients treated with paroxetine had significant weight gain, while those treated with sertraline and fluoxetine had non-significant weight gain and loss, respectively
Olguner Eker <i>et al.</i> ^[66]	Sertraline, escitalopram and fluoxetine	Case-control study on 40 patients and 32 control aged 29-49 years with symptoms of depression and anxiety, treated with SSRIs during 8 weeks, assessing metabolic variables	As a group, SSRI use was related to higher body weight, waist circumference and HDL-C, and lower insulin and HOMA index levels. Escitalopram was also associated with lower fasting glucose. Anthropometric changes were seen after treatment only in patients with depression and not anxiety. No anthropometric or biochemical changes were found in subjects on sertraline, fluoxetine or venlafaxine
Pigott <i>et al.</i> ^[114]	Escitalopram	Double-blind, randomized, placebo-controlled trial during 8 months, which aimed to compare the safety and efficacy of duloxetine and escitalopram in patients with MDD	Escitalopram was associated with increased systolic blood pressure and body weight in comparison to duloxetine. No significant changes were observed regarding diastolic blood pressure
Beyazyüz <i>et al.</i> ^[90]	Paroxetine, fluoxetine, citalopram and escitalopram	Prospective cohort study including 97 female patients with Generalized Anxiety Disorder treated with SSRIs during 16 weeks, evaluating various metabolic variables	After 16 weeks, subjects on paroxetine had significantly higher LDL-C, TC and TAG, while those on citalopram or escitalopram only had higher TAG. In contrast, subjects on fluoxetine showed lower TC and TAG
Serodio <i>et al.</i> ^[85]	All SSRIs	Cross-sectional study on 219 participants from the National Health and Nutrition Examination Survey treated with SSRIs, with the objective of evaluating their influence on obesity and cardiovascular risk	Independently of body mass index, subjects on SSRIs showed lower systolic blood pressure and higher HDL-C in comparison with subjects not on this medication
Wei <i>et al.</i> ^[89]	Paroxetine and sertraline	Observational cohort study on 2682 adults who received paroxetine or sertraline for at least 60 continuous days and had LDL-C measured twice, on and off the medication	Mixed regression model analyses adjusting for age, gender, comorbidities and hypolipemic medication, longer periods of time on paroxetine or sertraline were associated with increased LDL-C. Conversely, longer periods of time since suspending paroxetine and sertraline were related to lower LDL-C
Fjukstad <i>et al.</i> ^[99]	Paroxetine, fluoxetine, sertraline, escitalopram and citalopram	Cross-sectional study on 280 patients with schizophrenia or bipolar disorder treated with SSRIs, evaluating their effect on metabolic variables and prevalence of the metabolic syndrome	After adjusting for confounders, SSRI use was associated with increased TC, LDL-C and TAG, and increased incidence of metabolic syndrome. There were significant correlations between SSRI doses and TC and LDL-C levels
Yosmaoglu <i>et al.</i> ^[83]	Sertraline and citalopram	Experimental trial on 14 male subjects treated with SSRI at least 3 months. Resting metabolic rates, anthropometric measures and serum lipids were assessed	Resting metabolic rates decreased with increasing doses of SSRIs, yet no relation was found between this variable and body weight. Resting metabolic rates remained unchanged with constant doses of SSRIs. Body weight was reduced between the first and third weeks of treatment, but changes were non-significant by the third month. TC levels were significantly higher after 3 months of therapy

TAG: triacylglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; SSRI: selective serotonin reuptake inhibitor; MDD: major depressive disorder; OR: odds ratio

Dysglycemia

Although research on the effect of SSRIs on dysglycemia has been notoriously hindered by ample heterogeneity of study methodology, current evidence suggests each SSRI behaves distinctly regarding different outcome variables^[73,74]. In particular, fluvoxamine has shown the strongest association with the development of DM2, obtaining an odds ratio of 9.05 (95% CI 1.08-75.58) in a recent large case-control study by Andersohn *et al.*^[75]. On the other hand, sertraline has been reported to be associated with significant reductions in glycated

hemoglobin levels in patients with DM2 and MDD at 10 weeks of treatment^[76], and escitalopram has been linked with significant reductions in fasting glucose levels^[77]. Fluoxetine has been related to improved insulin sensitivity in patients with DM2, independently of weight loss^[78]; while citalopram does not appear to impact measures of insulin sensitivity^[79] or be associated with DM2^[58].

Evidence regarding paroxetine on this aspect is more controversial. Although this SSRI tends to be associated with increased risk of DM2 and obesity^[75], in short-term uses, it has proved to be innocuous, or even beneficial regarding glucose homeostasis: in a 5-week double-blind, randomized study by Weber-Hamann *et al.*^[80] on non-diabetic patients with MDD, paroxetine was associated with improved insulin sensitivity. Paile-Hyvärinen *et al.*^[81] echoed these findings in a 10-week, single-blind study on 15 patients with MDD. The time-dependent effects of paroxetine on glucose metabolism highlight the importance of long-term prospective clinical studies in the characterization of the effects of SSRIs in cardiometabolic risk.

In summary, although SSRIs as a drug class are typically believed to increase the risk of DM2^[82], current clinical evidence supports this notion predominantly for fluvoxamine and paroxetine^[75]. This underlines the importance of pondering the metabolic profiles for each individual SSRI, especially in subjects with risk factors for DM2 or CVD.

Dyslipidemia

Similar to dysglycemia, SSRIs have been frequently linked to dyslipidemia. In the large Hordaland Health Study, subjects who used SSRIs were more prone to presenting components of the MS in comparison to subjects who did not use any psychotropic drugs, especially high triacylglycerides (TAG), high LDL-C, and low HDL-C^[58]. Yosmaoğlu *et al.*^[83] obtained similar results in an Anatolian sample after 3 months of SSRI use, although comparable changes have been ascertained as early as after 5 weeks of treatment^[84]. Nevertheless, some findings also suggest SSRIs to have no impact on or be beneficial for lipid profiles^[85-87].

These discrepancies may reflect the distinct characteristics of each specific SSRI. For example, in an 8-week case-control study on patients with symptoms of depression and anxiety, use of escitalopram was linked with a significant increase in HDL-C^[66]. In contrast, in a 20-week follow-up study, both paroxetine and sertraline were related to higher total cholesterol and LDL-C^[88,89]. Similarly, after a 16-week follow-up, Beyazyuz *et al.*^[90] found paroxetine to increase total cholesterol and TAG levels, while fluoxetine lowered both parameters. Lastly, most SSRIs - including fluoxetine, paroxetine, sertraline, and citalopram, as well as venlafaxine - have been associated with high TAG after 8-12 weeks of therapy^[91-93].

Thus, in general, SSRI use tends to be associated with high TAG in the earlier weeks, and then with hypercholesterolemia in the later months; while the effect on HDL-C appears not to be significant. This outlines SSRIs as important promoters of atherogenic dyslipidemia, and may worsen cardiometabolic risk in conjunction with other factors.

Hypertension

In contrast to the previously discussed risk factors, the impact of SSRIs on the development of hypertension may be more negligible^[94-100]. Hypertension is notoriously not among the most frequent cardiovascular side effects of SSRIs, which include cardiac dysrhythmias, orthostatic hypotension, bradycardia, first-degree atrioventricular block, and syncope^[94-96]. Among these, dysrhythmias are the most common (4%), whether as a consequence of overdose or chronic use; in the case of the latter, the dysrhythmias tend to be well-tolerated^[97]. This profile renders SSRIs relatively safe regarding atherothrombotic risk^[98].

Various large-scale clinical epidemiological studies have reported that, as a drug class, SSRIs seem to be unrelated to significant increases in systolic or diastolic blood pressure, in patients with depression, anxiety

disorders, bipolar disorder and schizophrenia^[58,90,93,99]. At most, specific SSRIs such as sertraline and paroxetine may be linked to hypertension in < 1% of cases, while fluoxetine and citalopram appear to be innocuous in this regard^[94].

On the contrary, SSRIs may have a beneficial effect on blood pressure, especially by diminishing mean or systolic blood pressure, presumably via an inhibitory effect on the autonomic nervous system^[100]. Notably, a report by Serodio *et al.*^[85] found that, at any body mass index, systolic blood pressure was significantly lower in subjects treated with SSRIs than in those without antidepressant treatment, although this corresponded to a small 4 mmHg difference. In summary, although scarce, currently available evidence suggests no relationship between SSRIs and hypertension, and this might not be one of the principal contributions of these drugs in the development of MS.

CONCLUSIONS

At present, enough evidence is available to affirm SSRIs intervene in the pathogenesis of certain components of MS. However, this impact is not equal for all SSRIs; rather, each drug in this group has shown a particular cardiometabolic profile, differentially affecting body weight and composition, serum lipids, glycemia, blood pressure, and other parameters. These findings highlight the importance of holistic, patient-centered prescribing as a fundamental principle in clinical psychopharmacology. Further research is required to determine optimal approaches for the management of the metabolic effects of SSRIs. Future developments in psychopharmacology should consider the metabolic safety of novel drugs, in view of the burden CVD implies for public health, and the close association between mental and cardiometabolic disorders.

DECLARATIONS

Authors' contributions

Manuscript writing and editing, workgroup supervision, academic and methodological advisory: Chávez-Castillo M, Velasco M, Bermúdez V, Rojas-Quintero J

Data gathering and manuscript writing: Ortega Á, Nava M, Fuenmayor J, Lameda V

Financial support and sponsorship

This work was supported by Research Grant no. CC-0437-10-21-09-10 from the Technological, Humanistic, and Scientific Development Council (CONDES), University of Zulia, and Research Grant no. FZ-0058-2007 from Fundacite Zulia.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Lépine JP, Briley M. The increasing burden of depression. *Neuropsychiatr Dis Treat* 2011;7:3-7.
2. Reddy MS. Depression: the disorder and the burden. *Indian J Psychol Med* 2010;32:1-2.

3. Hare DL, Toukhsati SR, Johansson P, Jaarsma T. Depression and cardiovascular disease: a clinical review. *Eur Heart J* 2014;35:1365-72.
4. Colquhoun DM, Bunker SJ, Clarke DM, Glozier N, Hare DL, Hickie IB, Tatoulis J, Thompson DR, Tofler GH, Wilson A, Branagan MG. Screening, referral and treatment for depression in patients with coronary heart disease. *Med J Aust* 2013;198:483-4.
5. World Health Organization. Cardiovascular diseases. Media centre fact sheet. Updated May 2017. Available from: <http://www.who.int/mediacentre/factsheets/fs317/en/> [Last accessed on 10 Apr 2018]
6. Forman D, Bulwer BE. Cardiovascular disease: optimal approaches to risk factor modification of diet and lifestyle. *Curr Treat Options Cardiovasc Med* 2006;8:47-57.
7. Huang PL. A comprehensive definition for metabolic syndrome. *Dis Model Mec* 2009;2:231-7.
8. Olvera RL, Williamson DE, Fisher-Hoch SP, Vatcheva KP, McCormick JB. Depression, obesity, and metabolic syndrome: prevalence and risks of comorbidity in a population-based representative sample of Mexican Americans. *J Clin Psychiatry* 2015;76:e1300-5.
9. Kahl KG, Schweiger U, Correll C, Müller C, Busch ML, Bauer M, Schwarz P. Depression, anxiety disorders, and metabolic syndrome in a population at risk for type 2 diabetes mellitus. *Brain Behav* 2015;5:e00306.
10. McIntyre RS, Park KY, Law CW, Sultan F, Adams A, Lourenco MT, Lo AK, Soczynska JK, Woldeyohannes H, Alsuwaidan M, Yoon J, Kennedy SH. The association between conventional antidepressants and the metabolic syndrome: a review of the evidence and clinical implications. *CNS Drugs* 2010;24:741-53.
11. Chokka P, Tancer M, Yeragani VK. Metabolic syndrome: relevance to antidepressant treatment. *J Psychiatry Neurosci* 2006;31:414.
12. Mitchell AJ, Delaffon V, Vancampfort D, Correll CU, De Hert M. Guideline concordant monitoring of metabolic risk in people treated with antipsychotic medication: systematic review and meta-analysis of screening practices. *Psychol Med* 2012;42:125-47.
13. Riordan HJ, Antonini P, Murphy MF. Atypical antipsychotics and metabolic syndrome in patients with schizophrenia: risk factors, monitoring, and healthcare implications. *Am Health Drug Benefits* 2011;4:292-302.
14. Noordam R, Aarts N, Verhamme KM, Sturkenboom MC, Stricker BH, Visser LE. Prescription and indication trends of antidepressant drugs in the Netherlands between 1996 and 2012: a dynamic population-based study. *Eur J Clin Pharmacol* 2015;71:369-75.
15. Tripathi A, Avasthi A, Desousa A, Bhagabati D, Shah N, Kallivayalil RA, Grover S, Trivedi JK, Shinfuku N. Prescription pattern of antidepressants in five tertiary care psychiatric centres of India. *Indian J Med Res* 2016;143:507-13.
16. Santarsieri D, Schwartz TL. Antidepressant efficacy and side-effect burden: a quick guide for clinicians. *Drugs Context* 2015;4:212290.
17. Chittaranjan A, Chethan K, Sandarsh S. Cardiovascular mechanisms of SSRI drugs and their benefits and risks in ischemic heart disease and heart failure. *Int Clin Psychopharmacol* 2013;28:145-55.
18. Manabe I. Chronic inflammation links cardiovascular, metabolic and renal diseases. *Circ J* 2011;75:2739-48.
19. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, Allen NB, Stuart AL, Hayley AC, Byrne ML, Maes M. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med* 2013;11:200.
20. Bica T, Castelló R, Toussaint LL, Montesó-Curto P. Depression as a risk factor of organic diseases: an international integrative review. *J Nurs Scholarsh* 2017;49:389-99.
21. Chirinos DA, Murdock KW, LeRoy AS, Fagundes C. Depressive symptom profiles, cardio-metabolic risk and inflammation: results from the MIDUS study. *Psychoneuroendocrinology* 2017;82:17-25.
22. Mathews MJ, Mathews EH, Liebenberg L. The mechanisms by which antidepressants may reduce coronary heart disease risk. *BMC Cardiovasc Disord* 2015;15:82.
23. Maes M, Berk M, Goehler L, Song C, Anderson G, Galecki P, Leonard B. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC Med* 2012;10:66.
24. Cattaneo A, Gennarelli M, Uher R, Breen G, Farmer A, Aitchison KJ, Craig IW, Anacker C, Zunsztain PA, McGuffin P, Pariante CM. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'predictors' and longitudinal 'targets'. *Neuropsychopharmacology* 2013;38:377-85.
25. Taler M, Gil-Ad I, Lomnitski L, Korov I, Baharav E, Bar M, Zolokov A, Weizman A. Immunomodulatory effect of selective serotonin reuptake inhibitors (SSRIs) on human T lymphocyte function and gene expression. *Eur Neuropsychopharmacol* 2007;17:774-80.
26. Serebruany VL, Gurbel PA, O'Connor CM. Platelet inhibition by sertraline and n-desmethylsertraline: a possible missing link between depression, coronary events, and mortality benefits of selective serotonin reuptake inhibitors. *Pharmacol Res* 2001;43:453-61.
27. Kubera M, Maes M, Kenis G, Kim Y, Lasoñ W. Effects of serotonin and serotonergic agonists and antagonists on the production of tumor necrosis factor α and interleukin-6. *Psychiatry Res* 2005;134:251-8.
28. Young MR, Matthews JP. Serotonin regulation of T-cell subpopulations and of macrophage accessory function. *Immunology* 1995;84:148-52.
29. Hellstrands K, Hermodsson S. Serotonergic 5-HT_{1A} receptors regulate a cell contact-mediated interaction between natural killer cells and monocytes. *Scand J Immunol* 1993;37:7-18.
30. Nieuwland R, Berckmans RJ, McGregor S, Böing AN, Romijn FP, Westendorp RG, Hack CE, Sturk A. Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood* 2000;95:930-5.
31. Shajib M, Khan W. The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol (Oxf)* 2014;213:561-74.
32. Rudd ML, Nicolas AN, Brown BL, Fischer-Stenger K, Stewart JK. Peritoneal macrophages express the serotonin transporter. *J Neuroimmunol* 2005;159:113-8.
33. de las Casas-Engel M, Domínguez-Soto A, Sierra-Filardi E, Bragado R, Nieto C, Puig-Kroger A, Samaniego R, Loza M, Corcuera MT, Gómez-Aguado F, Bustos M, Sánchez-Mateos P, Corbí AL. Serotonin skews human macrophage polarization through HTR2B and HTR7. *J Immunol* 2013;190:2301-10.

34. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010;11:889-96.
35. Ahern GP. 5-HT and the immune system. *Curr Opin Pharmacol* 2011;11:29-33.
36. Kopp S, Alstergren P. Blood serotonin and joint pain in seropositive versus seronegative rheumatoid arthritis. *Mediators Inflamm* 2002;11:211-7.
37. Gobin V, Van Steendam K, Denys D, Deforce D. Selective serotonin reuptake inhibitors as a novel class of immunosuppressants. *Int Immunopharmacol* 2014;20:148-56.
38. Tynan R, Weidenhofer J, Hinwood M, Cairns M, Day T, Walker F. A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. *Brain Behav Immun* 2012;26:469-79.
39. St-Onge J, Joannis D, Simoneau J. The stimulation-induced increase in skeletal muscle glycogen synthase content is impaired in carriers of the glycogen synthase XbaI gene polymorphism. *Diabetes* 2001;50:195-8.
40. Levkovitz Y, Ben-shushan G, HersHKovitz A, Isaac R, Gil-Ad I, Shvartsman D, Ronen D, Weizman A, Zick Y. Antidepressants induce cellular insulin resistance by activation of IRS-1 kinases. *Mol Cell Neurosci* 2007;36:305-12.
41. Goldstein MR, Mascitelli L, Pezzetta F. Is the increase in LDL cholesterol induced by selective serotonin reuptake inhibitor therapy a blessing in disguise? *Med Hypotheses* 2010;74:955-6.
42. Avcikurt AS, Sinan S, Kockar F. Antidepressant and antipsychotic drugs differentially affect PON1 enzyme activity. *J Enzyme Inhib Med Chem* 2015;30:245-9.
43. Al-Asmari AK, Ullah Z, Al Masoudi AS, Ahmad I. Simultaneous administration of fluoxetine and simvastatin ameliorates lipid profile, improves brain level of neurotransmitters, and increases bioavailability of simvastatin. *J Exp Pharmacol* 2017;9:47-57.
44. Roose SP, Laghrissi-Thode F, Kennedy JS, Nelson JC, Bigger JT Jr, Pollock BG, Gaffney A, Narayan M, Finkel MS, McCafferty J, Gergel I. Comparison of paroxetine and nortriptyline in depressed patients with ischemic heart disease. *JAMA* 1998;279:287-91.
45. Kemp AH, Quintana DS, Gray MA, Felmingham KL, Brown K, Gatt J. Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol Psychiatry* 2010;67:1067-74.
46. Licht CM, de Geus EJ, Seldenrijk A, van Hout HP, Zitman FG, van Dyck R, Penninx BW. Depression is associated with decreased blood pressure, but antidepressant use increases the risk for hypertension. *Hypertension* 2009;53:631-8.
47. Serebruany V, O'Connor C, Gurbel P. Effect of selective serotonin reuptake inhibitors on platelets in patients with coronary artery disease. *Am J Cardiol* 2001;87:1398-400.
48. Serebruany VL, Gurbel PA. The relations of major platelet receptor expression during myocardial infarction. Monitoring efficacy of GPIIb/IIIa inhibitors by measuring P-selectin? *Thromb Haemost* 1999;81:314-6.
49. Andrade C, Sandarsh S, Chethan K, Nagesh K. Serotonin reuptake inhibitor antidepressants and abnormal bleeding. *J Clin Psychiatry* 2010;71:1565-75.
50. Serebruany V. Selective serotonin reuptake inhibitors and increased bleeding risk: are we missing something? *Am J Med* 2006;119:113-6.
51. Musselman D, Marzec U, Manatunga A, Penna S, Reemsnyder A, Knight BT, Baron A, Hanson SR, Nemeroff CB. Platelet reactivity in depressed patients treated with paroxetine. *Arch Gen Psychiatry* 2000;57:875-82.
52. Tseng Y, Chiang M, Huang T, Su K, Lane H, Lai Y. A selective serotonin reuptake inhibitor, citalopram, inhibits collagen-induced platelet aggregation and activation. *Thromb Res* 2010;126:517-23.
53. Lekakis J, Ikonomidis I, Papoutsi Z, Moutsatsou P, Nikolaou M, Parissis J, Kremastinos DT. Selective serotonin re-uptake inhibitors decrease the cytokine-induced endothelial adhesion molecule expression, the endothelial adhesiveness to monocytes and the circulating levels of vascular adhesion molecules. *Int J Cardiol* 2010;139:150-8.
54. Herr N, Mauler M, Witsch T, Stallmann D, Schmitt S, Mezger J, Bode C, Duerschmied D. Acute fluoxetine treatment induces slow rolling of leukocytes on endothelium in mice. *PLoS One* 2014;9:e88316.
55. Hiles S, Révész D, Lamers F, Giltay E, Penninx B. Bidirectional prospective associations of metabolic syndrome components with depression, anxiety, and antidepressant use. *Depress Anxiety* 2016;33:754-64.
56. Shrivastava A, Johnston M. Weight-gain in psychiatric treatment: risks, implications, and strategies for prevention and management. *Mens Sana Monogr* 2010;8:53-68.
57. Ferguson JM. SSRI antidepressant medications: adverse effects and tolerability. *Prim Care Companion J Clin Psychiatry* 2001;3:22-7.
58. Raeder M, Bjelland I, Vollset S, Steen V. Obesity, dyslipidemia, and diabetes with selective serotonin reuptake inhibitors. *J Clin Psychiatry* 2006;67:1974-82.
59. Serretti A, Mandelli L. Antidepressants and body weight. *J Clin Psychiatry* 2010;71:1259-72.
60. McIntyre RS, Park KY, Law CW, Sultan F, Adams A, Lourenco MT, Lo AK, Soczynska JK, Woldeyohannes H, Alsuwaidan M, Yoon J, Kennedy SH. The association between conventional antidepressants and the metabolic syndrome: a review of the evidence and clinical implications. *CNS Drugs* 2010;24:741-53.
61. Reekie J, Hosking S, Prakash C, Kao K, Juonala M, Sabin M. The effect of antidepressants and antipsychotics on weight gain in children and adolescents. *Obes Rev* 2015;16:566-80.
62. Wagner KD, Ambrosini P, Rynn M, Wohlberg C, Yang R, Greenbaum MS, Childress A, Donnelly C, Deas D; Sertraline Pediatric Depression Study Group. Efficacy of sertraline in the treatment of children and adolescents with major depressive disorder: two randomized controlled trials. *JAMA* 2003;290:1033-41.
63. Croft H, Settle E Jr, Houser T, Batey SR, Donahue RM, Ascher JA. A placebo-controlled comparison of the antidepressant efficacy and effects on sexual functioning of sustained-release bupropion and sertraline. *Clin Ther* 1999;21:643-58.
64. de Jonghe F, Ravelli DP, Tuynman-Qua H. A randomized, double-blind study of fluoxetine and maprotiline in the treatment of major

- depression. *Pharmacopsychiatry* 1991;24:62-7.
65. Michelson D, Amsterdam JD, Quitkin FM, Reimherr FW, Rosenbaum JF, Zajecka J, Sundell KL, Kim Y, Beasley CM Jr. Changes in weight due to a 1-year trial of fluoxetine. *Am J Psychiatry* 1999;156:1170-6.
 66. Olguner Eker Ö, Özsoy S, Eker B, Doğan H. Metabolic effects of antidepressant treatment. *Noro Psikiyatr Ars* 2017;54:49-56.
 67. Fava M, Judge R, Hoog SL, Nilsson ME, Koke SC. Fluoxetine versus sertraline and paroxetine in major depressive disorder: changes in weight with long-term treatment. *J Clin Psychiatry* 2000;61:863-7.
 68. Mansoor B, Rengasamy M, Hilton R, Porta G, He J, Spirito A, Emslie GJ, Mayes TL, Clarke G, Wagner KD, Shamseddeen W, Birmaher B, Ryan N, Brent D. The bidirectional relationship between body mass index and treatment outcome in adolescents with treatment-resistant depression. *J Child Adolesc Psychopharmacol* 2013;23:458-67.
 69. Emslie GJ, Heiligenstein JH, Hoog SL, Wagner KD, Findling RL, McCracken JT, Nilsson ME, Jacobson JG. Fluoxetine treatment for prevention of relapse of depression in children and adolescents: a double-blind, placebo-controlled study. *J Am Acad Child Adolesc Psychiatry* 2004;43:1397-405.
 70. Nilsson M, Joliat M, Miner C, Brown E, Heiligenstein J. Safety of subchronic treatment with fluoxetine for major depressive disorder in children and adolescents. *J Child Adolesc Psychopharmacol* 2004;14:412-7.
 71. Calarge CA, Mills JA, Janz KF, Burns TL, Coryell WH, Zemel BS. Body composition in adolescents during treatment with selective serotonin reuptake inhibitors. *Pediatrics* 2017;140:e20163943.
 72. Gibson-Smith D, Bot M, Milaneschi Y, Twisk J, Visser M, Brouwer IA, Penninx BW. Major depressive disorder, antidepressant use, and subsequent 2-year weight change patterns in the Netherlands study of depression and anxiety. *J Clin Psychiatry* 2015;77:e144-51.
 73. McIntyre R, Soczynska J, Konarski J, Kennedy S. The effect of antidepressants on glucose homeostasis and insulin sensitivity: synthesis and mechanisms. *Expert Opin Drug Saf* 2005;5:157-68.
 74. Derijks H, Meyboom R, Heerdink E, De Koning F, Janknegt R, Lindquist M, Egberts AC. The association between antidepressant use and disturbances in glucose homeostasis: evidence from spontaneous reports. *Eur J Clin Pharmacol* 2008;64:531-8.
 75. Andersohn F, Schade R, Suissa S, Garbe E. Long-term use of antidepressants for depressive disorders and the risk of diabetes mellitus. *Am J Psychiatry* 2009;166:591-8.
 76. Goodnick PJ, Kumar A, Henry JH, Buki VM, Goldberg RB. Sertraline in coexisting major depression and diabetes mellitus. *Psychopharmacol Bull* 1997;33:261-4.
 77. Dhavale HS, Panikkar V, Jadhav BS, Ghulghule M, Agari AD. Depression and diabetes: impact of antidepressant medications on glycaemic control. *J Assoc Physicians India* 2013;61:896-9.
 78. Maheux P, Ducros F, Bourque J, Garon J, Chiasson JL. Fluoxetine improves insulin sensitivity in obese patients with non-insulin-dependent diabetes mellitus independently of weight loss. *Int J Obes Relat Metab Disord* 1997;21:97-102.
 79. Kauffman RP, Castracane VD, White DL, Baldock SD, Owens R. Impact of the selective serotonin reuptake inhibitor citalopram on insulin sensitivity, leptin and basal cortisol secretion in depressed and non-depressed euglycemic women of reproductive age. *Gynecol Endocrinol* 2005;21:129-37.
 80. Weber-Hamann B, Gilles M, Lederbogen F, Heuser I, Deuschle M. Improved insulin sensitivity in 80 nondiabetic patients with MDD after clinical remission in a double-blind, randomized trial of amitriptyline and paroxetine. *J Clin Psychiatry* 2006;67:1856-61.
 81. Paile-Hyvärinen M, Wahlbeck K, Eriksson JG. Quality of life and metabolic status in mildly depressed women with type 2 diabetes treated with paroxetine: a single-blind randomised placebo controlled trial. *BMC Fam Pract* 2003;4:7.
 82. Yoon JM, Cho EG, Lee HK, Park SM. Antidepressant use and diabetes mellitus risk: a meta-analysis. *Korean J Fam Med* 2013;34:228-40.
 83. Yosmaoğlu A, Fıstıkcı N, Keyvan A, Hacıoğlu M, Erten E, Saatçioğlu Ö, Kora K. Correlation of selective serotonin re-uptake inhibitor use with weight gain and metabolic parameters. *Anadolu Psikiyatri Derg* 2013;14:245-51.
 84. Gabriel A. Changes in plasma cholesterol in mood disorder patients: does treatment make a difference? *J Affect Disord* 2007;99:273-8.
 85. Serodio KJ, Ardern CI, Rotondi M, Kuk J. Tricyclic and SSRI usage influences the association between BMI and health risk factors. *Clin Obes* 2014;4:296-302.
 86. Deisenhammer EA, Kramer-Reinstadler K, Liensberger D, Kemmler G, Hinterhuber H, Fleischhacker WW. No evidence for an association between serum cholesterol and the course of depression and suicidality. *Psychiatry Res* 2004;121:253-61.
 87. Ma Y, Balasubramanian R, Pagoto SL, Schneider KL, Hebert JR, Phillips LS, Goveas JS, Culver AL, Olendzki BC, Beck J, Smoller JW, Sepavich DM, Ockene JK, Uebelacker L, Zorn M, Liu S. Relations of depressive symptoms and antidepressant use to body mass index and selected biomarkers for diabetes and cardiovascular disease. *Am J Public Health* 2013;103:e34-43.
 88. Herran A, Ramirez ML, Carrera M, Garcia-Unzueta MT, Sierra-Biddle D, Rodríguez-Cabo B, Ayestarán A, Hoyuela F, Vázquez-Barquero JL. Panic disorder, treatment with selective serotonin reuptake inhibitors, and cholesterol levels. *J Clin Psychopharmacol* 2006;26:538-40.
 89. Wei F, Crain AL, Whitebird RR, Godlevsky OV, O'Connor PJ. Effects of paroxetine and sertraline on low-density lipoprotein cholesterol: an observational cohort study. *CNS Drugs* 2009;23:857-65.
 90. Beyazyüz M, Albayrak Y, Eğilmez OB, Albayrak N, Beyazyüz E. Relationship between SSRIs and metabolic syndrome abnormalities in patients with generalized anxiety disorder: a prospective study. *Psychiatry Investig* 2013;10:148-54.
 91. Teitelbaum M. Severe hypertriglyceridemia secondary to venlafaxine and fluoxetine. *Psychosomatics* 2001;42:440-1.
 92. Teitelbaum M. Severe and moderate hypertriglyceridemia secondary to citalopram and fluoxetine. *Psychosomatics* 2000;41:448-9.
 93. Kesim M, Tiryaki A, Kadioglu M, Muci E, Kalyoncu NI, Yaris E. The effects of sertraline on blood lipids, glucose, insulin and HbA1C levels: a prospective clinical trial on depressive patients. *J Res Med Sci* 2011;16:1525-31.
 94. Khawaja I, Feinstein R. Cardiovascular effects of selective serotonin reuptake inhibitors and other novel antidepressants. *Heart Dis*

- 2003;5:153-60.
95. Pacher P, Ungvari Z, Nanasi PP, Furst S, Kecskemeti V. Speculations on difference between tricyclic and selective serotonin reuptake inhibitor antidepressants on their cardiac effects. Is there any? *Curr Med Chem* 1999;6:469-80.
 96. Nezafati MH, Eshraghi A, Vojdanparast M, Abtahi S, Nezafati P. Selective serotonin reuptake inhibitors and cardiovascular events: a systematic review. *J Res Med Sci* 2016;21:66.
 97. Kostev K, Rex J, Eith T, Heilmaier C. Which adverse effects influence the dropout rate in selective serotonin reuptake inhibitor (SSRI) treatment? Results for 50,824 patients. *Ger Med Sci* 2014;12:Doc15.
 98. Katsi VK, Marketou M, Vamvakou G, Makris T, Tousoulis D, Stefanadis CI, Vardas P, Kallikazaros IE. Novel antidepressant drugs, arterial hypertension and cardiovascular disease. *Recent Pat Cardiovasc Drug Discov* 2013;8:178-85.
 99. Fjokstad KK, Engum A, Lydersen S, Dieset I, Steen NE, Andreassen OA, Spigset O. Metabolic abnormalities related to treatment with selective serotonin reuptake inhibitors in patients with schizophrenia or bipolar disorder. *J Clin Psychopharmacol* 2016;36:615-20.
 100. Licht C, Penninx B, Geus E. Effects of antidepressants, but not psychopathology, on cardiac sympathetic control: a longitudinal study. *Neuropsychopharmacology* 2012;37:2487-95.
 101. Hinze-Selch D, Schulz A, Kraus T, Kühn M, Uhr M, Haack M, Pollmächer T. Effects of antidepressants on weight and on the plasma levels of leptin, TNF-alpha and soluble TNF receptors: a longitudinal study in patients treated with amitriptyline or paroxetine. *Neuropsychopharmacology* 2000;23:13-9.
 102. Weber E, Stack J, Pollock BG, Mulsant B, Begley A, Mazumdar S, Reynolds CF3rd. Weight change in older depressed patients during acute pharmacotherapy with paroxetine and nortriptyline: a double-blind randomized trial. *Am J Geriatr Psychiatry* 2000;8:245-50.
 103. Nemeroff CB, Evans DL, Gyulai L, Sachs GS, Bowden CL, Gergel IP, Oakes R, Pitts CD. Double-blind, placebo-controlled comparison of imipramine and paroxetine in the treatment of bipolar depression. *Am J Psychiatry* 2001;158:906-12.
 104. Paile-Hyvärinen M, Wahlbeck K, Eriksson JG. Quality of life and metabolic status in mildly depressed patients with type 2 diabetes treated with paroxetine: a double-blind randomised placebo controlled 6-month trial. *BMC Fam Pract* 2007;8:34.
 105. Ye Z, Chen L, Yang Z, Li Q, Huang Y, He M, Zhang S, Zhang Z, Wang X, Zhao W, Hu J, Liu C, Qu S, Hu R. Metabolic effects of fluoxetine in adults with type 2 diabetes mellitus: a meta-analysis of randomized placebo-controlled trials. *PLoS One* 2011;6:e21551.
 106. O'Kane M, Wiles PG, Wales JK. Fluoxetine in the treatment of obese type 2 diabetic patients. *Diabet Med* 1994;11:105-10.
 107. Breum L, Bjerre U, Bak JF, Jacobsen S, Astrup A. Long-term effects of fluoxetine on glycemic control in obese patients with non-insulin-dependent diabetes mellitus or glucose intolerance: influence on muscle glycogen synthase and insulin receptor kinase activity. *Metabolism* 1995;44:1570-6.
 108. Fava M. Weight gain and antidepressants. *J Clin Psychiatry* 2000;61:37-41.
 109. Moon CA, Jesinger DK. The effects of psychomotor performance of fluvoxamine versus mianserin in depressed patients in general practice. *Br J Clin Pract* 1991;45:259-62.
 110. Nihalani N, Schwartz TL, Siddiqui UA, Megna JL. Weight gain, obesity, and psychotropic prescribing. *J Obes* 2011;2011:893629.
 111. Aberg-Wistedt A, Agren H, Ekselius L, Bengtsson F, Akerblad AC. Sertraline versus paroxetine in major depression: clinical outcome after six months of continuous therapy. *J Clin Psychopharmacol* 2000;20:645-52.
 112. Bouwer CD, Harvey BH. Phasic craving for carbohydrate observed with citalopram. *Int Clin Psychopharmacol* 1996;11:273-8.
 113. Emslie GJ, Ventura D, Korotzer A, Tourkodimitris S. Escitalopram in the treatment of adolescent depression: a randomized placebo-controlled multisite trial. *J Am Acad Child Adolesc Psychiatry* 2009;48:721-9.
 114. Pigott TA, Prakash A, Arnold LM, Aaronson ST, Mallinckrodt CH, Wohlreich MM. Duloxetine versus escitalopram and placebo: an 8-month, double-blind trial in patients with major depressive disorder. *Curr Med Res Opin* 2007;23:1303-18.

Case Report

Open Access



Endovascular treatment of an iatrogenic superior mesenteric arteriovenous fistula after Nissen fundoplication

S. Keisin Wang, Jie Xie, Raghu L. Motaganahalli

Division of Vascular Surgery, Department of Surgery, Indiana University School of Medicine, Indianapolis, IN 46202, USA.

Correspondence to: Dr. Raghu L. Motaganahalli, Division of Vascular Surgery, Department of Surgery, Indiana University School of Medicine, 1801 N. Senate Blvd MPC2-3500, Indianapolis, IN 46202, USA. E-mail: rmotagan@iupui.edu; Dr. S. Keisin Wang, Division of Vascular Surgery, Department of Surgery, Indiana University School of Medicine, 1801 N. Senate Blvd MPC2-3500, Indianapolis, IN 46202, USA. E-mail: wangkei@iupui.edu

How to cite this article: Wang SK, Xie J, Motaganahalli RL. Endovascular treatment of an iatrogenic superior mesenteric arteriovenous fistula after Nissen fundoplication. *Vessel Plus* 2018;2:7. <http://dx.doi.org/10.20517/2574-1209.2018.15>

Received: 27 Mar 2018 **First Decision:** 24 Apr 2018 **Revised:** 24 Apr 2018 **Accepted:** 26 Apr 2018 **Published:** 27 Apr 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

We present a case of a superior mesenteric arteriovenous fistula (SMAVF) diagnosed four years after index Nissen fundoplication and examine the associated imaging, clinical course, and surgical treatment followed by a review of the limited, available literature. From a transbrachial approach, a covered stent was successfully deployed in the superior mesenteric artery to exclude the fistula after confirmation of the site of pathology on both digital subtraction angiography and intravascular ultrasound. Follow-up imaging demonstrated continued exclusion of the anomalous fistula with complete resolution of his symptoms at both his postprocedure and 1-year follow-up visits. SMAVFs are usually encountered secondary to previous surgical dissection or trauma and presents with nondescript abdominal pain making early diagnosis difficult; however, they can be successfully treated with minimally-invasive stent exclusion.

Keywords: Superior mesenteric artery, iatrogenic, fistula, endovascular, stent, treatment

INTRODUCTION

A superior mesenteric arteriovenous fistula (SMAVF) is a rare pathology that can lead to gastrointestinal bleeding, portal hypertension, and hepatic dysfunction if allowed to progress^[1]. Although limited outcomes data is available, endovascular therapy is preferred secondary to a lower postoperative morbidity compared to open surgical repair^[2].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



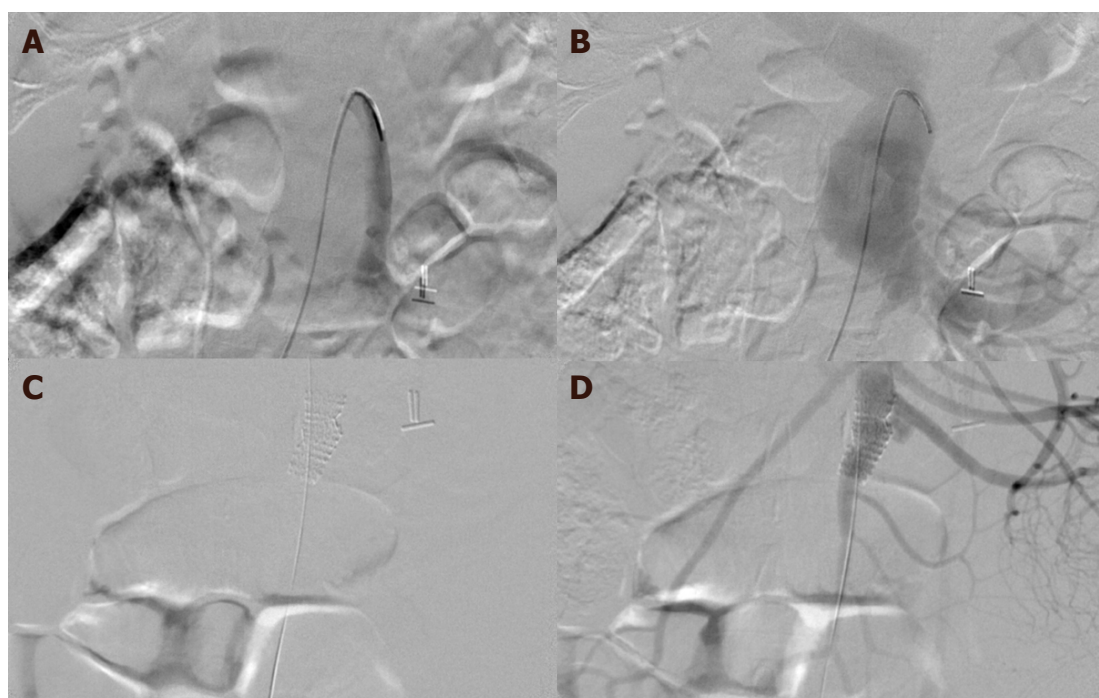


Figure 1. Initial selective angiography demonstrates flow through the proximal SMA (A) before redirection of contrast through the SMV (B) and the venous system. After accessing the brachial artery, a 10 mm × 2.5 cm Viabahn stent was deployed (C) over the identified SMA defect (via IVUS) successfully excluding the pathologic anatomy (D). SMA: superior mesenteric artery; SMV: superior mesenteric vein; IVUS: intravascular ultrasound

CASE REPORT

A 44-year-old gentleman presented to us following a 4-year history of nonspecific abdominal discomfort and a newly discovered peri-umbilical bruit. He recalled developing abdominal discomfort immediately following laparoscopic Nissen fundoplication that was complicated by a large peritoneal bleed upon trocar insertion. This bleeding event during the index operation resolved with copious application of surgical clips by the general surgeon. A mesenteric duplex was performed which demonstrated turbulence in the superior mesenteric vein (SMV) distribution. The pathologic connection was confirmed on a computed tomographic angiogram (CTA), but it was difficult to distinguish the exact location of fistula formation.

At this point, we elected to proceed with a diagnostic angiogram via a transfemoral approach. The presence and location of the fistula was established but no intervention was undertaken secondary to the significant angulation of the superior mesenteric artery (SMA). The following week, the patient returned to the operating theatre where a cutdown of the left arm was performed to expose the brachial artery to ease SMA cannulation and stent deployment. The brachial artery was accessed with a micropuncture needle and a long 6F sheath was positioned into the distal thoracic aorta with the assistance of an OmniFlush angled catheter (AngioDynamics, Latham, NY). Once the SMA was successfully cannulated, digital subtraction angiography (DSA), once again, confirmed an anomalous connection between the SMA and the hypertrophied SMV; identification of the exact point of fistula formation was achieved with the assistance of intravascular ultrasound (IVUS). The previously positioned 6F sheath was upsized to a long 12F sheath in preparation of stent deployment. After exchanging the wire for a stiff Amplatz (Cook Medical, Bloomington, IN), a 10 mm × 2.5 cm Viabahn self-expanding, covered stent (W.L. Gore and Associates, Flagstaff, AZ) was deployed to cover the marked anomalous arteriovenous connection [Figure 1].

The patient tolerated the procedure well without any postoperative complications and was discharged home the following day. His repeat CTA at 1-month postoperatively exhibited a patent stent, absence of any

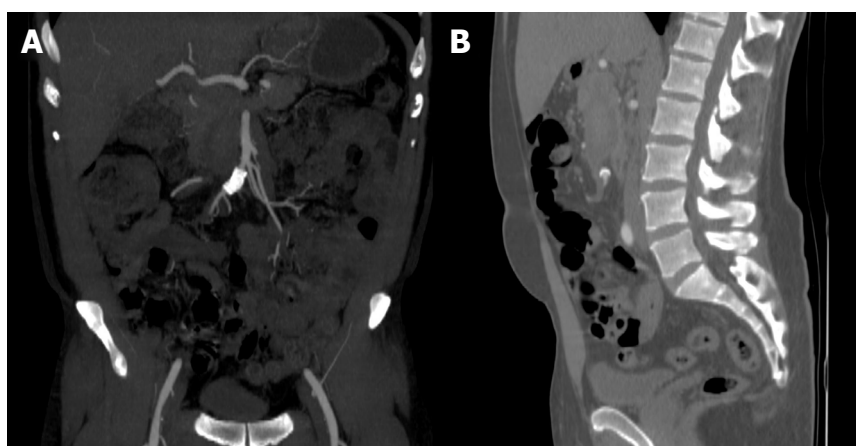


Figure 2. Postoperative surveillance CTA demonstrated successful exclusion of the SMAVF and loss of venous collaterals on both coronal (A) and sagittal (B) projections. CTA: computed tomographic angiogram; SMAVF: superior mesenteric arteriovenous fistula

communicating fistulas, and reduced SMV and portal vein size [Figure 2]. Most importantly, his abdominal discomfort resolved. The patient was again seen in clinic at 14-month postoperatively where a mesenteric duplex demonstrated a widely patent SMA stent in an asymptomatic patient.

DISCUSSION

SMAVF is most commonly caused by penetrating abdominal trauma or iatrogenic injury during abdominal surgery. Specifically, this complication has been observed after small bowel resection, colectomy, aortobifemoral bypass, and kidney-pancreas transplantation^[3,4]. The clinical manifestations of SMAVF may vary from completely asymptomatic to nonspecific abdominal pain, anorexia, nausea, diarrhea, gastrointestinal hemorrhage, signs of portal hypertension, and even congestive heart failure secondary to a persistent high-flow state^[5]. The most common physical findings are the presence of a machine-like bruit, palpable abdominal mass, or a thrill over the abdomen.

Mesenteric duplex can be used as the initial diagnostic imaging modality as it does not have any associated risks related to radiation or contrast administration. While specificity may be high with this study, sensitivity can be lacking - particularly in the morbidly obese patient. Therefore, CTA with both an arterial and venous phase should also be considered as the confirmatory diagnostic modality. However, the gold standard for diagnosis, continues to be intraoperative DSA which carries the highest sensitivity and specificity. In our patient, DSA was used to define the area of vascular defect intraoperatively. Unfortunately, in the setting of significant venous congestion, near-instant filling of arterial and venous collaterals can make delineation of vascular anatomy difficult, even with the benefit of orthogonal angles. In these situations, IVUS in the suspected vascular territories can help identify the exact site of defect for stent coverage.

Endovascular treatment has supplanted open ligation secondary to low morbidity and a desire to avoid laparotomy in a reoperative abdomen. Coil embolization, vascular plugs, and covered stents have all been deployed with varying success. A literature review demonstrates at least 25 instances of endovascular SMAVF repair consisting of 16 cases with coil embolization, two cases with vascular plugs, six cases with covered stents, and one patient repaired with a combination of both coil embolization and covered stent exclusion^[5-7]. Coil embolization, while effective at fistula exclusion, is associated with a high risk of postoperative migration and inadvertent thrombotic events and should be avoided if possible. For example, three of the coil embolization procedures were complicated by portal thrombosis. While two were self-limited and resolved after heparinization^[8,9], the third patient died from multiorgan failure^[1]. Additionally,

there was one reported case of bowel ischemia requiring intestinal resection after coil embolization^[10]. Not surprisingly, it appears that the risk of coil migration is increased in patients with a short, wide fistula neck.

Although no complications were reported in the seven patients treated with covered stents, the drawbacks associated with SMA stent grafting should not be neglected; these potential complications include the need for large access sheaths, difficulty cannulating the SMA ostia, intraoperative thromboembolism, and risk of subacute or late stent thrombosis which could lead to acute or chronic bowel ischemia. Therefore, the risks and benefits for each patient should be individually characterized before an operative decision is made. We empirically recommend dual antiplatelet therapy in the three-month postimplantation period with subsequent life-long treatment with aspirin and regular duplex surveillance for stenosis/occlusion.

In conclusion, SMAVF is a rare pathology observed after abdominal injury. Although severe morbidity can occur if left untreated, these anomalous connections can be safely intervened upon to relieve symptoms with covered stents.

DECLARATIONS

Authors' contributions

Concept and design, writing the manuscript, and critical revision: Wang SK, Xie J, Motaganahalli RL
Data collection: Wang SK

Financial support and sponsorship

None.

Conflicts of interest

The authors have no relevant financial conflicts of interest to disclose.

Patient consent

Written informed consent was obtained previous to the preparation and submission of this manuscript and is available upon request.

Ethics approval

This report was exempt from institutional review board protocols.

Copyright

© The Author(s) 2018.

REFERENCES

1. Zhao Y, Li Z, Zhang L, Wei B, Zeng X, Fu P. Portal vein thrombosis secondary to embolization of superior mesenteric arteriovenous fistula. *Ann Vasc Surg* 2014;28:490.e9-12.
2. Bulut T, Oosterhof-Berkas R, Geelkerken RH, Brusse-Keizer M, Stassen EJ, Kolkman JJ. Long-term results of endovascular treatment of atherosclerotic stenoses or occlusions of the coeliac and superior mesenteric artery in patients with mesenteric ischaemia. *Eur J Vasc Endovasc Surg* 2017;53:583-90.
3. Khan TF, Ciancio G, Burke GW 3rd, Sfakianakis GN, Miller J. Pseudoaneurysm of the superior mesenteric artery with an arteriovenous fistula after simultaneous kidney-pancreas transplantation. *Clin Transplant* 1999;13:277-9.
4. Grujic D, Knezevic A, Vojvodic S, Grujic B. Superior mesenteric arteriovenous fistula presenting with massive lethal upper gastrointestinal bleeding 14 years after small bowel resection. *Balkan Med J* 2015;32:214-7.
5. Weinstein D, Altshuler A, Belinki A, Peer A, Gayer G, Halevy A, Bass A. Superior mesenteric artery to superior mesenteric vein arteriovenous fistula presenting as abdominal pain and gastrointestinal bleeding 3 years after an abdominal gunshot wound: report of a case and review of the literature. *J Trauma* 2009;66:E13-6.
6. Wang C, Zhu X, Guo GH, Shu X, Wang J, Zhu Y, Li B, Wang Y. Superior mesenteric arteriovenous fistula presenting as gastrointestinal

- bleeding: case report and literature review. *Rev Esp Enferm Dig* 2016;108:503-7.
7. An T, Zhou S, Song J, Jiang T, Li X, Wang W. Massive gastrointestinal bleeding secondary to superior mesenteric arteriovenous fistula. *Am J Gastroenterol* 2013;108:1662-5.
 8. Mick SL, Bush HL Jr, Barie PS. Superior mesenteric arteriovenous fistula causing massive hematemesis. *Surgery* 2003;134:102-4.
 9. Purow DB, Maltz C. Superior mesenteric arteriovenous fistula: a rare cause of esophageal variceal bleeding. *J Clin Gastroenterol* 2002;35:284-5.
 10. Hussein M, Issa G, Muhsen S, Haydar A. Superior mesenteric arteriovenous fistula embolisation complicated by bowel ischaemia. *BMJ Case Rep* 2013;2013:bcr2013009521.

Review

Open Access



Ruptured isolated descending thoracic aortic aneurysm: open or endovascular repair?

Amer Harky^{1,2}, Nichola Manu¹, Rafal Al Nasiri³, Dilan Sanli⁴, Ciaran Grafton-Clarke⁵, Jeffrey Shi Kai Chan⁶, Chris Ho Ming Wong⁶

¹Department of Vascular Surgery, Countess of Chester Hospital, Chester CH2 1UL, UK.

²School of Medicine, Cardiff University, Cardiff CF14 4XN, UK.

³Department of General Surgery, Countess of Chester Hospital, Chester CH2 1UL, UK

⁴Faculty of Medicine, Bulent Ecevit University, Zonguldak 67100, Turkey.

⁵School of Medicine, University of Liverpool, Liverpool L69 3GE UK.

⁶Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China.

Correspondence to: Dr. Amer Harky, Department of Vascular Surgery, Countess of Chester Hospital, Chester CH2 1UL, UK.
E-mail: aaharky@gmail.com

How to cite this article: Harky A, Manu N, Al Nasiri R, Sanli D, Grafton-Clarke C, Chan JSK, Wong CHM. Ruptured isolated descending thoracic aortic aneurysm: open or endovascular repair? *Vessel Plus* 2018;2:8.
<http://dx.doi.org/10.20517/2574-1209.2018.12>

Received: 18 Mar 2018 **First Decision:** 9 Apr 2018 **Revised:** 27 Apr 2018 **Accepted:** 3 May 2018 **Published:** 7 May 2018

Science Editor: Nikolaos Patelis, Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

Descending thoracic aortic aneurysm management has gained momentum and became a topic of many debates at international levels since the evolution of endovascular repair. Ruptured descending thoracic aortic aneurysm is a clinical emergency which is associated with high mortality and morbidity rates if not managed properly. Prior to thoracic endovascular aortic repair (TEVAR), open repair (OR) was the gold standard management, however since the evolution of TEVAR, this has changed. Several centers have reported many of their experiences and published that TEVAR can provide equal or even better perioperative outcomes when compared to OR, although the evidences can be of only short term and could be biased at different levels at the time of publication. This review article is aimed to examine current literature evidences behind the use of TEVAR *vs.* OR and the reported comparative clinical outcomes.

Keywords: Open repair, aorta, endovascular repair, ruptured aorta, descending thoracic aorta

INTRODUCTION

Descending thoracic aortic aneurysm (DTAA) is a clinical entity that gained a lot of international attention in the current era. Currently, the presence of such aneurysms is mandated for elective repair to prevent



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



rupture or dissection, however the clinical emergency occurs when such aneurysms rupture. Although it is a rare pathology, ruptured descending thoracic aortic aneurysm (rDTAA) carries high mortality and morbidity rates with the majority of patients dying prior to arrival to hospital^[1]. The incidence of rupture is estimated to be 5 in 100,000 of population.

Just over a decade ago, the gold standard management of rDTAA was open repair (OR) requiring thoracotomy, aortic cross-clamping, aneurysm resection and replacement with an interposition of a prosthetic graft and cardiopulmonary bypass^[2-4]. However, ORs were associated with high perioperative mortality and neurological adverse events^[5]; nevertheless with advancement in clinical practice and evolving minimal access surgical interventions, the role of endovascular repair in such emergencies has been explored. Thoracic endovascular aortic repair (TEVAR) has appealing qualities including a minimally invasive procedure with rapid deployment with decreased operative time and decreased blood loss^[3]. However, this approach is subject to anatomical and logistical limitations, including anatomical requirements and variations, the quality of the landing zones, ease of iliac access, availability of the wide range of stent graft sizes in an emergency setting and available expertise on site^[4]. The major benefits of endovascular repair reported are lower mortality and morbidity rates associated with such a complex procedure^[5]. The advantage of TEVAR was not only limited to such perioperative outcomes but TEVAR was also used in patients who were not surgical candidates, which has resulted in an alternate management option as opposed to conservative management in those patients with almost 100% mortality rates^[5].

Despite TEVAR being an attractive alternative option for OR, it still remains a high-risk procedure. The current literature has many limitations in that the majority of evidence comes from case series and there is a lack of appropriate randomized controlled trials or long term comparative data that confirms the accuracy of the data and thus globalization of the results^[6-8]. Therefore, the aim of this paper is to review current literature and the evidence behind using TEVAR in the emergency setting of rDTAA and comparing the clinical outcomes with OR in such cohort of patients.

EVIDENCES BEHIND TEVAR IN rDTAA

The use of endovascular repair goes back to as early as 1991, when first performed on an abdominal aortic aneurysm^[9]. Since then, the technology has evolved with advancement in using endovascular repair for aortic aneurysms repair. Most of the patients who experience rDTAA do not survive to present to hospital. Hence why of those that do, open repair remains a strong choice for managing such patients. Yet, open repair is associated with significantly higher rates of mortality (ranging 14%-45% in specialized centers) and morbidity peri- and post-operatively^[10-13].

Currently, TEVAR is the standard management plan for elective cases of DTAA^[14], however the evidences behind using TEVAR in the emergency setting are scarce and limited^[7]. There are however several published reports from international centers about the clinical outcomes for the use of TEVAR in emergency situations for rDTAA^[2,6-8,15], but most of these are limited to a relatively small number of cases and are observational studies. These studies have been summarized in [Table 1](#).

A study by Jonker *et al.*^[7] analysed 87 patients that underwent emergency TEVAR for rDTAA between 2002 and 2009. The majority of the cases (> 90%) were in critical condition and immediate intervention was required. Forty percent of the patients were haemodynamically unstable and 22% were in shock. In their study, they have noted a 30-day mortality rate of 18.4%, whilst the rate of stroke and neurological complications were 8% in both. Eighteen percent of the patients were diagnosed with an endoleak within 30 days of the procedure. It is important to note that the presence of shock and haemothorax at the time of admission were the two contributing factors for increased mortality rates in these groups of patients. The same group^[16] published their data of 161 patients, of which 92 patients were treated with OR and 69 with

Table 1. Findings of large-scale comparative studies on open versus endovascular repair of rDTAA

Study	Type	Population	Sample size	Age (years)		Male		30-day mortality		Long term survival		Re-intervention rate ^a	
				Open	TEVAR	Open	TEVAR	Open	TEVAR	Open	TEVAR	Open	TEVAR
Jonker <i>et al.</i> ^[18] , 2010	Meta-analysis	United States	Open: 81 TEVAR: 143 Total: 244	70.2	70	66.7%	70.8%	33.3%	18.9% (<i>P</i> = 0.016)	3 years 82%	70.6%	30 days 2.3% (<i>P</i> = 0.169)	9.1% ^b
Jonker <i>et al.</i> ^[16] , 2011	Retrospective cohort	United States	Open: 69 TEVAR: 92 Total: 161	64.8	69.4	51%	62%	24.6%	17.4% (<i>P</i> = 0.26)	4 years 64.3%	75.2% (<i>P</i> = 0.191)	30 days 2.9%	7.6% ^b
Jonker <i>et al.</i> ^[7] , 2010	Retrospective cohort	United States	Open: n/a TEVAR: 87 Total: 87	n/a	69.8	n/a	69%	n/a	18.4% (<i>P</i> = 0.014)	4 years n/a	74.6%	30 days n/a	18.4% ^b
Piffaretti <i>et al.</i> ^[17] , 2015	Retrospective analysis		Open: n/a TEVAR: 56 Total: 56	n/a	62	n/a	71.2%	n/a	12.5%	2 years n/a	81%	30 days n/a	0%
Kilic <i>et al.</i> ^[15] , 2014	Retrospective analysis	United States	Total: 2788 1998-2004: 1596 2005-2008: 1192	68.6		60%		36.6%	21.5%	n/a		n/a	
Minami <i>et al.</i> ^[8] , 2015	Retrospective analysis	Japan	Open: 14 TEVAR: 23 Conservative: 13 Total: 50	n/a	76.8	n/a	62.5%	7.7%	4.3%	2 years n/a	57.8%	30 days n/a	17.4% ^b

^aIncludes re-exploration for bleeding, open repair and additional TEVAR; ^brate of Endoleak within 30 days. TEVAR: thoracic endovascular aortic repair; rDTAA: ruptured descending thoracic aortic aneurysm; n/a: not available

TEVAR. The outcomes were different, however in favour of TEVAR. There was a 30-day mortality of 25% in the OR group *vs.* 17% following TEVAR, although this was not deemed to be statistically significant. On the other hand, the 4-year survival rate was 75% in TEVAR *vs.* 64% in OR group; moreover the postoperative neurological complications were much less in TEVAR than the OR group. In a further study by Piffaretti *et al.*^[17], who studied 56 patients that underwent TEVAR for rDTAA found that early evacuation of a haemothorax reduced postoperative mortality significantly in patients with cardiorespiratory compromise at the time of presentation.

All these findings were supported by a larger study from Kilic *et al.*^[15], who analysed 2788 patients that had rDTAA and underwent either open or endovascular repair in an emergency setting. They identified an operative mortality reduction from 52.6% to 23.4% primarily related to the use of an endovascular repair approach in the majority of patients. Whilst their study is reflective of a large database, they have only demonstrated the short-term outcome with operative mortality rather any mid or long term outcomes.

In a further study by Minami *et al.*^[8] in 2015, 23 patients that underwent emergency TEVAR for rDTAA showed that the mortality rate is much lower when benchmarked with OR at an international center. They reported operative mortality of 26% whilst the rate of neurological complications postoperatively was 26%.

Lastly, a meta-analysis of 224 patients that had rDTAA by Jonker *et al.*^[18], of which 143 patients underwent TEVAR and 81 patients underwent OR have concluded that TEVAR is a safe and effective alternate option to OR in selected patients. The 30-day mortality was 19% *vs.* 33% in TEVAR and OR group of patients respectively. Although the rate of postoperative neurological complications was higher in OR than TEVAR, this again was not statistically significant.

The overall current published literature shows that TEVAR is a feasible option in managing patients with rDTAA and the results are promising in the short term when compared to open repair.

IS EVERYONE A CANDIDATE FOR TEVAR?

Although TEVAR seems an attractive option for managing patients with rDTAA, it cannot be used as a standard management in every patient at the time of presentation due to many factors^[3]. Initially the gold standard method for managing such patients was OR^[19], however this method has been challenged by the evolution of TEVAR and the favourable short-term outcomes^[16,20,21]. One of the key factors in choosing TEVAR over OR, is the anatomic variations and suitability for TEVAR. To assess such anatomical variation, thorough imaging studies are required such as computerized tomography and magnetic resonance imaging to assess such anatomical suitability^[3]. Cross-sectional imaging of the aorta is essential, alongside detailed aortic pathology assessment using computerized tomography aortogram. Although obtaining such imaging can be time consuming and may delay the immediate management, they are crucial to determine the location and extent of the pathology so that appropriate interference can be implicated^[19]. It is not only important to obtain knowledge about the pathology itself, but rather the assessment of the neck vessels, vertebral arteries and access point vessels are of paramount to evaluate for suitability of endovascular repair, which provides a sufficient amount of information for a rapid decision about whether to perform TEVAR or OR.

An important factor considering TEVAR for rDTAA is the quality of the landing zones. The application for traditional TEVAR requires a proximal and distal landing zone of at least 2 cm. However, patients who present with thoracic aortic pathology can have disease extending to the aortic arch, resulting in an unsuitable proximal landing zone distal to the left subclavian artery. In order to optimize outcome and reduce complications following the graft placement in zones 0, 1 or 2 of the aortic arch, planning for revascularization of the aortic vessels is essential to prevent neurovascular compromise and risk of stroke. Therefore, when TEVAR is extended into zone 2 further procedures such as left carotid-subclavian artery bypass or left carotid-subclavian artery transposition is warranted. When TEVAR is extended into zone 1 of the aortic arch, the left common carotid artery requires revascularization via carotid-carotid crossover bypass^[22].

Traditionally, patients with calcified vessels, difficult anatomy and an inability to identify suitable access points, as well as patients with connective tissue disorders, are offered open repair over TEVAR^[7,16,23].

OPEN OR ENDOVASCULAR REPAIR FOR rDTAA?

The choice of OR or TEVAR in patients presenting with rDTAA remains debatable at present. In many centers internationally TEVAR is offered as the first line treatment for these patients unless contraindicated, such as patients with connective tissue disorders, except as a temporizing solution until definitive surgery can be performed^[24]. The choice of TEVAR also depends largely on the available expertise and the anatomic limitations of the DTA, as discussed above.

TEVAR itself offers a minimal access procedure and thus saving major operating time and reducing perioperative complications associated with OR. Stabilization of the patient through aggressive resuscitation is a key step in preparing the patient for either OR or TEVAR. This includes potentially controlling of the source of bleeding through either application of a clamp at the proximal aorta in OR or balloon inflation in the case of TEVAR; however the latter seems to be less efficient technically in providing adequate control of the bleeding^[3]. An advantage of TEVAR is the selection of anaesthetic technique. It is possible to perform TEVAR under either local, regional or general anaesthesia, in contrary to OR where it can be performed only under general anaesthesia. Therefore, patients with advanced cardiopulmonary comorbidities and those who are unfit for OR may potentially be a suitable candidate for TEVAR and thus a life saving procedure can be performed^[25].

Neuroprotection in patients undergoing repair of rDTAA is a key step for a favourable outcome. This includes stabilization of spinal cord perfusion pressure through placement of a lumbar drain to avoid the

effect of fluctuated blood pressure during repair^[26,27]. The current paraplegia rate following TEVAR is around 2%-6%^[28] in contrary to OR that has a 8.7% rate^[16]. This rate in TEVAR can go as high as 15% depending on the presence of many cofounding factors such as hypotension, concomitant repair of abdominal aortic aneurysm, long standing aortic disease and renal failure^[25,27,28].

Although the current trend in managing patients with rDTAA is shifting towards TEVAR, the experience is limited to case series and based on centers of excellence and the presence of experienced operators. Nevertheless, the studies are confined to short-term outcomes. There is a lack of data related to long-term outcomes, on the contrary to OR, where the evidence behind its success and long-term outcomes has been well reported in literature. The surge in using TEVAR is pushed forward by the satisfactory short-term outcomes in TEVAR itself, providing lower morbidity and mortality rates when compared with OR^[7,16,23].

In a meta-analysis by Jonker *et al.*^[18], they have identified that the mortality rate is much lower in TEVAR group than OR, 18.9% vs. 33.3% respectively ($P = 0.16$), whilst the stroke rate is reported to be higher in OR than TEVAR (10.2% vs. 4.1% respectively), however this is not statistically significant ($P = 0.11$). Similarly, the paraplegia rate was higher in OR than TEVAR (5.5% vs. 3.1%), yet this is also not statistically significant ($P = 0.40$). Whilst vascular complications were higher in the TEVAR group than OR (9.1% vs. 2.3%, $P = 0.17$), interestingly, the survival rate from aneurysm related complications was 70.6% in TEVAR patients compared with 100% in the OR patients.

Despite the success of TEVAR, it carries many limitations. A key consideration in patients undergoing TEVAR is the rate of re-intervention. A study by Desai *et al.*^[29] has reported a survival rate of TEVAR equivalent to OR in patients with rDTAA at 8-10 years of follow up. In a later study by Botsios *et al.*^[21], the rate of re-intervention is thought to be between 4.5%-16% at 1.5-44 months follow up time. Interestingly this rate reported to be as high as 45.5% after rDTAA in some other studies^[6,18]. Such re-interventions can be very drastic and require further major intervention and hence affect the long-term outcomes overall. Another limitation of TEVAR is the rate of graft infection, although rare, it is associated with a high mortality rate of up to 50% and often requires surgical intervention for definitive management^[30].

Moreover, current vascular surgery practice guidelines suggest considering several factors prior to TEVAR in patients presenting with rDTAA. These factors include anatomical consideration, surgical urgency and the presence of surgical expertise to perform the procedure^[18].

At this current stage, there is no randomized controlled trial found to provide comparative clinical outcomes and cost effectiveness comparison between OR and TEVAR in patients presenting with rDTAA. Therefore, the choice of procedure in these patients is based on the experience of the center and the operator^[3].

CONCLUSION

TEVAR serves a feasible and attractive option for patients presenting with rDTAA. It is being used in many centers as the first line management of such acutely unwell patients, primarily due to its promising short-term outcomes. However, the published data behind this recommendation is limited and is composed of only case series with retrospective observational studies and lacks any randomized data trials. Regular follow up of patients that undergo a TEVAR is required for early identification and management of TEVAR-related complications such as endoleaks.

DECLARATIONS

Authors' contributions

Design: Harky A, Chan JSK, Wong CHM

Draft: Harky A, Al Nasiri R, Sanli D, Chan JSK, Wong CHM

Proofreading: Manu N, Grafton-Clarke C

Revising: Harky A, Manu N, Grafton-Clarke C

Approved the final manuscript: all authors

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Johansson G, Swedenborg J. Ruptured abdominal aortic aneurysms: a study of incidence and mortality. *Br J Surg* 1986;73:101-3.
2. Gaudino M, Lau C, Munjal M, Girardi LN. Open repair of ruptured descending thoracic and thoracoabdominal aortic aneurysms. *J Thorac Cardiovasc Surg* 2015;150:814-23.
3. Farlough CL, Eskandari MK. Thoracic endovascular aneurysm repair (TEVAR) for ruptured thoracic aortic aneurysms. *J Vasc Endovasc Surg* 2017;2:14.
4. Gabriel SA, Rinaldi E, Leopardi M, Melissano G, Chiesa R. TEVAR for ruptured descending thoracic aortic aneurysm: case report. *J Vasc Surg* 2016;15:322-7.
5. Keulen JWV, Jonker FHW, Indes J, Muhs BE. Endovascular repair of ruptured descending thoracic aneurysms. *Endovasc Today* 2010;9:64-6.
6. Choi JS, Oh SJ, Sung YW, Moon HJ, Lee JS. Early experiences with the endovascular repair of ruptured descending thoracic aortic aneurysm. *Korean J Thorac Cardiovasc Surg* 2016;49:73-9.
7. Jonker FH, Verhagen HJ, Lin PH, Heijmen RH, Trimarchi S, Lee WA, Moll FL, Athamneh H, Muhs BE. Outcomes of endovascular repair of ruptured descending thoracic aortic aneurysms. *Circulation* 2010;121:2718-23.
8. Minami T, Imoto K, Uchida K, Karube N, Yasuda S, Choh T, Suzuki S, Masuda M. Thoracic endovascular aortic repair for ruptured descending thoracic aortic aneurysm. *J Card Surg* 2015;30:163-9.
9. Parodi JC, Palmaz JC, Barone HD. Transfemoral intraluminal graft implantation for abdominal aortic aneurysms. *Ann Vasc Surg* 1991;5:491-9.
10. Minatoya K, Ogino H, Matsuda H, Sasaki H, Yagihara T, Kitamura S. Replacement of the descending aorta: recent outcomes of open surgery performed with partial cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 2008;136:431-5.
11. Barbato JE, Kim JY, Zenati M, Abu-Hamad G, Rhee RY, Makaroun MS, Cho JS. Contemporary results of open repair of ruptured descending thoracic and thoracoabdominal aortic aneurysms. *J Vasc Surg* 2007;45:667-76.
12. Girardi LN, Krieger KH, Altorki NK, Mack CA, Lee LY, Isom OW. Ruptured descending and thoracoabdominal aortic aneurysms. *The Ann Thorac Surg* 2002;74:1066-70.
13. Schermerhorn ML, Giles KA, Hamdan AD, Dalhberg SE, Hagberg R, Pomposelli F. Population-based outcomes of open descending thoracic aortic aneurysm repair. *J Vasc Surg* 2008;48:821-7.
14. Walsh SR, Tang TY, Sadat U, Naik J, Gaunt ME, Boyle JR, Hayes PD, Varty K. Endovascular stenting versus open surgery for thoracic aortic disease: systematic review and meta-analysis of perioperative results. *J Vasc Surg* 2008;47:1094-8.
15. Kilic A, Shah AS, Black JH, Whitman GJR, Yuh DD, Cameron DE, Conte JV. Trends in repair of intact and ruptured descending thoracic aortic aneurysms in the United States: a population-based analysis. *J Thorac Cardiovasc Surg* 2014;147:1855-60.
16. Jonker FH, Verhagen HJ, Lin PH, Heijmen RH, Trimarchi S, Lee WA, Moll FL, Athamneh H, Rampoldi V, Muhs BE. Open surgery versus endovascular repair of ruptured thoracic aortic aneurysms. *J Vasc Surg* 2011;53:1210-6.
17. Piffaretti G, Menegolo M, Kahlberg A, Mariscalco G, Rinaldi E, Castelli P, Grego F, Chiesa R, Antonello M. Hemothorax management after endovascular treatment for thoracic aortic rupture. *Eur J Vasc Endovasc Surg* 2015;50:608-13.

18. Jonker FH, Trimarchi S, Verhagen HJ, Moll FL, Sumpio BE, Muhs BE. Meta-analysis of open versus endovascular repair for ruptured descending thoracic aortic aneurysm. *J Vasc Surg* 2010;51:1026-32.
19. Coselli JS, Gopaladas RR. Ruptured thoracic aneurysms: to stent or not to stent? *Circulation* 2010;121:2705-7.
20. Goldstein LJ, Ramaiah VG, McKinsey JF. TEVAR for the ruptured thoracic aorta. *Endovasc Today* 2007;6:74-8.
21. Botsios S, Fromke J, Walterbusch G, Schuermann K, Reinstadler J. Endovascular treatment for non-traumatic rupture of the descending thoracic aorta: long-term results. *J Card Surg* 2014;29:353-8.
22. Han DK, Jokisch C, McKinsey J. Expanding the landing zone for TEVAR. *Endovasc Today* 2016;15:85-90.
23. Cambria RP, Crawford RS, Cho JS, Bavaria J, Farber M. A multicenter clinical trial of endovascular stent graft repair of acute catastrophes of the descending thoracic aorta. *J Vasc Surg* 2009;50:1255-64.
24. Shah A, Khojenezhad A. Thoracic repair for acute type A aortic dissection: operative technique. *Ann Cardiothorac Surg* 2016;5:389-96.
25. Hogendoorn W, Schlosser FJ, Muhs BE, Popescu WM. Surgical and anesthetic considerations for the endovascular treatment of ruptured descending thoracic aortic aneurysms. *Curr Opin Anaesthesiol* 2014;27:12-20.
26. Lam CH, Vatakencherry G. Spinal cord protection with a cerebrospinal fluid drain in a patient undergoing thoracic endovascular aortic repair. *J Vasc Interv Radiol* 2010;21:1343-6.
27. Scali ST, Wang SK, Feezor RJ, Huber TS, Martin TD. Preoperative prediction of spinal cord ischemia after thoracic endovascular repair. *J Vasc Surg* 2014;60:1481-90.
28. Eskandari MK, Daly CM. Management of Late TEVAR Failures. In: Eskandari MK, Pearce WH, Yao JST, editors. *Current Vascular Surgery*, 2012. Shelton, CT: PMPH-USA; 2012. p. 393-406.
29. Desai ND, Burtch K, Moser W, Moeller P, Szeto WY. Long-term comparison of thoracic endovascular aortic repair (TEVAR) to open surgery for the treatment of thoracic aortic aneurysms. *J Thorac Cardiovasc Surg* 2012;144:604-9.
30. Cernohorsky P, Reijnen MM, Tielliu IF, van Sterkenburg SM, van den Dungen JJ. The relevance of aortic endograft prosthetic infection. *J Vasc Surg* 2011;54:327-33.

Case Report

Open Access



Repair of mitral subvalvular apparatus and a calcified left ventricle aneurysm

Kasra Shaikhrezai¹, Sanjeet Singh Avtaar Singh¹, Karim Morcos¹, Steve Hunter²

¹Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK.

²Department of Cardiothoracic Surgery, Northern General Hospital, Sheffield S5 7AU, UK.

Correspondence to: Dr. Sanjeet Singh Avtaar Singh, Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK. E-mail: sanjeetsingh@nhs.net

How to cite this article: Shaikhrezai K, Singh SSA, Morcos K, Hunter S. Repair of mitral subvalvular apparatus and a calcified left ventricle aneurysm. *Vessel Plus* 2018;2:9. <http://dx.doi.org/10.20517/2574-1209.2018.17>

Received: 29 Mar 2018 **First Decision:** 28 Apr 2018 **Revised:** 4 May 2018 **Accepted:** 7 May 2018 **Published:** 10 May 2018

Science Editor: Cristiano Spadaccio, Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

Left ventricle (LV) myocardial infarction may result in changes to the structure of the subvalvular apparatus. This may lead to a functional regurgitation if accompanied by annular dilatation preventing coaptation of leaflets. Scar tissue formation in the left ventricle may also lead to aneurysm of the left ventricle. This can then calcify, making repair of the leaflet technically challenging. We present a case of a mitral valve repair with concomitant repair of left ventricle aneurysm in a 75-year-old gentleman who suffered an ST-segment elevation myocardial infarction to the lateral wall 20 years ago. He presented with breathlessness on minimal activity, severe mitral regurgitation with a posteriorly oriented regurgitant jet and calcification of LV aneurysm on chest X-ray and computed tomography scan. Despite the challenging nature, it is possible to repair a mitral valve with concomitant calcified LV aneurysm formation. Long term outcomes are still unknown for this cohort of patients.

Keywords: Transmural infarct, left ventricular dilatation, ischaemic mitral valve repair, left ventricular aneurysm with calcification

INTRODUCTION

Left ventricle (LV) myocardial infarction (MI) may initiate a series of configurational remodelling of the ventricle which leads to changes in the 3D geometry of the subvalvular apparatus^[1]. This chain of events accompanied by annular dilatation may cause functional mitral regurgitation (MR). Another well-recognised post MI complication is the LV aneurysm which may result in calcification of the aneurysm that can be visualised on a chest radiograph^[2]. Generally LV aneurysm is considered as a deleterious outcome



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



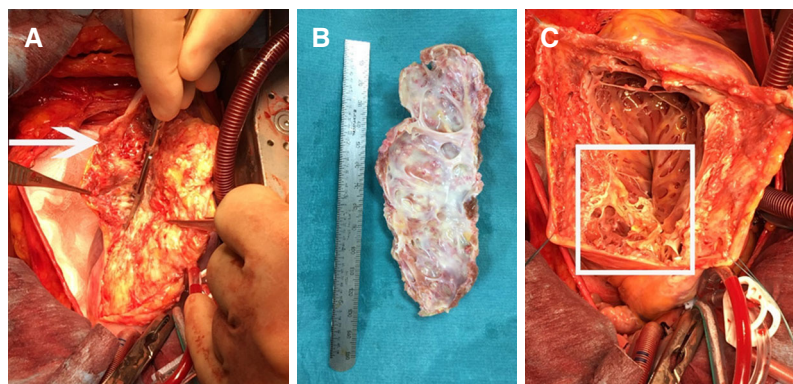


Figure 1. A: Shaving the calcified patch off the epicardium (arrow); B: the giant calcified patch; C: ventriculotomy with distorted and damaged mitral subvalvular apparatus (inside the square)

of MI with an increased risk of congestive heart failure, thromboembolism, unstable angina as well as ventricular tachycardia^[3]. In symptomatic patients, aneurysmectomy is predominantly offered as an adjunct to surgical revascularisation however in this group the survival is more dependent on the revascularisation rather than the aneurysmectomy^[3]. Combination of LV aneurysm calcification and severe MR remains a rare and challenging post-MI pathology while the failing heart on maximal medical therapy makes complex surgery the primary treatment choice.

CASE REPORT

A 75-year-old Caucasian male was referred with severe symptomatic MR 20 years following lateral ST-elevation MI. He presented with breathlessness on minimal activity, severe MR with a posteriorly oriented regurgitant jet and calcification of LV aneurysm on chest X-ray and computed tomography scan. His calculated EuroSCORE II mortality was 21%.

Following institution of aorto-bicaval cardiopulmonary bypass (CPB), cooling to 32 °C and cardioplegic arrest, the heart was positioned to expose the lateral surface where the aneurysm was located. Using a skin knife (blade size 24), the lateral surface of the LV was incised longitudinally parallel to the course of posterior descending artery. An incision was made whereby the blade met the outer surface of calcification which was extended to endocardium and myocardium sparing the epicardium. The epicardium was shaved off the calcified patch [Figure 1A]. The patch measuring 15 cm × 5 cm was entirely excised completing the ventriculotomy [Figure 1B and C]. The mitral subvalvular apparatus needed reconstruction due to excision of the calcified patch and intrinsic disease. The construction of subvalvular apparatus started with reimplantation of healthy parts of the posteromedial papillary muscle using pledgeted CV-4 polytetrafluoroethylene (PTFT) sutures (GORE-TEX, WL Gore & Associates Inc, Flagstaff, AZ, USA) followed by attachment of neo-chordae to the edge of both mitral valve leaflets where detached native chordae were located. For papillary muscle reimplantation the fibrosed segment of papillary muscle was used to anchor a pledgeted box stitch to the stable part of papillary muscle already located on the LV. Subsequently the PTFT sutures were passed through the LV [Video 1] and tied on the outside of the heart using pledgets for more strength.

The neo-chordae to the edge of leaflets were left untied on the atrial side because prior to closure of ventriculotomy accurate length measurement of neo-chordae was impossible. Satisfactory reconstruction of subvalvular apparatus was confirmed by firm and stable reimplantation of papillary muscle and attachment of neo-chordae between papillary muscle and the leaflets for each detached native chordae. The ventriculotomy was closed by bovine pericardial patch which was sewn by running polypropylene suture around the edge of ventriculotomy [Figure 2A]. The epicardium flap from which the calcified patch was

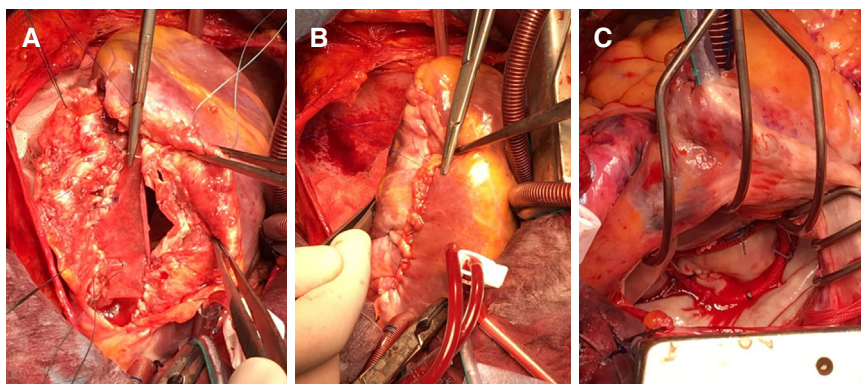


Figure 2. A: Closing the left ventricle by bovine pericardial patch; B: reinforcement of ventriculotomy closure by epicardium; C: mitral valve with ring annuloplasty is satisfactorily tested

shaved off was used to cover bovine pericardial patch for haemostasis [Figure 2B].

The atrial surface of mitral valve was approached via left atriotomy. The length of neo-chordae attached to the leaflets was accurately measured for an optimised coaptation before tying on the atrial side of the leaflets. A flexible 31-mm annuloplasty ring (St. Jude Medical Inc, St. Paul, MN, USA) was implanted after sizing the annulus from trigone to trigone and considering the area of anterior leaflet. The mitral valve was tested using a cardioplegia catheter into the LV through the valve [Figure 2C]. This was confirmed following weaning from CPB. The patient was asymptomatic at 6 weeks and 3 months follow-up with an echocardiogram demonstrating mitral valve competence with improved LV function.

The overall cardiopulmonary bypass time was 158 min with an aortic cross clamp time of 133 min.

DISCUSSION

LV aneurysm in the context of ischemic heart disease and subsequent calcification has been reported previously^[4]. However, there is a paucity of literature regarding the surgical treatment of calcified LV aneurysm in a symptomatic patient with severe MR. LV aneurysm calcification can be attributed to chronic renal failure^[5] or other hypercalcemic conditions, but this was absent in our patient. He was also suffering from concomitant severe MR which was structurally distorted by the calcification of LV aneurysm. Although severe MR in a symptomatic patient would be an indication for surgery, we could not ignore the role of calcification, impairing valve function and its impact on the planned repair as without excision of the calcified patch reimplantation of papillary muscle and subvalvular apparatus repair was not feasible. Although concomitant mitral valve repair and excision of LV aneurysm calcification is possible with desirable short-term results, longer follow-up is required to evaluate outcomes.

DECLARATIONS

Authors' contributions

Performed the operation: Shaikhrezai K, Hunter S

Wrote the case report: Shaikhrezai K

Reviewed and made changes to the structure and format of the manuscript: Singh SSA

Reviewed the manuscript: Morcos K

Served as the primary supervisor of the manuscript: Hunter S

Financial support and sponsorship

None.

Conflicts of interest

The authors do not have any conflicts of interest to declare.

Patient consent

Patient consent (images/case history) was obtained during the consent process.

Ethics approval

The study was approved by the local Clinical Governance and Research and Development Unit.

Copyright

© The Author(s) 2018.

REFERENCES

1. Otsuji Y, Handschumacher MD, Schwammenthal E, Jiang L, Song JK, Guerrero JL, Vlahakes GJ, Levine RA. Insights from three-dimensional echocardiography into the mechanism of functional mitral regurgitation: direct in-vivo demonstration of altered leaflet tethering geometry. *Circulation* 1997;96:1999-2008.
2. MacGregor JH, Chen JT, Chiles C, Kier R, Godwin JD, Ravin CE. The radiographic distinction between pericardial and myocardial calcifications. *AJR Am J Roentgenol* 1987;148:675-7.
3. Friedman BM, Dunn MI. Postinfarction ventricular aneurysms. *Clin Cardiol* 1995;18:505-11.
4. Lee BK, Atwood E. Images in clinical medicine. Calcified left ventricular aneurysm. *N Engl J Med* 2003;348:918.
5. Kempf AE, Momeni MG, Saremi F. Myocardial calcinosis in chronic renal failure. *J Radiol Case Rep* 2009;3:16-9.

Case Report

Open Access



The first INSPIRIS RESILIA Aortic Valve™ replacement (Edwards Lifesciences) in endocarditis

Sanjeet Singh Avtaar Singh¹, Gwyn Beattie¹, David Reid², Philip Curry¹

¹Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK.

²Department of Anaesthesia and Intensive Care, Golden Jubilee National Hospital, Glasgow G81 4DY, UK.

Correspondence to: Dr. Sanjeet Singh Avtaar Singh, Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK. E-mail: sanjeetsingh@nhs.net

How to cite this article: Singh SSA, Beattie G, Reid D, Curry P. The first INSPIRIS RESILIA Aortic Valve™ replacement (Edwards Lifesciences) in endocarditis. *Vessel Plus* 2018;2:10. <http://dx.doi.org/10.20517/2574-1209.2018.18>

Received: 29 Mar 2018 **First Decision:** 10 Apr 2018 **Revised:** 4 May 2018 **Accepted:** 7 May 2018 **Published:** 15 May 2018

Science Editor: Cristiano Spadaccio, Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

There is an increasing number of patients who have surgery during the active phase of infective endocarditis. Despite the decreasing in-hospital mortality and increasing early intervention rate, optimal timing for surgery remains a difficult decision. For patients with mental illnesses, the choice of valve is another factor to consider as non-adherence may lead to serious adverse events. Antipsychotic medications may also alter the metabolism of vitamin K antagonists increasing the risk of stroke or major haemorrhage. We report a case of a 19-year-old man with a history of Ehlers-Danlos syndrome and aortic regurgitation, who required management of aortic valve bacterial endocarditis. This is the first report describing the use of the new RESILIA INSPIRIS valve which has increased durability and does not require anticoagulation.

Keywords: Endocarditis, bio-prosthetic valve, anti-coagulation, Ehler-Danlos syndrome

INTRODUCTION

Infective endocarditis is inflammation of the endocardium, usually of the valves, usually by bacterial infections. Indications for surgery include symptomatic heart failure, uncontrollable infection, embolic events, large vegetations, severe valvular and perivalvular lesions and infections by virulent microorganisms^[1]. Risk factors include structural abnormalities of the cardiac valve which alter flow dynamics (connective tissue disorders, prosthetic valves) for adherence of bacteria. This allows adhesion of bacteria to the valvular surface and propagates as vegetation or systemic emboli^[1].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





Figure 1. Computed tomography showing a pyramidal wedge of affected splenic tissue with the apex pointing towards the hilum, and the base on the splenic capsule with areas of heterogenous enhancement

CASE REPORT

We report a case of a 19-year-old man who was referred to the valvular multidisciplinary team (MDT) meeting. He was an inpatient in the coronary care unit for 2 weeks with a background of cardiac-valvular Ehlers-Danlos syndrome (cvEDS) with severe aortic regurgitation. His other comorbidities included Asperger's syndrome, borderline autism, psychotic depression and asthma. His regular medications included aripiprazole, fluoxetine, and bronchodilators. He had multiple admissions to psychiatric services due to non-adherence to medications.

He presented to the general surgeons with increasing abdominal pain, vomiting and raised inflammatory markers. There were no subcutaneous stigmata of infective endocarditis but a collapsing pulse was present on examination. A computed tomography scan showed embolic infarcts in the spleen [Figure 1] and blood cultures were positive for *Streptococcus mutans*.

He later developed cellulitis of his right foot in keeping with a septic embolism. A transthoracic echocardiography showed a large vegetation on the non-coronary cusp (NCC) of the aortic valve with no other valvular abnormalities and a preserved ventricular function. A subsequent trans-oesophageal echocardiograph revealed a 1.3 cm × 1.05 cm vegetation on the NCC with a perforation of the NCC and severe aortic regurgitation with two jets. The mitral valve was clean with no evidence of tricuspid valve regurgitation nor pulmonary valve insufficiency and there was no aortic root dilatation. His electrocardiogram incidentally was normal [Figure 2].

He received intravenous benzylpenicillin, clindamycin and gentamicin resulting in a C-reactive protein reduction from 134 to 26 mg/L. His dental checks were satisfactory at the time of referral. Due to the patient's comorbidities and previous history of non-adherence, there were concerns about the use of anticoagulation in the setting of a mechanic valve implantation. Therefore, the MDT outcome favoured the use of a bio-prosthetic valve. The INSPIRIS RESILIA Aortic Valve™ (Edwards Lifesciences, One Edwards Way, Irvine CA 92614 USA) was chosen partly due to the young age of the patient.

A median sternotomy was performed. The patient was cooled to 32 °C. Hockey stick aortotomy was done to access the native aortic valve. Cardioplegia was administered into the coronaries. On inspection, there was a large perforation on the non-coronary leaflet. The annulus was sized to 27 mm. The INSPIRIS RESILIA valve (model no. 11500A) was inserted using pledgeted Ti-Cron (Ti-Cron™, Covidien 555 Long Wharf Drive New Haven, CT 06511) mattress sutures. A Goretex™ (© 2012 W. L. Gore & Associates) patch was applied between the pulmonary artery and aorta as a wrap. The pericardium was closed using a Goretex patch. The patient was weaned off bypass without difficulty. The total ischaemic time was 91 min with a bypass time of 110 min. An intraoperative transoesophageal echocardiography revealed a well-seated valve with no

19 yr	Vent. rate	87	BPM	Normal sinus rhythm
Male	PR interval	146	ms	No previous ECGs available
	QRS duration	84	ms	
Room:	QT/QTc	382/459	ms	
Loc:99	P-R-T axes	63 50 31		

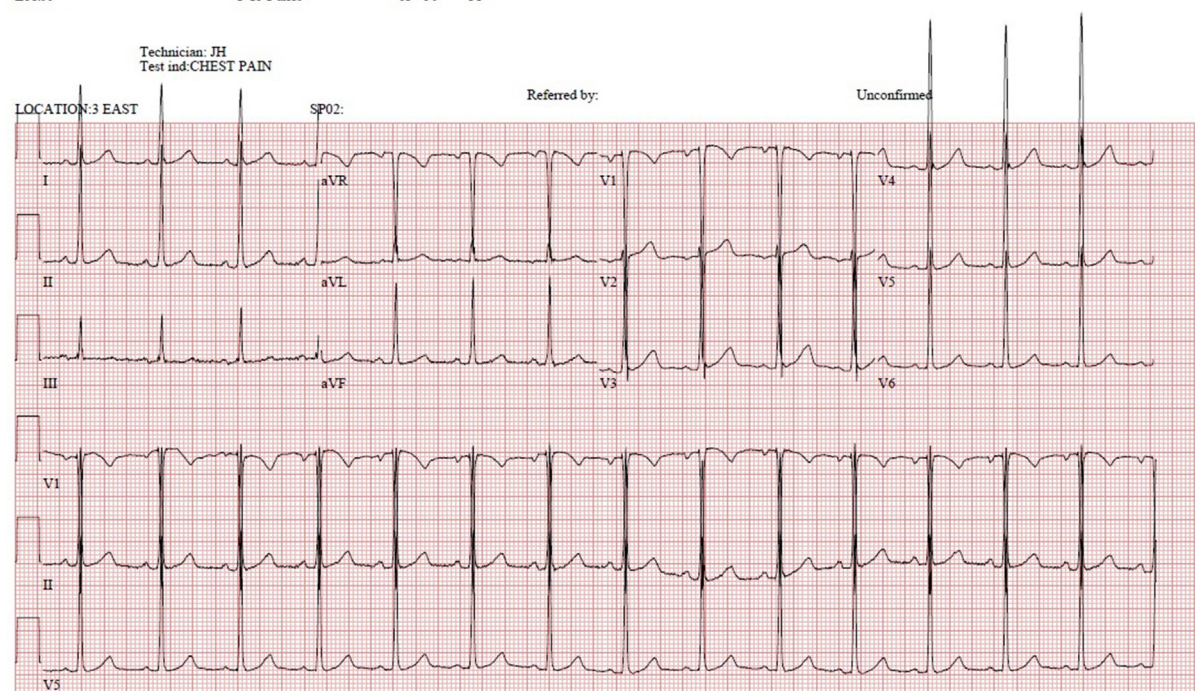


Figure 2. Preoperative electrocardiogram showing normal sinus rhythm

paravalvular leak.

He was extubated within 6 h of arrival to the intensive care unit, discharged to the high dependency unit on post-operative day 1 and to the ward the following day. A peripherally inserted central catheter line was inserted for subsequent doses of IV antibiotics post-operatively [Figure 3]. He was discharged home with the remainder of his antibiotic course administered as an outpatient on the 9th post-operative day. He attended the follow-up cardiology clinic with well-healed wounds and a transthoracic echocardiography revealed a well-seated valve with no regurgitant flow.

DISCUSSION

Bio-prosthetic tissue valves are advantageous due to the lower frequency of thromboembolism thereby avoiding long-term anticoagulation. Long-term durability, however, limits its usage in younger patients. Calcification of these valves causes structural valve degeneration. The type of bio-prosthetic tissue used also influences the rate of calcification. Liao *et al.*^[2] proved in rat models that bovine pericardium valves calcified less than porcine valves. This, however, did not have any effect on long-term survival. Most bio-prosthetic valves are fixed in glutaraldehyde to prevent early degeneration after implantation. This process in itself can cause in-vivo calcification of tissue. Various fixation tissue treatments have been developed to circumvent this effect. Carpentier *et al.*^[3] proved that the rate of calcification could be reduced by decreasing the phosphate content or by blocking calcification binding sites with magnesium ions and using surfactant. The enhancement of such properties may facilitate the use of tissue valves in younger patients. In one particular single center experience utilizing the Resilia™ technology, revealed excellent haemodynamic performance and safety outcomes at one year of follow-up with a paravalvular leak rate of < 1% at one year and no reduction in effective orifice area^[4]. Flameng *et al.*^[5] looked at the anti-calcification efficacy of using a novel method of treatment vs. standard anti-calcification in juvenile sheep. The novel technology involved:

1. Functional group capping which reduces the presence of aldehydes in glutaraldehyde-fixed tissues



Figure 3. Post-operative chest X-ray showing valve in the aortic position and peripherally inserted central catheter line

preventing oxidation and mitigating calcification;

2. Glycerolization with additional ethanol rinsing to reduce the residual chemicals and allowing the valves to be packed dry as the glycerol and ethanol mixture displaces most of the water;
3. Sterilisation with ethylene oxide, thus valves need not be rinsed prior to usage.

In this randomized study, the valves were placed in the mitral position. The sheep with the novel preservation had a lower mean gradient across the valve and less leaflet calcification. They concluded that the novel preservation significantly improved the hemodynamic and anti-calcification properties compared to current standard of care. This novel treatment led to the development of the INSPIRIS RESILIA valve. In its recently published safety and efficacy trial (COMMENCE 2), the INSPIRIS RESILIA valve showed improved breathlessness, increased effective orifice area, reduced mean gradient and low paravalvular leak rate at two years. One-year actuarial freedom from all-cause mortality for all patients was 97.6%^[6].

Our patient also has a long history of non-adherence to medication. This was taken into consideration prior to deciding the choice of the valve to use. Retrospective analysis of the Veterans Affairs Study to Improve Anticoagulation Registry demonstrated that patients with mental health conditions had a lower time in therapeutic ratio after adjusting for other covariates^[7]. Mechanical heart valves also have an audible click which has been shown to affect a patient's health-related quality of life raising the likelihood of non-adherence^[8].

Ehler-Danlos syndrome is a genetic defect of collagen and connective tissue synthesis and structure. Our patient had a recessively inherited form of EDS with an increased risk of cardiac valvular dysfunction. This results in failure to synthesize pro α 2(I) chains of type I procollagen^[9]. Aortic regurgitation occurs by either proximal aortic dilatation or laxity and redundancy of the aortic leaflets and mitral valve regurgitation secondary to mitral valve prolapse. This alteration in valvular flow dynamics increases the risk of sub-acute bacterial endocarditis (SBE). SBE has an insidious onset characterized by low-grade fever, night sweats, chills, fatigability, malaise, weight loss and valvular insufficiency. Septic emboli may disseminate causing distal infarcts or infections. Our patient had classical symptoms and a right foot cellulitis alongside splenic infarcts from emboli. SBE is usually caused by the viridans group of streptococci. However, *Streptococcus mutans* is rarely reported due to difficulties isolating and culturing the bacteria^[10]. Weinstein and Moellering^[11] first published a case series of four patients with SBE and two with meningitis who were successfully treated with penicillin and gentamicin. A similar treatment plan was employed in our patient with clindamycin for better skin penetration due to the cellulitis that emerged.

In conclusion, durable bioprosthetic valves can be safely used in patients with contraindications to lifelong anticoagulation. Further studies are needed to evaluate its long-term efficacy. With advances made in transcatheter valve insertions, this may be a viable option for younger patients in the near future.

DECLARATIONS

Authors' contributions

Performed the operation: Beattie G, Curry P

Performed the transoesophageal echocardiography on the patient: Reid D

Wrote the case report, reviewed and made changes to the structure and format of the manuscript: Singh SSA

Served as the primary supervisor of the manuscript: Curry P

Financial support and sponsorship

None.

Conflicts of interest

The authors do not have any conflicts of interest to declare.

Patient consent

Patient consent (images/case history) was obtained during the consent process.

Ethics approval

The study was approved by the local Clinical Governance Unit after consulting the Local Valve Multidisciplinary Team.

Copyright

© The Author(s) 2018.

REFERENCES

1. Hoen B, Duval X. Clinical practice. Infective endocarditis. *N Engl J Med* 2013;368:1425-33.
2. Liao K, Seifter E, Hoffman D, Yellin EL, Frater RW. Bovine pericardium versus porcine aortic valve: comparison of tissue biological properties as prosthetic valves. *Artif Organs* 1992;16:361-5.
3. Carpentier A, Nashef A, Carpentier S, Ahmed A, Goussef N. Techniques for prevention of calcification of valvular bioprostheses. *Circulation* 1984;70:165-8.
4. Bartuś K, Litwinowicz R, Kuśmierczyk M, Bilewska A, Bochenek M, Stapór M, Woźniak S, Różański J, Sadowski J, Kapelak B. Primary safety and effectiveness feasibility study after surgical aortic valve replacement with a new generation bioprosthesis: one-year outcomes. *Kardiologia Polska* 2018;76:618-24.
5. Flameng W, Hermans H, Verbeken E, Meuris B. A randomized assessment of an advanced tissue preservation technology in the juvenile sheep model. *J Thorac Cardiovasc Surg* 2015;149:340-5.
6. Puskas JD, Bavaria JE, Svensson LG, Blackstone EH, Griffith B, Gammie JS, Heimansohn DA, Sadowski J, Bartus K, Johnston DR, Rozanski J, Rosengart T, Girardi LN, Klodell CT, Mumtaz MA, Takayama H, Halkos M, Starnes V, Boateng P, Timek TA, Ryan W, Omer S, Smith CR; COMMENCE Trial Investigators. The COMMENCE trial: 2-year outcomes with an aortic bioprosthesis with RESILIA tissue. *Eur J Cardiothorac Surg* 2017;52:432-9.
7. Paradise HT, Berlowitz DR, Ozonoff A, Miller DR, Hylek EM, Ash AS, Jasuja GK, Zhao S, Reisman JI, Rose AJ. Outcomes of anticoagulation therapy in patients with mental health conditions. *J Gen Intern Med* 2014;29:855-61.
8. Koertke H, Hoffmann-Koch A, Boethig D, Minami K, Breymann T, El-Arousy M, Seifert D, Koerfer R. Does the noise of mechanical heart valve prostheses affect quality of life as measured by the SF-36® questionnaire? *Eur J Cardiothorac Surg* 2003;24:52-7; discussion 57-8.
9. Hata R, Kurata S, Shinkai H. Existence of malfunctioning pro α 2(I) collagen genes in a patient with a pro α 2(I)-chain-defective variant of Ehlers-Danlos syndrome. *Eur J Biochem* 1988;174:231-7.
10. McGhie D, Hutchison JG, Nye F, Ball AP. Infective endocarditis caused by *Streptococcus mutans*. *Br Heart J* 1977;39:456-8.
11. Weinstein AJ, Moellering RC Jr. Penicillin and gentamicin therapy for enterococcal infections. *JAMA* 1973;223:1030-2.

Case Report

Open Access



Bortezomib-induced posterior reversible encephalopathy syndrome: a case report

Paolo Candelaresi¹, Maria Chiara Casorio²

¹Neurology and Stroke Unit, Department of Emergency Medicine, San Carlo Borromeo Hospital, Milano 20147, Italy.

²Department of Anaesthesiology and Intensive Care, IRCCS San Matteo, Pavia 27100, Italy.

Correspondence to: Dr. Paolo Candelaresi, Neurology and Stroke Unit, Department of Emergency Medicine, San Carlo Borromeo Hospital, via Pio II 3, Milano 20147, Italy. E-mail: paolocandelaresi@gmail.com

How to cite this article: Candelaresi P, Casorio MC. Bortezomib-induced posterior reversible encephalopathy syndrome: a case report. *Vessel Plus* 2018;2:11. <http://dx.doi.org/10.20517/2574-1209.2018.09>

Received: 7 Mar 2018 **First Decision:** 28 Apr 2018 **Revised:** 29 Apr 2018 **Accepted:** 11 May 2018 **Published:** 18 May 2018

Science Editor: Aaron S. Dumont **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

Posterior reversible encephalopathy syndrome (PRES) is an uncommon neurological syndrome due to autoregulation breakthrough with subsequent predominantly vasogenic oedema associated with several clinical conditions. It is being increasingly reported in antineoplastic-treated patients. Here we report the case of a 72-year-old man who developed PRES during the second cycle of bortezomib treatment for multiple myeloma. Unlike usual PRES cases, only moderate hypertension was present at symptom onset, supporting the hypothesis that alterations of the vascular endothelium and blood-brain-barrier are the principal pathophysiological mechanisms involved in bortezomib-induced PRES. Prompt recognition of this potentially serious neurological adverse event is paramount to prevent mortality and long-term sequelae.

Keywords: Bortezomib, chemotherapy, multiple myeloma, posterior reversible encephalopathy syndrome

INTRODUCTION

Posterior reversible encephalopathy syndrome (PRES) is a clinico-radiological entity characterized by variable associations of headache, altered mental status, seizures, visual disturbances and less frequently other neurological signs. It may develop in association with many clinical conditions, such as acute hypertension, infections, pre-eclampsia/eclampsia, autoimmune disorders, neoplastic disease, exposure to toxins or drugs^[1]. Regardless of the underlying cause, cerebral vasogenic edema usually develops due to impaired autoregulation, blood-brain-barrier damage, and endothelial dysfunction.



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



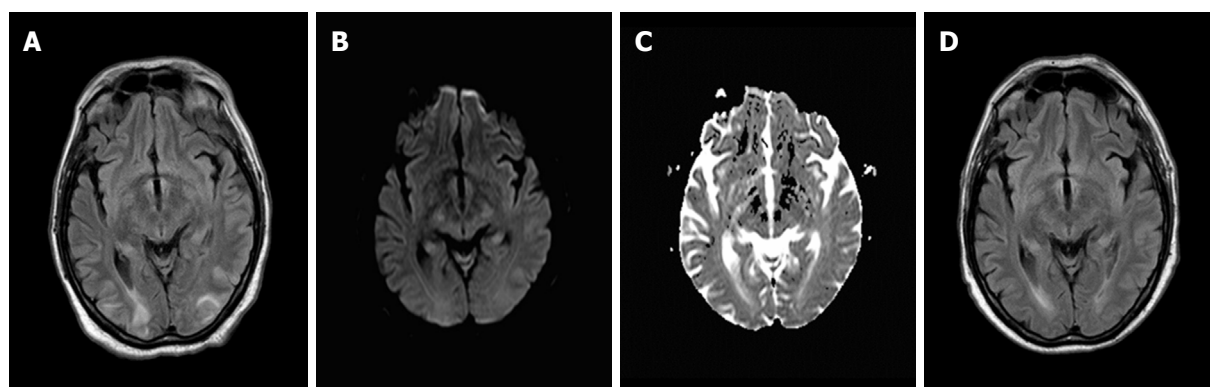


Figure 1. Brain magnetic resonance imaging scans demonstrating: symmetrical high intensity signals in the subcortical white matter of the occipital lobes at onset on FLAIR images (A), whereas a very mild changes on DWI and ADC sequences (B and C), and imaging remission after 10 days on FLAIR images (D)

Bortezomib is a proteasome inhibitor approved for the treatment of multiple myeloma and mantle cell lymphoma. It acts through the inhibition of nuclear factor kappa B (NF- κ B) activity. Few neurological complications have been reported in association with the use of bortezomib, predominantly peripheral neuropathies, and to date, only six cases^[2-7] of PRES.

CASE REPORT

In November 2016, a 72-year-old hypertensive man received a diagnosis of multiple myeloma with IgG κ /IgA λ monoclonal component. He was initially treated with bortezomib, thalidomide and dexamethasone. In March 2017, he was switched on melphalan, prednisone and bortezomib.

At the end of March 2017, he acutely developed altered mental status and visual disturbances. At symptom onset, his blood pressure was 170/80 mmHg, slightly higher than his typical values (140-150/70-80 mmHg). A neurological examination showed nonfluent aphasia with altered comprehension and cortical blindness.

No acute changes were revealed on a computed tomography scan, as well as in any laboratory test, including renal function. His blood pressure was controlled adding nifedipine to his habitual enalapril. On a brain magnetic resonance imaging (MRI) scan extensive vasogenic oedema in the subcortical white matter of the occipital lobes and left frontal lobe was demonstrated [Figure 1].

The clinical history, neurological examination and MRI findings were highly suggestive of PRES, attributable to the use of bortezomib. Further administration of bortezomib was withheld and a short course of dexamethasone 8 mg daily was started and tapered over 5 days.

After 10 days from onset, the patient completely recovered. He was switched on lenalidomide and dexamethasone, and at 6-month follow-up he is experiencing a serological remission of the monoclonal component.

DISCUSSION

PRES was originally described as the sudden onset of headache, altered mental status, seizures, visual disturbances and less frequently other focal neurological signs in the setting of uncontrolled hypertension, associated with the imaging findings of bilateral parieto-occipital vasogenic brain oedema, characterized by favourable prognosis with improvement or complete resolution. Since the first reports in the mid-90s, much has changed in the spectrum of the disease. The same clinical syndrome has been described in association with atypical radiological patterns, involving frontal and temporal lobes, basal ganglia and even

the brainstem. Persistent disability and life-threatening complications such as severe cerebral hemorrhage, cerebellar herniation and refractory status epilepticus have been described. Likewise, an increasing number of different inciting entities have been identified, such as haemodialysis, glomerulopathies and other autoimmune disorders, sepsis, neoplastic disease, and drugs. Among the latter, increasing attention is being directed to antineoplastic therapies. The syndrome has been described in association with induction chemotherapy in childhood acute lymphoblastic leukaemia, specifically with oxaliplatin, L-asparaginase, gemcitabine, bevacizumab, and sunitinib. Multiple myeloma, a neoplastic proliferation of an aberrant colony of plasma cells, has historically been difficult to treat with remission rates as low as 5%. Recent research identified the ubiquitin-proteasome pathway as a potential target for chemotherapeutic agents as its inhibition blocks cellular growth and division, ultimately leading to a proapoptotic state. Bortezomib is a potent, specific and reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome, leading to the inhibition of NF- κ B. NF- κ B acts as a transcription factor, turning on genes implicated in cell growth, surface molecules expression and vascular endothelial growth factor (VEGF)-mediated angiogenesis. By inhibiting NF- κ B, bortezomib leads to the inhibition of cell growth, tumor-associated angiogenesis, and the promotion of apoptosis. The most common adverse events reported in patients receiving bortezomib include thrombocytopenia, fatigue, peripheral neuropathy and neutropenia. To date, only 6 cases of bortezomib-induced PRES have been reported. The exact mechanisms of vasogenic brain oedema in such patients have not been completely delineated. In all cases, imaging studies show prominent posterior poles involvement. Local more sparse posterior circulation sympathetic innervation, compared to that in the anterior circulation, may explain the increased susceptibility of the posterior circulation to haemodynamic stress. Furthermore, as it happens in cases of granulomatous vasculitis and capillary leak syndrome^[8], endothelial cell damage and perivascular lymphomononuclear infiltration may further contribute to the development of brain oedema. There have been several cases of anti-VEGF agent induced PRES reported so far^[9]. Bortezomib induces decreased transcription of cellular growth factors, including VEGF. Therefore, bortezomib might indirectly alter the integrity of the vascular endothelium through the NF- κ B pathway.

In our case, as in other 4/6 cases of bortezomib-induced PRES, only a small increase in blood pressure levels has been detected, suggesting that in bortezomib-treated patients hypertension might be a contributor but not the originator of the cascade leading to brain oedema.

The concomitant use of melphalan is to be considered. In a case series^[10], 9/451 of high-dose melphalan treated patients developed acute encephalopathy. Eight of them developed changes in mental status ranging from drowsiness and confusion to loss of consciousness, while one patient had tonic-clonic seizures. Only one patient had imaging abnormalities compatible with posterior reversible encephalopathy syndrome. Melphalan-induced encephalopathy is usually associated with renal impairment. It is not the case in our patient, who was treated with low-dose melphalan and did not experience renal impairment. However, we cannot completely rule out a minor contribution of melphalan in the development of PRES. Our patient received a short course of dexamethasone, which should theoretically improve vasogenic edema, even though robust evidence for their use is lacking.

In conclusion, PRES must be considered when a bortezomib-treated patient develops altered mental status associated with other neurological signs, such as visual alterations or language impairment as in our case, even in the absence of an excessive rise in blood pressure levels, particularly if concomitant potentially neurotoxic drugs are used. Brain MRI scan is the preferred imaging modality to diagnose vasogenic oedema, and posterior poles seem to show a particular susceptibility.

Prompt withdrawal of the offending drug and control of blood pressure are associated with neurological recovery in all cases of bortezomib-induced PRES reported so far.

DECLARATIONS

Authors' contributions

Conceived the idea of the manuscript and wrote the first draft: Candelaresi P
Searched for the literature and critically revised the manuscript: Casorio MC
Approved the final version: both authors

Financial support and sponsorship

None.

Conflicts of interest

The authors confirm that this case content has no conflicts of interest.

Patient consent

The authors declare that a written informed consent has been obtained from the patient to publish anonymous information.

Ethics approval

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Fugate JE, Rabinstein AA. Posterior reversible encephalopathy syndrome: clinical and radiological manifestations, pathophysiology, and outstanding questions. *Lancet Neurol* 2015;14:914-25.
2. Terwiel E, Hanrahan R, Lueck C, D'Rozario J. Reversible posterior encephalopathy syndrome associated with bortezomib. *Intern Med J* 2010;40:69-71.
3. Kelly K, Kalachand R, Murphy P. Bortezomib-induced reversible posterior leucoencephalopathy syndrome. *Br J Haematol* 2008;141:566.
4. Kager LM, Kersten MJ, Kloppenborg RP, Van Oers R, Van den Born BJ. Reversible posterior leucoencephalopathy syndrome associated with bortezomib in a patient with relapsed multiple myeloma. *BMJ Case Rep* 2009;2009:bcr06.2009.1926.
5. Oshikawa G, Kojima A, Doki N, Kobayashi T, Kakihana K, Tsuda H, Endo I, Kamata N, Ohashi K, Sakamaki H. Bortezomib-induced posterior reversible encephalopathy syndrome in a patient with newly diagnosed multiple myeloma. *Intern Med* 2013;52:111-4.
6. Nixon NA, Parhar K. Posterior reversible encephalopathy syndrome resulting from repeat bortezomib usage. *BMJ Case Rep* 2014;2014:bcr2014204592.
7. Ho CH, Lo CP, Tu MC. Bortezomib-induced posterior reversible encephalopathy syndrome: clinical and imaging features. *Intern Med* 2014;53:1853-7.
8. Hsiao SC, Wang MC, Chang H, Pei SN. Recurrent capillary leak syndrome following bortezomib therapy in a patient with relapsed myeloma. *Ann Pharmacother* 2010;44:587-9.
9. Tlemsani C, Mir O, Boudou-Rouquette P, Huillard O, Maley K, Ropert S, Coriat R, Goldwasser F. Posterior reversible encephalopathy syndrome induced by anti-VEGF agents. *Target Oncol* 2011;6:253-8.
10. Najera JE, Sudhakar T, Bashir Q, Shah N, Champlin RE, Qazilbash MH, Giralt S, Hosing C, Popat UR, Ciurea SO. Neurotoxicity after high-dose melphalan. *J Clin Oncol* 2012;30 suppl 15:abstr6546.

Review

Open Access



Research into biodegradable polymeric stents: a review of experimental and modelling work

Tianyang Qiu, Liguozhao

Wolfson School of Mechanical, Electrical, and Manufacturing Engineering, Loughborough University, Loughborough LE11 3TU, UK.

Correspondence to: Dr. Liguozhao, Wolfson School of Mechanical, Electrical, and Manufacturing Engineering, Loughborough University, Loughborough LE11 3TU, UK. E-mail: L.Zhao@Lboro.ac.uk

How to cite this article: Qiu T, Zhao L. Research into biodegradable polymeric stents: a review of experimental and modelling work. *Vessel Plus* 2018;2:12. <http://dx.doi.org/10.20517/2574-1209.2018.13>

Received: 19 Mar 2018 **First Decision:** 18 Apr 2018 **Revised:** 26 Apr 2018 **Accepted:** 14 May 2018 **Published:** 5 Jun 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Bioresorbable stents (BRSs) are regarded as the next-generation medical devices to treat blocked or diseased arteries. The use of BRSs aims to reduce the risk of late stent thrombosis and long-term tissue inflammation associated with permanent metallic stents. BRSs are designed to relieve symptoms immediately and also provide mechanical support for an appropriate time period before they are fully absorbed by human body. To promote clinical adoption of BRSs or even to substitute metallic stents, the mechanical performance of BRSs needs to be thoroughly investigated and quantitatively characterised, especially over the full period of degradation. This paper offers a review of current research status of polymeric BRSs, covering both experimental and modelling work. Review of experimental studies highlighted the effects of stent designs and materials on the behaviour of polymeric BRSs. Computational work was able to simulate crimping, expansion and degradation of polymeric BRSs and the results were useful for performance assessment. In summary, the development of polymeric BRSs is still at an early stage, and further research is urgently required for a better understanding and control of their mechanical performance.

Keywords: Bioresorbable stents, mechanical performance, degradation, experimental studies, computational work

INTRODUCTION

Since the implantation of the first coronary artery stent in 1986, stent deployment has become a standard medical procedure to treat coronary stenosis, a leading cause of heart attack. The worldwide coronary stent market is worth over \$7 billion and forecasted to grow by more than 5% annually^[1]. Over the past three decades, there have been significant improvements made in stent materials and designs, especially for the



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



drug-eluting stents (DESs) which were firstly approved by Food and Drug Administration (FDA) of USA in 2002. DESs are coated with non-degradable polymer embedded with anti-proliferative drugs, to avoid the occurrence of in-stent restenosis (ISR) which represents a major drawback of bare metal stents (BMSs)^[2]. Although the use of DESs can substantially reduce the ISR rates, especially in high-risk patients (lowered by 74%), other clinical problems arise, particularly the increase of late stent thrombosis (ST, with a mortality rate of about 45%) after implantation of DESs^[3,4]. The development of deadly ST is concertedly facilitated by hypersensitivity to the permanent presence of alloy and by incomplete repair of the endothelium at the site of vascular wall injury due to the anti-proliferative drug coatings^[5]. Also, the presence of permanent stent could cause the increasing risk of strut fracture and failure under complicated loading and corrosive conditions, leading to heart attack or failure, unstable angina and other clinical complications in patients^[6].

To overcome these issues, it becomes mandatory to develop next-generation bioresorbable stents (BRs) to replace metallic ones. Biodegradable polymers have attracted the most interests for manufacturing BRs^[7-9]. Fully expandable biodegradable polymeric stents provide the mechanical support to the vessel wall with appropriate radial strength, to prevent mechanical recoil after immediate implantation. When arterial remodelling achieves a relatively stable phase at 6 months, the mechanical support is no more required. As a result, stents are supposed to be designed to dissolve after 6 months in human body, leaving behind the intact vessel with no pro-inflammatory substances or obstacles^[10]. During the process, the biodegradable polymers gradually soften, thus allowing a smooth transfer of the load from the stent to the healing artery, and eventually removing the significantly high stresses induced to the stented artery.

Earlier development of polymeric BRs includes the Igaki-Tamai stent (Kyoto Medical Planning Co. Ltd, Japan), the Abbott BVS vascular drug-eluting stent (Abbott Vascular, Santa Clara, California, USA) and the REVA stent (Reva Medical, San Diego, California, USA)^[8,9]. The Igaki-Tamai stent, made of poly-L-lactic acid (PLLA), has a zig-zag design with straight connective struts. It was the first fully biodegradable polymeric stent ever implanted in humans, and degraded over 18 to 24 months with good performance^[8]. The Abbott BVS stent has a bioresorbable polymer backbone of PLLA, incorporated with a polymer coating of poly-DL-lactic acid (PDLLA) containing and controlling the release of the anti-proliferative drug. The BVS 1.0 has out-of-phase sinusoidal rings connected either directly or by straight bridging struts. The first in-man study of BVS 1.0 stents not only highlighted the efficacy and safety of using a biodegradable scaffold, but also provided vital data that help develop the BVS 1.1 stent which has in-phase sinusoidal rings connected by straight bridging struts, which can provide radial support for a longer period of time^[9]. This is also the case for REVA stent, which is made of absorbable tyrosine-derived polycarbonate polymer and has a distinctive slide-and-lock design. The preclinical trials of REVA stents have led to the development of the second-generation REZOLVE bioresorbable stent with a helical design^[9].

Development of next-generation polymeric BRs still faces many challenges and unsolved issues, such as stent material and design, mechanical support, *in vivo* performance, biodegradation rate and manufacturing process. One of the major uncertainties for polymeric BRs is the effective control of mechanical properties during the biodegradation process. It is very important for BRs to relieve symptoms immediately and also offer mechanical support for an appropriate period of times. Currently, there is a critical shortage of such data and information, severely limiting the application and further development of BRs. Designers must use a trial-and-error approach to work out the appropriate material formulation and geometrical parameters of BRs that can sustain mechanical loads during the degradation process. As an evidence, the concept of a biodegradable polymeric stent dates back to the 1980s, yet currently there are no designs with the FDA approval on the market. Abbott BVS PLLA stent, also named as ABSORB, is a first-of-its-kind device used initially in Europe and parts of Asia Pacific and Latin America to treat arteries with mild restenosis. To promote clinical application at a large scale or even to replace metallic stents, the mechanical performance of BRs needs to be thoroughly investigated and quantitatively characterised, especially over the full period

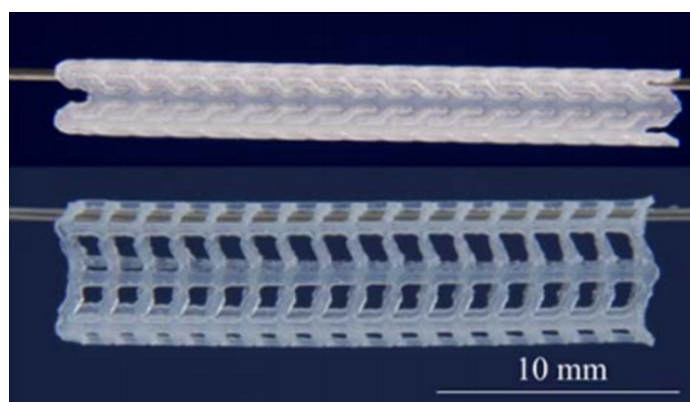


Figure 1. Stent in crimped and deployed configurations^[11]

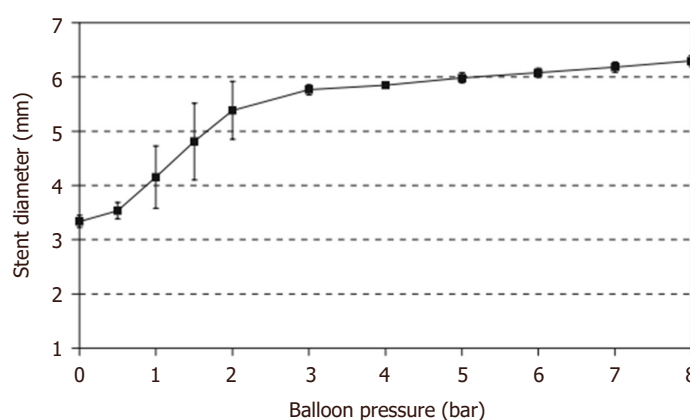


Figure 2. Stent diameter change against balloon pressure during expansion^[11]

of degradation. This paper offers a review of current research status of biodegradable polymeric scaffolds, covering both experimental and modelling work.

EXPERIMENTAL STUDIES OF BRSS

Mechanical behaviour

Stents, as medical devices used in human bodies, impose difficulties and challenges for experimental studies due to their tiny size and complex design. Despite the challenges, various methods have already been developed to investigate the behaviour of stents, including polymeric stents. Grabow *et al.*^[11] studied the expansion behaviour of a biodegradable slotted tube stent, made of PLLA and poly-hydroxybutyric acid (PHB), by stent bench testing. As shown in Figure 1, the crimped stent was mounted on a balloon catheter, and then expanded by balloon inflation. All stents were expanded successfully without the occurrence of strut fracture, and Figure 2 shows the stent diameter change as a function of balloon pressure. The stent initially expanded rapidly at a balloon pressure of 1-2 bars and then achieved full expansion at a pressure of 6 bars. The measured recoil effect and collapse pressure were 4.2% and 1.1 bars, respectively. These results revealed that the PLLA/PHB slotted tube stent exhibited adequate mechanical properties during rapid expansion process. The same method was also used by Grabow *et al.*^[12] to investigate the mechanical performance of two types of biodegradable balloon-expandable stents, made of PLLA and PLLA/PCL/TEC, respectively. Both types of stents were able to expand fully by balloon inflation under a pressure of 8 bars, but differences were observed for expansion process as shown in Figure 3. Specifically, the PLLA/polycaprolactone (PCL)/TEC stent expanded immediately with the balloon inflation while the pure PLLA stent started to expand at

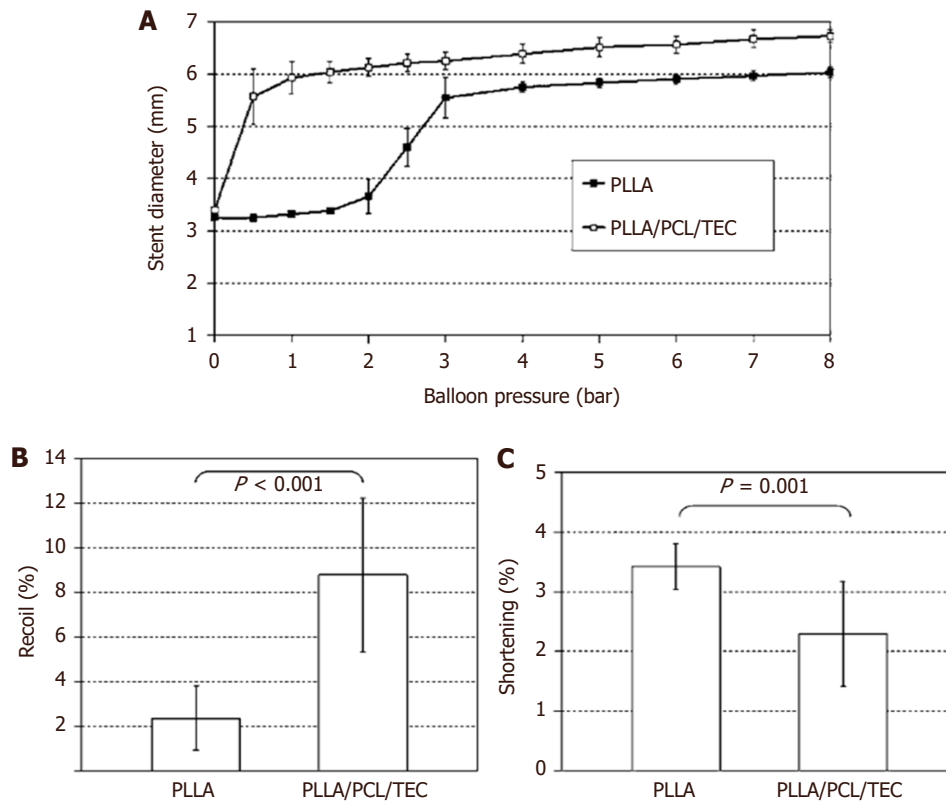


Figure 3. Stent diameter change against balloon pressure (A), elastic recoil (B) and stent shortening (C)^[12]

a balloon pressure of ~2 bars. As a result, the pure PLLA stent achieved a smaller diameter (6.0 mm) than that of PLLA/PCL/TEC stent (6.7 mm) at peak balloon pressure. Moreover, the pure PLLA stent exhibited smaller elastic recoil (2.4%) and larger shortening (3.4%) compared to PLLA/PCL/TEC stent (8.8% and 2.3%, respectively). These results suggested that material properties affected the mechanical behaviour of stents.

Schmidt *et al.*^[13] characterized the *in vitro* mechanical performance of bioresorbable scaffolds (i.e., Absorb GT1, Elixir DESolve and Biotronik Dreams 2G) using the same method as described above. The crimped stent had an outer diameter of 1.38 mm, 1.39 mm and 1.44 mm for Absorb GT1, Elixir DESolve and Dreams 2G, respectively. The Dreams 2G exhibited a pushability of 45.41% compared to 33.77% for Absorb GT1 and 36.27% for Elixir DESolve, but no significant difference was found for trackability of the three stents which were 0.68 N, 0.75 N and 0.64 N for Dreams 2G, Absorb GT1 and Elixir DESolve stents, respectively. Moreover, they examined and compared expansion behaviour of the three stents within a mock vessel and a rigid vessel model. The Dreams 2G showed smaller recoil (5.6% and 5.0%) in both two cases without change over time, while Absorb GT1 and Elixir DESolve stents showed time-dependent recoil. These results highlighted the differences between metallic and polymeric BRSs due to different material properties and designs. Ormiston *et al.*^[14] performed expansion and post-dilatation experiments for two commercially available bioresorbable stents (Absorb and DESolve) and compared to typical metallic DES Xience Xpedition. They examined the mechanical characteristics, such as crossing profile, recoil and radial strength, by imaging and intravascular ultrasound techniques. The crossing profile had a diameter of 1.14 mm, 1.43 mm and 1.44 mm for Xpedition, Absorb and DESolve, respectively. The radial strength of stent, measured in terms of pressure required to reduce 25% of cross-sectional area, was found to be 1.6 atm for Xpedition, 1.4 atm for Absorb and 1.1 atm for DESolve. All three stents showed elastic recoil after expansion with a slight change over time. Initially, all three stents significantly recoiled by approximately 0.1 mm. Afterwards, Absorb and Xpedition continued slight recoiling whereas the diameter of DESolve showed an increase (self-correction). Their results revealed

that the metallic stent had thinner strut with smaller profile but greater radial strength compared to polymeric stents. Welch *et al.*^[15] studied the effect of thermal annealing on the mechanical behaviour of helical coiled PLLA stents. The stents were annealed at a temperature of 70 °C, 80 °C and 90 °C for 25 min after manufacturing, and then expanded by balloon catheter up to a pressure of 12 atm. Experimental results showed that the stent had higher stiffness and greater collapse resistance with the increase of annealing temperature, whereas the elastic recoil had a decrease. Probably, thermal annealing induced some changes in crystalline structure of PLLA, and thus affected the stress-strain behaviour and stent expansion behaviour.

The experimental work mainly studied the expansion behaviour of polymeric stents, such as stent diameter change against inflation pressure, recoil and shortening effect, which are greatly affected by stent materials and designs. Polymeric BRSSs, as the latest generation of stent, are mostly made of biodegradable polymer PLLA. It is well known that PLLA has low mechanical strength and poor elongation compared to metal, which limits its application for stents. Consequently, studies have also been performed to investigate and improve the mechanical behaviour of poly-lactic acid (PLA), including blending PLA with ductile biodegradable polymers as discussed above. In order to further improve the performance of BRSSs and compete with metallic stents, research into processing and characterisation of biodegradable polymers is particularly required.

Degradation behaviour

One of key features for polymeric stents is their degradation behaviour over time. There were some experimental studies available in terms of characterization of stent degradation *in vitro* and *in vivo*. Xu *et al.*^[16] evaluated *in vitro* and *in vivo* degradation behaviour of biodegradable tubular stents, made of poly-lactic-co-glycolic acid (PLGA) with five different molar weight ratios of LA/GA (50/50, 60/40, 71/29, 80/20 and 88/12), for application in common bile duct (CBD) repair and reconstruction. For *in vitro* test, stents were placed in bile degradation medium (pH = 7.2-7.6). Morphological results showed that the stents slightly expanded, deformed and then cracked meanwhile their colour changed from initially transparent to yellow and opaque due to the bile and water absorption. Weight loss, molecular weight change and water uptake were also observed, indicating the PLGA stents exhibited different degradation rates due to different composition ratios. For *in vivo* test, PLGA (71/29) stents were implanted in the rat CBDs. Results showed that the polymeric stents can provide the same biomedical functions as typical T tubes and completely disappeared after 5 weeks.

Hadaschik *et al.*^[17] investigated the degradation behaviour of a new biodegradable ureteral stent (Uriprene) made of copolymer PLGA (LA/GA = 80/20) using porcine models. Uriprene stents were implanted in 20 pigs while standard biostable stents were implanted in 16 pigs as a control. Compared to control stents, Uriprene stents caused significantly less ureteral dilatation and urinary tract infections. Their results also showed that the Uriprene stents began degradation at week 3, and completely degraded at week 10. The novel stent was proved to be biocompatible *in vivo*. Yang *et al.*^[18] carried out degradation experiments on a novel biodegradable PGLA ureteral stent, with multilayer design immersed by microsphere zein and BaSO₄, in human urine *in vitro*. The scanning electron microscope (SEM) results showed that the novel stent started degradation at week 2 and fully degraded after 4 weeks, and the degradation happened layer by layer from outer to inner surface. The stent weight and mechanical strength (i.e., tensile strength, elastic recovery and radial compression load) showed a decrease over the degradation time.

Currently, commercial degradable polymeric stents are made of PLLA, which breaks down to natural by-products, i.e., water, gases (CO₂ and N₂), biomass and inorganic salts. These natural by-products are non-toxic, so there are no negative effects on the blood or vessel wall. Degradation behaviour of polymeric stents was mainly characterized by *in vitro* and *in vivo* methods, focusing on variation of physical (e.g., mass weight and molecular weight) and mechanical properties (e.g., tensile strength and elastic modulus) over degradation times. Key parameters used to assess degradation behaviour of biodegradable polymers

Table 1. Key parameters used in assessing degradation behaviour of polymer scaffolds

References	Gong <i>et al.</i> ^[19] (2007)	Liu <i>et al.</i> ^[20] (2014)	Rodrigues <i>et al.</i> ^[21] (2016)	Zamiri <i>et al.</i> ^[22] (2010)	Grabow <i>et al.</i> ^[12] (2007)
Materials	PLLA scaffold	PLLA scaffold	Porous PLA scaffold	PLLA scaffold/ PLGA scaffold	PLLA scaffold
Media	PBS	PBS	PBS	PBS	PBS
Degradation times	39 weeks	200 days	8 weeks	25 weeks	24 weeks
Mass loss	30%	8%	Remain relatively constant	5%/64%	-
Molecular weight loss	From 177,000 to 80,000	From 179,000 to 146,000	No significant change (120,000-150,000)	-	28%
Water uptake	Decreased dramatically with degradation time, and lowest value appeared between week 9 and 12	900%	-	-	-
Morphology change	Microcracks at week 9; more cracks and big holes after week 23	Some little holes on - the surface of the porous walls	-	-	-

PLLA: poly-L-lactic acid; PBS: poly-butylene-succinate; PLA: poly lactic acid; PLGA: poly-lactic-co-glycolic acid

include mass loss, molecular weight change, water uptake and morphology change. Gong *et al.*^[19] carried out *in vitro* degradation study of porous PLLA scaffolds over 39 weeks, and the scaffolds degraded at a slow rate due to its highly porous structure in terms of weight water uptake and structure change. As reported by Liu *et al.*^[20], PLLA porous scaffolds exhibited a reduction in mass and molecular weight during an *in vitro* degradation time of 200 days. Rodrigues *et al.*^[21] studied degradation process of porous PLA scaffold immersed in phosphate-buffered-saline solution, and revealed that there was no significant change in molecular weight over 8 weeks. Zamiri *et al.*^[22] compared the *in vitro* degradation behaviour of PLLA and PLLA/PLGA braided scaffolds over 25 weeks, and the mass loss was found very limited for PLLA braided scaffolds whereas PLLA/PLGA braided scaffolds experienced a mass loss of 64%. Grabow *et al.*^[12] reported a gradual and steady reduction in molecular weight for a PLLA scaffold prototype during 24 weeks of *in vitro* degradation. Table 1 gives a summary of these key parameters used in assessing degradation behaviour of polymer scaffolds.

Mechanical properties (i.e., ductility, toughness and strength) of biodegradable polymers change significantly during degradation due to hydrolytic chain scission at molecule level. It is of importance to understand the mechanical behaviour of bioresorbable polymeric scaffolds during degradation period. The earliest assessment of the mechanical performance of biodegradable stents was conducted by Agrawal *et al.*^[23], who tested *in vitro* the pressure-diameter behaviour of the Duke biodegradable stents made of PLLA fibres. They reported that with a careful balance between fibre mechanical properties (varied with draw ratio and thermal treatments) and stent designs, it was possible to achieve a successful biodegradable stent. Zilberman *et al.*^[24] reported a loss in radial compression strength for their PLLA stent designs with degradation time, which is also associated with reductions in elastic modulus and yield strain of the PLLA fibres. Nuutinen *et al.*^[25] carried out *in vitro* tests of a woven fibre polymeric braided stent subjected to radial compression in a pressurized chamber. The stent design did not perform well enough when made of biodegradable polymer, and the collapse pressure was still lower than its metal counterpart even with thicker fibres. Specifically, the stent lost structural integrity after 36 weeks of degradation (immersed in saline at 37 °C) and the collapse pressure decreased by half at 30 weeks. The *in vitro* degradation study by Liu *et al.*^[26] showed that PLLA porous scaffolds exhibited a reduction in compressive modulus and strength during a degradation time of 200 days. Recently, Rodrigues *et al.*^[21] carried out an *in vitro* degradation study of porous PLA scaffold immersed in phosphate-buffered-saline solution for 8 weeks. The study revealed that compressive properties (i.e., compressive modulus and stress at yield) of the scaffolds maintained constant during the initial 6 weeks and increased significantly at week 8. Similarly, Grabow *et al.*^[12] conducted *in vitro* degradation study for a PLLA scaffold prototype in a deployed

shape, and they found that collapse pressure increased firstly within 12 weeks and then decreased. From the material point of view, the initial increase of compressive properties might be caused by the recrystallization of biodegradable polymer PLA/PLLA due to the water absorption and temperature increase (from room temperature 20 °C to human body temperature 37 °C). It was reported that crystallization can significantly improve the mechanical strength and stiffness of PLLA. It should be pointed out that there is a lack of efforts in assessing the discontinuity of the scaffold during the late stage of degradation, due to the difficulties in imaging the scaffold. However, such studies can be accomplished by using the micro computed tomography (CT) technology to investigate the internal damage associated with both biodegradation and mechanical deformation wherever feasible. Also the microstructures of all specimens can also be investigated using SEM both before and after the tests, by fracturing the specimens (e.g., in liquid nitrogen) and coating the fracture surfaces with a thin layer of gold.

In addition, clinical trials have also been carried out extensively and long-term follow up indicated the safety and efficacy of polymeric stents in treatment of coronary artery disease. Follow-up studies, based on invasive imaging methods such as CT, angiography and intravascular ultrasound (IVUS), were normally carried out to assess the stented vessel after the implantation of bioresorbable scaffolds. As reported in Ormiston *et al.*^[27], the bioresorbable vascular scaffold (BVS) absorb showed an overall 16.8% reduction of lumen area at 6-month follow-up of 30 patients implanted with the scaffolds. Serruys *et al.*^[28] evaluated the outcomes of Absorb for treating coronary artery stenosis in 45 patients. The mean lumen area was found to decrease by 3.1% (6.60 to 6.37 mm²) according to IVUS analysis at 6-month follow up whereas it increased to 6.85 mm² at 2-year follow up. Recent 5-year follow-up results after Absorb implantation concluded that the mean lumen area tended to increase from 6 months to 1 year and 5 years^[29]. Progressive lumen gain was also observed after Absorb implantation in porcine coronary arteries^[30], for which the lumen area kept stable up to 6 months and then showed a progressive increase from one to 3.5 years. These studies confirmed the lumen gain after scaffold implantation over the degradation times, especially from 6 months onwards.

However, studies are still limited and challenging due to the microscale geometry of stent and complex environment of human artery. To complement experimental work, finite element simulations were consequently used to study the stent performance during deployment, which is reviewed in the next section.

COMPUTATIONAL WORK

Stent deployment

Finite element (FE) modelling is of particular help in evaluating stent performance. However, most FE modelling of stent implantation, including recent ones, were dealing with metallic stents such as the effects of design and material on stent expansion, recoiling and dogboning^[31-33]. FE modelling of polymeric BRSs is very limited, especially how they compare with metallic stents in terms of mechanical performance. There are only a few papers in literature. For instance, Pauck and Reddy^[34] simulated the mechanical performance of PLLA stents with different designs and varying polymer stiffness. Material stiffness and geometrical design affects the radial strength of polymeric stent significantly. Debusschere *et al.*^[35] studied the free-expansion behaviour of bioresorbable Absorb stent by considering the viscoplasticity of the material. They simulated stent expansion by increasing inflation pressure linearly or in a stepwise, and linear inflation method was found to induce higher stress concentration in the U-bend struts. Inflation rate affected stent expansion mainly during inflation process, while not much difference was shown after balloon deflation. Wang *et al.*^[36] studied the mechanical deformation of a PLLA biodegradable coronary stent using experimental and computational methods. They modelled the crimping of stent to an outer diameter of 1.41 mm and subsequent expansion to different inner diameters. Stent deformation profiles were in good agreement with experimental ones during both crimping and expansion processes, and large deformation occurred at sharp curvature of struts in all cases. The radial recoil ratio showed a decrease

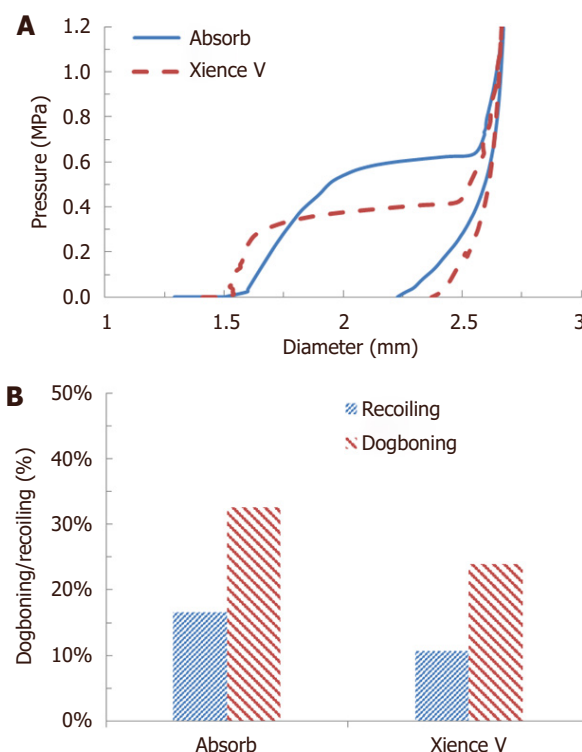


Figure 4. (A) Stent outer diameter change against pressure and (B) recoiling and dogboning effects for Absorb scaffold and Xience V stent in concentric lesion^[38]

trend with the increase of stent expansion, which was 4.19%, 2.92% and 1.81% obtained for stent with expanding to 3.1 mm, 3.7 mm and 3.92 mm, respectively. They also modelled re-crimping of the expanded stent to evaluate the radial strength and stiffness, and calculated radial strength and stiffness was 1.46 N/mm (close to the experimental value of 1.55 N/mm) and 1.40%, respectively. Shanahan *et al.*^[37] developed a viscoelastic material model for a biodegradable polymeric braided stent, and evaluated the time-dependent viscoelastic behaviour using finite element method. They simulated the crimping of stent by enforcing radial displacement on 8 rigid plates, and the linear viscoelastic material model was validated against their experimental data. However, no significant difference was observed for the crimping behaviour of braided stent modelled as either linear elastic or viscoelastic material. However, all these simulation work neglected the diseased artery in their models and was unable to assess the performance of polymeric stents comprehensively.

Very recently, Schiavone *et al.*^[38] carried out a comparative study for polymeric Absorb stent and metallic Xience stent by including diseased artery in finite element simulations. The Absorb stent showed a lower rate of expansion in diseased artery, with higher dog-boning and recoiling when compared to Xience stent [Figure 4]. This was due to the difference in material property and stent design. It was suggested that post-dilatation of Absorb stent is required in order to achieve an effective treatment of stenosis, especially for patients with stiffer vessels and highly calcified plaques. However, significantly lower stress was induced to the plaque-artery system treated with the Absorb stent, which is a clinical benefit in terms of causing less injury to the vessels. Effect of plaque eccentricity was also evaluated in their simulations, and higher stress was found for the media and adventitia tissue layers with the increase of plaque eccentricity [Figure 5]. Eccentric plaque also caused complications to stent implantation, such as non-uniform stress distribution and artery expansion. As such, the selection of stents, in terms of designs and materials, will be of high importance for patients in order to achieve the most effective clinical outcomes.

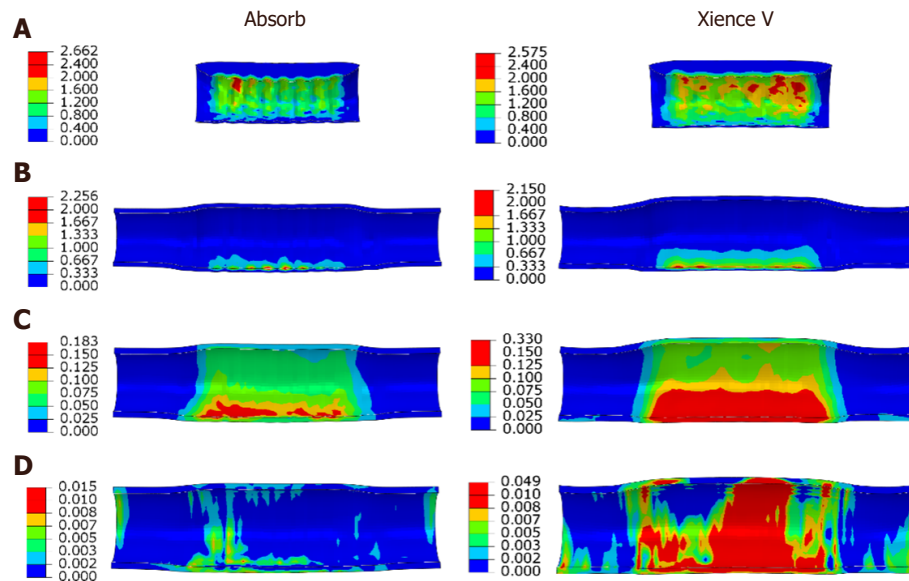


Figure 5. Contour plot of the maximum principal stress (MPa) on the (A) plaque, (B) intima layer, (C) media layer and (D) adventitia layer, after deployment of Absorb scaffold and Xience V stent in eccentric lesion^[38]

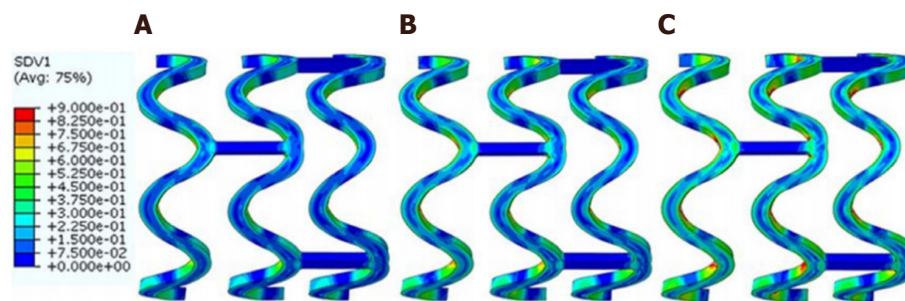


Figure 6. Degradation degree distribution on the stent over time^[40]. (A) 10 days degradation; (B) 20 days degradation; (C) 30 days degradation

Stent degradation

BRSs have advantages in overcoming clinical complications (e.g., restenosis and ST) associated with permanent metallic stents due to their biodegradation characteristics. Generally, constitutive models are required to describe the mechanical response of stent materials during the process of degradation. For instance, Soares *et al.*^[39] applied a thermodynamically consistent constitutive model to predict degradation behaviour of PLLA stents. This model was suitable for simulating deformation-induced degradation, especially for biodegradable PLLA. The model is based on a degradation-dependent Helmholtz potential (material properties decrease as a function of degradation) and the rate of dissipation. Degradation of three different stent designs was modelled, and results showed that high risk of degradation was mainly observed at the bends of stent rings and junction points. Luo *et al.*^[40] developed a numerical model to study the degradation behaviour of bioabsorbable PLLA stent. The constitutive degradation model combined the degree of degradation with pre-stretched deformation, and the degree of degradation was used to describe the change of material property as a function of degradation time. The degradation model was validated against experimental tensile data at different degradation times. Results showed that degradation happened throughout the whole stent over 30 days, but the degradation rate was not uniform [Figure 6]. The maximum degradation was observed at the inner surface of U-bend strut where the stress concentration/maximum strain occurred during stent expansion, which was in good agreement with experimental result. This indicated that the degradation rate of material was influenced by the initial deformation.

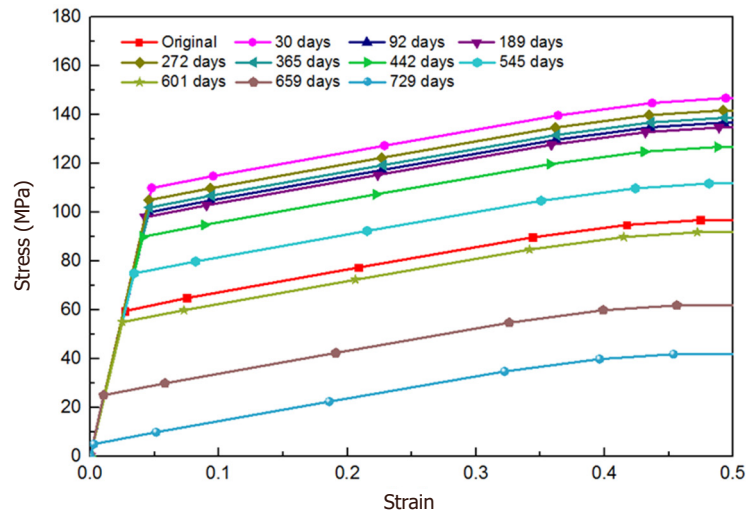


Figure 7. Constructed poly-L-lactic acid stress-strain curves for Absorb scaffold at different degradation time points^[41]

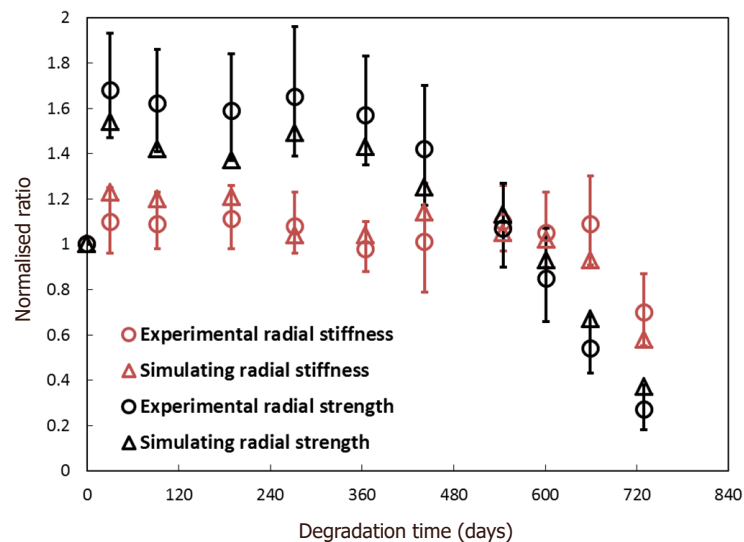


Figure 8. Radial strength and stiffness data obtained from simulation for Absorb scaffold compared to experimental data at different degradation time points^[41]

Material property of biodegradable polymer shows a difference during degradation, and therefore mechanical behaviour of polymeric stents is also influenced by stent degradation. Several studies have been carried out to predict the degradation behaviour of polymeric stents by developing constitutive models for degradable material, but neglected stent-artery interaction during degradation. For the first time, Qiu *et al.*^[41] evaluated the mechanical behaviour of a polymeric BRS using computational method, with a focus on stent-artery interaction during the process of degradation and artery remodelling. In particular, stress-strain responses were calibrated for polymeric stents over 2-year *in vitro* degradation period, according to experimentally measured strength and stiffness [Figures 7 and 8]. Effect of degradation on stent behaviour was simulated by considering the change of material's property over time. In addition, vessel remodelling was simulated by manually changing the geometry of diseased artery, following those published clinical data. Over 2-year degradation times, stresses in the plaque and vessel layers showed a consistent decrease; while the stent experienced an increase in stress at the beginning followed by a gradual decrease, corresponding to the changing properties of the polymeric material [Figure 9]. In addition, vessel remodelling led to a reduction

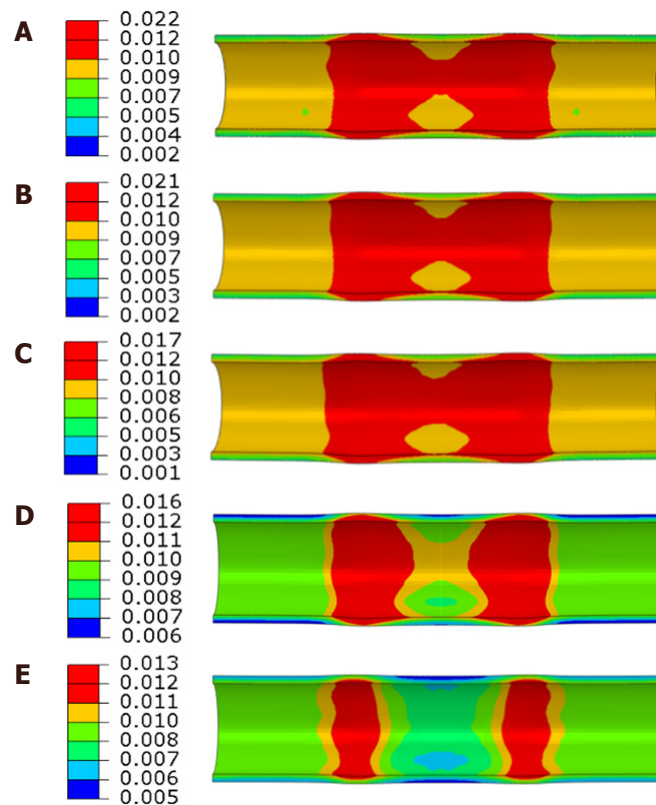


Figure 9. Contour plot of the maximum principal stress on the media layer at a degradation time of 0 day (A), 189 days (B), 365 days (C), 659 days (D) and 729 days (E)^[41]

of stress for the whole scaffold-plaque-artery system. This work offered valuable insights into interactive behaviour between a bioresorbable stent and diseased artery during a 2-year period of *in vitro* degradation, which is helpful for developing new-generation BRSs for treating artery stenosis.

The work of Qiu *et al.*^[41] highlighted that for percutaneous coronary intervention, stent deployment causes severe stresses to the artery, which will induce negative influence such as damage to arterial tissue. Thus, it is of importance to assess the stress state produced in the artery-plaque system during stent implantation process. A desirable process of stent implantation requires an outcome of expansion which is enough to relieve vessel obstruction and also causes least damage to the arterial walls^[42], as unsuccessful deployment may trigger ISR. Here, unsuccessful deployment includes injury and excessive stretch of arterial walls, under-expansion of stent and structural failure of struts. A strong correlation between severe wall stresses and high restenosis rate has been confirmed by literature^[43]. Clearly, it is also necessary to assess the safety and efficacy of polymeric stents during degradation, especially stent-artery interaction. The work of Qiu *et al.*^[41] is the first attempt to model the mechanical interaction of BRS with stenotic artery over the full process of stent degradation. A general reduction of stresses was shown for the plaque and vessel layers over stent degradation, which can be beneficial for the natural healing of stented artery.

So far, computational work has focused on the development and validation of material constitutive models that can predict the mechanical behaviour of degradable polymer or implant^[44,45]. There is not a model available yet to model the degradation process as well as property changes, especially to predict both biodegradable implant mechanics and its interaction with blood vessel. For instance, finite-element models were used to predict the recoil and collapse behaviour of polymer stent design by considering the elastic and/or inelastic behaviour of the polymer^[46,47], but neglecting any changes of material properties that occur

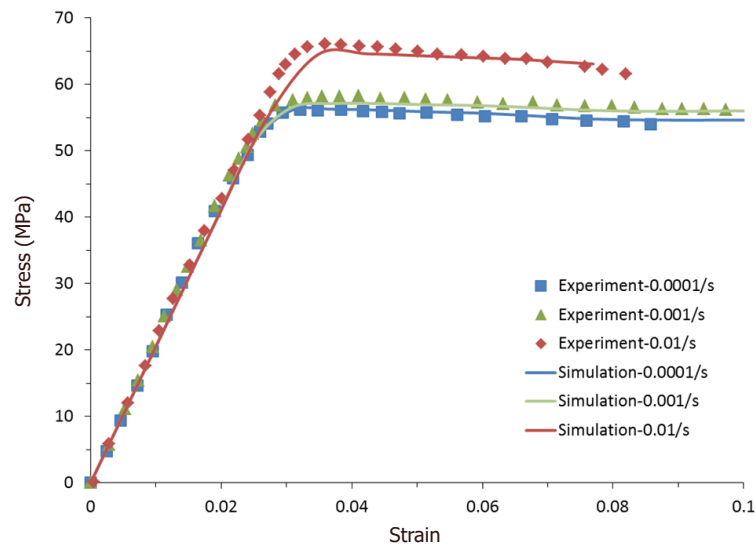


Figure 10. Experimental and simulated stress-strain behaviours of blended PLA/PBS (70/30) for different strain rates^[50]

with degradation^[48]. At human-body temperature, the biodegradation process is a combined effect of time and local deformation. To study this process appropriately, it is necessary to couple the visco-deformation model with biodegradation. In this case, a damage tensor needs to be introduced into the model to simulate the material degradation process, linked with the multi-axial stress/strain states in the implanted stent. The evolution of this damage tensor will depend on mechanical deformation and subsequently affect the mechanical properties as a result of material degradation^[49]. In this way, the interplay between deformation and degradation can be modelled, including the influence of deformation rate and material composition, which has not been previously reported for biodegradable polymeric stents^[44]. This type of model, although not available yet, can best simulate the degradation process and help predict the biological outcome due to progressively changing load-bearing property of vascular implant during degradation process.

Loading rate dependency

It is well known that PLLA has low mechanical strength and poor elongation compared to metal, which limits its application for stents. Based on literature review, a number of experimental studies have been performed to investigate and improve the mechanical behaviour of PLA, including adding plasticizers to PLA and blending PLA with ductile biodegradable polymers. Also, different constitutive material models (e.g., elastic-plastic model and viscoelastic model) were applied to model the deformation of PLLA stent. It should be noted that the stress-strain behaviour of polymers is affected by strain rate, which indicates the time-dependent deformation nature of the materials (an increase of stress level with the increase of loading rate). Consequently, consideration of the time-dependent or viscous behaviour of the materials should be given during the process of designing and manufacturing of polymeric devices, including computational studies.

Only recently, Qiu et al.^[50] applied an elastic-plastic constitutive model with time dependency to model free expansion of Elixir stent under a loading rate of 1.4, 14 and 140 MPa/s, respectively. Loading-rate dependence was described by inputting the ratio of yield stress as a function of plastic-strain rate. As shown in Figure 10, the rate-dependent stress-strain response of a PLA and poly-butylene-succinate (PBS) copolymer (with a weight ratio of 70% to 30%) was simulated very well by the rate-dependent elastic-plastic model^[50]. After expansion, stresses developed in the stent was dependent on the loading rates, for both magnitude and distribution. Stent recoiling showed a significant reduction when the loading rate was changed from 1.4 to 140 MPa/s, as

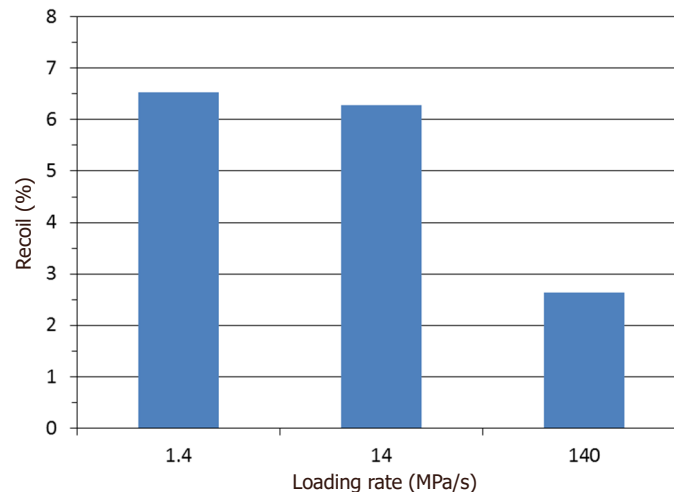


Figure 11. Recoiling effect of the Elixir stent for different loading rates^[50]

shown in Figure 11. These results proved that polymeric stent behaved differently during expansion process if the loading rate was different. With the increase of inflating rate, stent intended to expand further, with reduced recoiling but higher level of stresses. These results suggest that loading rate should be taken as an additional factor in the surgical practice of stent deployment.

CONCLUSIONS

Stents have been developed from BMSs, to DESs and then BRSs to prevent various complications and improve their clinical efficacy. Materials are also extended from metal (e.g., stainless steel and cobalt-chromium alloy) to biodegradable polymer (PLLA). For polymeric stents, experimental studies showed stent designs and materials played an important role in the stent behaviour during expansion. Physical properties (e.g., mass weight and molecular weight) and mechanical properties (e.g., tensile strength and elastic modulus) showed a difference during stent degradation. Computational approaches have also been used to study the stent performance during expansion and degradation. However, the development of bioresorbable polymeric stents is still ongoing, and studies are extremely limited due to the microscale geometry of stent and complex environment of human artery. Therefore, further research is urgently required for a better understanding of the mechanical performance of polymeric stents, including long term clinical outcomes.

DECLARATIONS

Authors' contributions

Carried out the review and drafted the paper: Qiu T

Discussed about the technical contents and also contributed to the writing of the review paper: Zhao L

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Moore JE Jr, Soares JS, Rajagopal KR. Biodegradable stents: biomechanical modelling challenges and opportunities. *Cardiovasc Eng Technol* 2010;1:52-65.
2. Hoffmann R, Mintz GS. Coronary in-stent restenosis - predictors, treatment and prevention. *Eur Heart J* 2000;21:1739-49.
3. Pfisterer ME. Late stent thrombosis after drug-eluting stent implantation for acute myocardial infarction: a new red flag is raised. *Circulation* 2008;118:1117-9.
4. Iakovou I, Schmidt T, Bonizzoni E, Ge L, Sangiorgi GM, Stankovic G. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *J Am Med Assoc* 2005;293:2126-30.
5. Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, Kutys R, Skorija K, Gold HK, Virmani R. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. *J Am Coll Cardiol* 2006;48:193-202.
6. Yang TH, Kim DI, Park SG, Seo JS, Cho HJ, Seol SH, Kim SM, Kim DK, Kim DS. Clinical characteristics of stent fracture after sirolimus- eluting stent implantation. *Int J Cardiol* 2009;131:212-6.
7. Flege C, Vogt F, Höges S, Jauer L, Borinski M, Schulte VA, Hoffmann R, Poprawe R, Meiners W, Jobmann M, Wissenbach K, Blindt R. Development and characterization of a coronary polylactic acid stent prototype generated by selective laser melting. *J Mater Sci Mater Med* 2013;24:241-55.
8. Ormiston JA, Serruys PW. Bioabsorbable coronary stents. *Circ Cardiovasc Interv* 2009;2:255-60.
9. Onuma Y, Serruys PW. Bioresorbable scaffold: the advent of a new era in percutaneous coronary and peripheral revascularization? *Circulation* 2011;123:779-97.
10. Waksman R. Biodegradable stents: they do their job and disappear. *J Invasive Cardiol* 2006;18:70-4.
11. Grabow N, Bünger CM, Schultze C, Schmohl K, Martin DP, Williams SF, Sternberg K, Schmitz KP. A biodegradable slotted tube stent based on poly (L-lactide) and poly (4-hydroxybutyrate) for rapid balloon-expansion. *Ann Biomed Eng* 2007;35:2031-8.
12. Grabow N, Bünger CM, Sternberg K, Mews S, Schmohl K, Schmitz KP. Mechanical properties of a biodegradable balloon-expandable stent from poly (L-lactide) for peripheral vascular applications. *J Med Devices* 2007;1:84-8.
13. Schmidt W, Behrens P, Brandt-Wunderlich C, Siewert S, Grabow N, Schmitz KP. In vitro performance investigation of bioresorbable scaffolds-standard tests for vascular stents and beyond. *Cardiovasc Revasc Med* 2016;17:375-83.
14. Ormiston JA, Webber B, Ubod B, Darremont O, Webster MW. An independent bench comparison of two bioresorbable drug-eluting coronary scaffolds (Absorb and DESolve) with a durable metallic drug-eluting stent (ML8/Xpedition). *EuroIntervention* 2015;11:60-7.
15. Welch TR, Eberhart RC, Reisch J, Chuong CJ. Influence of thermal annealing on the mechanical properties of PLLA coiled stents. *Cardiovasc Eng Technol* 2014;5:270-80.
16. Xu X, Liu T, Zhang K, Liu S, Shen Z, Li Y, Jing X. Biodegradation of poly (l-lactide-co-glycolide) tube stents in bile. *Polym Degrad Stab* 2008;93:811-7.
17. Hadaschik BA, Paterson RF, Fazli L, Clinkscales KW, Shalaby SW, Chew BH. Investigation of a novel degradable ureteral stent in a porcine model. *J Urol* 2008;180:1161-6.
18. Yang G, Xie H, Huang Y, Lv Y, Zhang M, Shang Y, Zhou J, Wang L, Wang JY, Chen F. Immersed multilayer biodegradable ureteral stent with reformed biodegradation: an in vitro experiment. *J Biomater Polym* 2017;31:1235-44.
19. Gong Y, Zhou Q, Gao C, Shen J. In vitro and in vivo degradability and cytocompatibility of poly (l-lactic acid) scaffold fabricated by a gelatin particle leaching method. *Acta Biomaterials* 2007;3:531-40.
20. Liu YS, Huang QL, Kienle A, Müller WEG, Feng QL. In vitro degradation of porous PLLA/pearl powder composite scaffolds. *Mater Sci Eng C* 2014;38:227-34.
21. Rodrigues N, Benning M, Ferreira AM, Dixon L, Dalgarno K. Manufacture and characterisation of porous PLA scaffolds. *Procedia CIRP* 2016;49:33-8.
22. Zamiri P, Kuang Y, Sharma U, Ng TF, Busold RH, Rago AP, Core LA, Palasis M. The biocompatibility of rapidly degrading polymeric stents in porcine carotid arteries. *Biomaterials* 2010;31:7847-55.
23. Agrawal CM, Haas KF, Leopold DA, Clark HG. Evaluation of poly(L-lactic acid) as a material for intravascular polymeric stents. *Biomaterials* 1992;13:176-82.
24. Zilberman M, Nelson KD, Eberhart RC. Mechanical properties and in vitro degradation of bioresorbable fibers and expandable fiber-based stents. *J Biomed Mater Res B* 2005;74:792-9.
25. Nuutinen JP, Clerc C, Reinikainen R, Törmälä P. Mechanical properties and in vitro degradation of bioabsorbable self-expanding

- braided stents. *J Biomater Sci* 2003;14:255-66.
26. Liu G, Zhang X, Wang D. Tailoring crystallization: towards high-performance poly (lactic acid). *Adv Mater* 2014;26:6905-11.
27. Ormiston JA, Serruys PW, Regar E, Dudek D, Thuesen L, Webster MW, Onuma Y, Garcia-Garcia HM, McGreevy R, Veldhof S. A bioabsorbable everolimus-eluting coronary stent system for patients with single de-novo coronary artery lesions (ABSORB): a prospective open-label trial. *Lancet* 2008;371:899-907.
28. Serruys PW, Onuma Y, Ormiston JA, de Bruyne B, Regar E, Dudek D, Thuesen L, Smits PC, Chevalier B, McClean D, Koolen J. Evaluation of the second generation of a bioresorbable everolimus drug-eluting vascular scaffold for treatment of de novo coronary artery stenosis clinical perspective. *Circulation* 2010;122:2301-12.
29. Serruys PW, Ormiston J, van Geuns RJ, de Bruyne B, Dudek D, Christiansen E, Chevalier B, Smits P, McClean D, Koolen J, Windecker S. A polylactide bioresorbable scaffold eluting everolimus for treatment of coronary stenosis. *J Am Coll Cardiol* 2016;67:766-76.
30. Lane JP, Perkins LE, Sheehy AJ, Pacheco EJ, Frie MP, Lambert BJ, Rapoza RJ, Virmani R. Lumen gain and restoration of pulsatility after implantation of a bioresorbable vascular scaffold in porcine coronary arteries. *Cardiovas Interv* 2014;7:688-95.
31. Chua SD, Mac Donald BJ, Hashmi MSJ. Finite element simulation of stent and balloon interaction. *J Mater Pro Tech* 2003;143:591-7.
32. Lally C, Dolan F, Prendergast PJ. Cardiovascular stent design and vessel stresses: a finite element analysis. *J Biomech* 2005;38:1574-81.
33. Schiavone A, Zhao LG, Abdel-Wahab AA. Effects of material, coating, design and plaque composition on stent deployment inside a stenotic artery -- finite element simulation. *Mater Sci Eng C Mater Biol Appl* 2014;42:479-88.
34. Pauck RG, Reddy BD. Computational analysis of the radial mechanical performance of PLLA coronary artery stents. *Med Eng Phys* 2015;37:7-12.
35. Debusschere N, Segers P, Dubrue P, Verhegghe B, De Beule M. A finite element strategy to investigate the free expansion behaviour of a biodegradable polymeric stent. *J Biomech* 2015;48:2012-8.
36. Wang Q, Fang G, Zhao Y, Wang G, Cai T. Computational and experimental investigation into mechanical performances of poly-L-lactide acid (PLLA) coronary stents. *J Mech Behav Biomed Mater* 2017;65:415-27.
37. Shanahan C, Tofail SA, Tiernan P. Viscoelastic braided stent: finite element modelling and validation of crimping behaviour. *Mater Design* 2017;121:143-53.
38. Schiavone A, Abunassar C, Hossainy S, Zhao LG. Computational analysis of mechanical stress-strain interaction of a bioresorbable scaffold with blood vessel. *J Biomech* 2016;49:2677-83.
39. Soares JS, Moore JE, Rajagopal KR. Modeling of deformation-accelerated breakdown of polylactic acid biodegradable stents. *J Med Devices* 2010;4:410-7.
40. Luo Q, Liu X, Li Z, Huang C, Zhang W, Meng J, Chang Z, Hua Z. Degradation model of bioabsorbable cardiovascular stents. *PLoS One* 2014;9:e110278.
41. Qiu T, He R, Abunassar C, Hossainy S, Zhao LG. Effect of two-year degradation on mechanical interaction between a bioresorbable scaffold and blood vessel. *J Mech Behav Biomed Mater* 2017;78:254.
42. Farooq V, Gogas BD, Serruys PW. Restenosis delineating the numerous causes of drug-eluting stent restenosis. *Circ Cardiovasc Interv* 2010;4:195-205.
43. Imani SM, Goudarzi AM, Ghasemi SE, Kalani A, Mahdinejad J. Analysis of the stent expansion in a stenosed artery using finite element method: application to stent versus stent study. *Proc Inst Mech Eng H* 2014;228:996-1004.
44. Soares JS, Moore JE Jr, Rajagopal KR. Constitutive framework for biodegradable polymers with applications to biodegradable stents. *Asaio* 2008;54:295-301.
45. Vieira AC, Vieira JC, Ferra JM, Magalhães FD, Guedes RM, Marques AT. Mechanical study of PLA-PCL fibers during in vitro degradation. *J Mechan Behav Biomed Mater* 2011;4:451-60.
46. Grabow N, Büniger CM, Schultze C, Schmohl K, Martin DP, Williams SF, Sternberg K, Schmitz KP. A biodegradable slotted tube stent based on poly(L-lactide) and poly(4-hydroxybutyrate) for rapid balloon-expansion. *Ann Biomed Eng* 2007;35:2031-8.
47. Grabow N, Schlun M, Sternberg K, Hakansson N, Kramer S, Schmitz KP. Mechanical properties of laser cut poly(L-lactide) micro-specimens: implications for stent design, manufacture, and sterilization. *ASME J Biomechan Eng* 2008;127:25-31.
48. Moore JE Jr, Soares JS, Rajagopal KR. Biodegradable stents: biomechanical modeling challenges and opportunities. *Cardiovas Eng Tech* 2015;1:52-65.
49. Wu W. Experimental data confirm numerical modeling of the degradation process of magnesium alloys stents. *Acta Biomaterialia* 2013;9:8730-9.
50. Qiu TY, Song M, Zhao LG. A computational study of crimping and expansion of bioresorbable polymeric stents. *Mech Time-Depen Mater* 2017;6:1-18.

Original Article

Open Access



Plasmatic biomarkers of inflammation correlate with ^{18}F FDG-PET-CT and microembolic signals in patients with carotid stenosis

Hubertus Mueller¹, Loraine Fisch¹, Christophe Bonvin², Karl Lovblad³, Osman Ratib⁴, Patrice Lalive¹, Stephane Pagano⁵, Nicolas Vuilleumier⁵, Jean-Pierre Willi⁴, Roman Sztajzel¹

¹Department of Neurology, University Hospital of Geneva and Medical School, Geneva 1211, Switzerland.

²Department of Neurology, Hospital of Sion, Sion 1950, Switzerland.

³Department of Neuroradiology, University Hospital of Geneva and Medical School, Geneva 1211, Switzerland.

⁴Department of Nuclear Medicine, University Hospital of Geneva and Medical School, Geneva 1211, Switzerland.

⁵Department of Laboratory Medicine, University Hospital of Geneva and Medical School, Geneva 1211, Switzerland.

Correspondence to: Dr. Roman Sztajzel, Department of Neurology, University Hospital of Geneva and Medical School, Rue Gabrielle Perret Gentile 4, Geneva 1211, Switzerland. E-mail: roman.sztajzel@hcuge.ch

How to cite this article: Mueller H, Fisch L, Bonvin C, Lovblad K, Ratib O, Lalive P, Pagano S, Vuilleumier N, Willy JP, Sztajzel R. Plasmatic biomarkers of inflammation correlate with ^{18}F FDG-PET-CT and microembolic signals in patients with carotid stenosis. *Vessel Plus* 2018;2:13. <http://dx.doi.org/10.20517/2574-1209.2018.19>

Received: 8 Apr 2018 **First Decision:** 23 Apr 2018 **Revised:** 2 May 2018 **Accepted:** 11 May 2018 **Published:** 5 Jun 2018

Science Editor: Aaron S. Dumont **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Aim: To determine whether plasmatic biomarkers correlate with ^{18}F fluoro-2-deoxy-D-glucose (FDG) positron emission tomography/computed tomography (PET-CT) and presence of microembolic signals (MES) detected by transcranial Doppler in patients with carotid stenosis.

Methods: ^{18}F FDG-PET-CT and MES detection was performed in consecutive patients with 50% to 99% symptomatic or asymptomatic carotid stenosis. Uptake index was defined by a target to background ratio (TBR) between maximum standardized uptake value of the carotid plaque and the average uptake of the jugular veins. The analysis of biomarkers included adhesion molecules [intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule 1, P-selectin and E-selectin], interleukins (IL-1, IL-6), chemokines (RANTES, monocyte chemoattractant protein 1), cytokines (tumor necrosis factor α), matrix-metalloproteases (MMP), myeloperoxidase, and lipoprotein-associated phospholipase A2.

Results: There were 54 symptomatic and 57 asymptomatic patients. TBR values were significantly higher in the symptomatic compared to the asymptomatic (median 2.1 *vs.* 1.8, $P = 0.002$) and in the MES positive (MES+) compared to the MES negative (MES-) group (MES+, $n = 19$, median 2.3 and MES-, $n = 88$, median 1.8, $P = 0.01$). The best threshold for TBR values was of 1.9. We found a significant correlation between higher ^{18}F FDG uptake (TBR ≥ 1.9) and the



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



plasmatic levels of chemokine RANTES ($P = 0.03$) and higher levels of ICAM-1 in MES+ patients ($P = 0.03$). Interestingly MMP-2 levels were more important in patients with lower TBR values ($P = 0.02$) and MMP-3 and P-selectin in those who were MES- (respectively $P = 0.001$ and $P = 0.009$).

Conclusion: In the present study, ICAM-1 was associated with the presence of thrombotically active atherosclerotic plaques, while RANTES mainly correlated with the inflammatory process. MMP-2, MMP-3 and P-selectin levels were more important in patients with stable plaques.

Keywords: Carotid plaque, biomarkers of inflammation, microemboli detection, transcranial Doppler, ^{18}F FDG-PET-CT

INTRODUCTION

Inflamed carotid plaques play a key role in the occurrence of distal embolic cerebral infarcts^[1-3]. Intensive research has been performed in the last few decades aimed at optimizing different imaging modalities with adequate spatial resolution to precisely analyse the arterial wall morphology, plaque composition, and degree of local inflammation^[4-6]. Among them, positron emission tomography (PET) using the glucose analogue ^{18}F fluoro-2-deoxy-D-glucose (^{18}F FDG) as a radiotracer reflecting glycolytic activity has shown promise for non-invasive functional detection of local inflammation in atherosclerotic plaques and is considered as an emergent marker of plaque vulnerability^[7-9]. Furthermore, ultrasound-based imaging modalities demonstrated that the presence of microembolic signals (MES) detected by means of transcranial Doppler downstream the stenosis is associated with an increased risk of embolic stroke^[10-12].

In the present study we investigated in patients with symptomatic or asymptomatic carotid stenosis the relationship between ^{18}F FDG-PET-computed tomography (CT), MES and plasmatic biomarkers of inflammation including adhesion molecules, interleukins (ILs), chemokines, cytokines, matrix-metalloproteases (MMP) and lipoprotein-associated phospholipase A2 (lp-PLA2).

METHODS

We included patients with unilateral symptomatic or asymptomatic carotid disease with 50% to 99% degree of stenosis according to the ECST criteria. Symptomatic stenosis was defined as any recent (< 6 months) neurological or retinal deficit, persisting or transient ischemic attack (TIA), which could be plausibly attributed to the ipsilateral carotid artery. Assessment of clinical parameters was performed upon study inclusion. Symptomatic patients had a complementary work up including cardiac ultrasound examination and long duration electrocardiogram for 7 days in order to exclude a cardio-embolic origin of stroke. Asymptomatic stenosis was defined as no history of recent (within the last 6 months) neurological or retinal deficit and/or presence of ipsilateral ischemic magnetic resonance imaging (MRI) lesions. All patients gave their written consent.

^{18}F FDG-PET/CT angiography

All patients underwent ^{18}F FDG-PET-CT with contrast angiography within 2-3 days after admission when symptomatic, and within 10 days after assessment of the diagnosis of carotid stenosis when asymptomatic. We used a standard protocol as described previously^[13]. Analysis of PET-CT was done by two experienced investigators (JPW, HM) blinded to the clinical and biological data. The target to background ratio (TBR) was assessed by dividing the SUVmax of the carotid plaque wherever highest by the average SUVmean of the jugular veins.

Ultrasound analysis

Standard examination included duplex ultrasound of the carotid arteries (SIEMENS) with assessment of degree of stenosis according to the ECST criteria^[14], plaque surface morphology and plaque echogenicity.

For MES detection, bilateral transcranial doppler (TCD) recording was performed during 60 min. In symptomatic patients, this examination was performed within 7 days after stroke onset and within 10 days when asymptomatic. We used a standard protocol as described elsewhere^[13]. Embolic signal interpretation was done manually by an experienced ultrasonographer based on the criteria of the International Consensus group on Microembolus Detection^[15,16]. Detection of at least 1 MES ipsilateral of the stenosis resulted in a positive exam, and those patients were defined as MES+.

Imaging

Symptomatic patients underwent a CT scan with contrast angiography of vessels of the neck and brain. Additionally, MRI study was performed with T1, T2, diffusion weighted (DWI) and fluid attenuated inversion recovery (FLAIR) sequences.

Plasmatic biomarkers

Venous blood samples taken on the day of ¹⁸FDG-PET-CT were analyzed for 111 patients. Plasma levels of MMP-2, -3, -8, -9, IL-1, IL-6, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, P-selectin, E-selectin, tumor necrosis factor α (TNF α), RANTES, monocyte chemoattractant protein (MCP)-1, were performed using a bioplex 200 array reader (Bio-Rad Laboratories; Hercules, CA, USA) with Luminex MAPTM Technology (Luminex Corporation, Austin, TX, USA). MPO levels were measured using the colorimetric enzyme-linked immunosorbent assay (ELISA), a commercial kit purchased from R&D Systems (Minneapolis, MN, USA), according to manufacturer's instructions. The lp-PLA2 concentration (mass) was measured using PLAC Test ELISA Kit.

Statistical analysis

All continuous variables were summarized by mean and median. For testing of significant difference between groups, the *U*-Mann-Whitney test was applied. Receiver operating characteristic (ROC) curve analysis was used to determine the prognostic accuracy of the plasmatic biomarkers with respect to TBR values and presence or absence of MES. For correlation we used Spearman's rho method. Statistical analysis was performed using MedCalc (MedCalc Software, Ostend, Belgium) software.

RESULTS

From 2009 to 2015, 111 patients were analyzed. Fifty-four patients presented with symptomatic and 57 with asymptomatic carotid disease. All demographical data of the patients are summarized in Table 1. The CV risk factor profile was similar in the two groups, for the exception of degree of stenosis which was significantly higher in symptomatic patients. The male gender predominated in both groups.

MES

MES detection could be carried out in 107 patients. In 4 patients the investigation was not possible because of insufficient temporal bone window. Nineteen of 107 (18%) patients presented microembolic signals ipsilaterally during TCD recording. The mean number of emboli was 10 (range 1-50). The proportion of MES+ patients was higher in the symptomatic (26%, $n = 14/54$) when compared to the asymptomatic group (9%, $n = 5/57$; $P = 0.01$) [Table 1].

¹⁸FDG-PET

One hundred eleven patients with 111 carotid plaques were analyzed. Hundred-one plaques presented with partial calcification whereas 10 showed exclusively a soft component on CTA. ¹⁸FDG uptake was significantly higher in the symptomatic group as compared to the asymptomatic one (TBR: median 2.1 vs. 1.8, $P = 0.002$) [Table 1]. When confronting presence of MES to ¹⁸FDG uptake, those plaques producing

Table 1. Baseline characteristics of the whole cohort

	Symptomatic (<i>n</i> = 54)	Asymptomatic (<i>n</i> = 57)	<i>P</i> values
Age	Mean 71.7	Mean 72.1	0.7
Gender (male)	44 (81%)	43 (75%)	0.5
Degree of stenosis	Mean 77.3	Mean 73.2	0.03
High blood pressure	41 (76%)	42 (74%)	0.9
Diabetes	17 (31%)	16 (28%)	0.5
Dyslipidemia	25 (46%)	34 (60%)	0.2
Tobacco	30 (56%)	31 (54%)	0.3
Coronary disease	10 (19%)	10 (18%)	0.9
Family history	4 (7.4%)	5 (8.7%)	0.9
Antiplatelet	7 (17%)	4 (3.5%)	0.3
Statins	18 (33%)	26 (48%)	0.2
Stroke	42 (78%)	-	
TIA	12 (22%)	-	
Lesion MRI (ipsilateral to stenosis)	41 (76%)	-	
*MES	14 (19%)	5 (9.2%)	0.02
TBR	Median 2.1	Median 1.8	0.002

*MES detection performed in 107 patients. TIA: transitory ischemic attack; MRI: magnetic resonance imaging; MES: microembolic signal; TBR: target to background ratio

Table 2. Inflammatory plasmatic biomarkers in symptomatic and asymptomatic patients (median values)

	Symptomatic (<i>n</i> = 54), pg/mL	Stroke only (<i>n</i> = 42), pg/mL	TIA only (<i>n</i> = 12), pg/mL	<i>P</i> value (stroke vs. TIA)	Asymptomatic (<i>n</i> = 57), pg/mL	<i>P</i> value (sympt vs. asympt)
MMP-9	283,302	291,844	186,353	0.07	195,299	0.03
MMP-8	11,292	11,574	9302	0.7	8001	0.03
MMP-3	17,909	18,745	17,485	0.6	24,434	0.18
MMP-2	344,126	326,695	394,951	0.1	345,067	0.26
TNF- α	5.2	5.1	5.8	0.8	5.3	0.94
ICAM-1	277,016	280,808	256,042	0.7	285,594	0.56
VCAM-1	1,024,550	1,024,550	1,014,682	0.8	953,996	0.87
P-selectin	96,102	102,334	87,809	0.9	99,868	0.40
E-selectin	36,714	37,362	34,503	0.3	41,567	0.12
RANTES	45,188	45,992	41,941	0.2	44,278	0.49
MCP-1	296	310	251	0.3	258	0.17
*MPO	107	107	80	0.5	63	0.07
IL-6	1.35	1.3	2.07	0.7	1.35	0.24
IL-1	1943	1946	1840	0.7	1707	0.10
*lp-PLA2	126	118	131	0.4	138	0.25

**n* = 51. MMP: matrix-metalloproteases; TNF: tumor necrosis factor; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; MCP: monocyte chemoattractant protein; MPO: myeloperoxidase; IL: interleukin; lp-PLA2: lipoprotein-associated phospholipase; TIA: transitory ischemic attack

emboli, showed also an increased inflammatory activity (TBR: median 2.3 vs. 1.8, *P* = 0.01). The best TBR threshold value for the distinction between symptomatic, asymptomatic, MES+ and MES- negative patients was 1.9^[13].

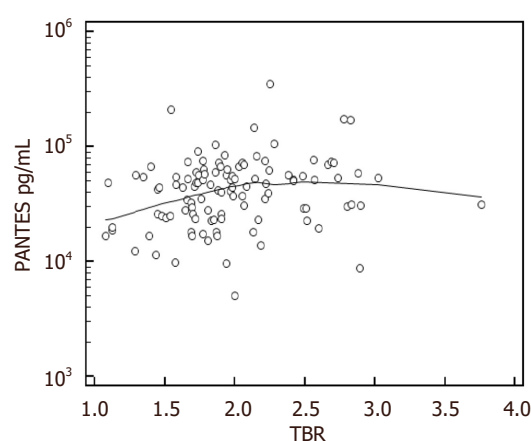
Plasmatic biomarkers

Analysis of plasmatic biomarkers was performed in 111 patients. When symptomatic and asymptomatic patients were compared, levels of MMP-8 and MMP-9 were significantly higher in the symptomatic ones (*P* = 0.03 for both) [Table 2]. In a subgroup analysis of the symptomatic patients differentiating between stroke and TIA, only stroke patients maintained a difference of MMP-9, however without reaching significance. MPO showed a trend towards higher levels in stroke patients but did not attain statistical significance [Table 2].

Table 3. Inflammatory plasmatic biomarkers according to TBR values of ^{18}F FDG-PET-CT in symptomatic or asymptomatic patients with carotid stenosis (median values)

	TBR ≥ 1.9 (<i>n</i> = 56), pg/mL	TBR < 1.9 (<i>n</i> = 55), pg/mL	<i>P</i> value
MMP-9	227,612	253,273	0.9014
MMP-8	10,263	10,181	0.83
MMP-3	22,183	20,670	0.8273
MMP-2	328,626	365,796	0.0268
TNF- α	4.8600	5.5600	0.1018
ICAM-1	276,728	290,987	0.85
VCAM-1	959,190	1,020,700	0.4242
P-selectin	102,491	87,460	0.5672
E-selectin	38,700	38,744	0.5514
RANTES	51,396	41,367	0.0387
MCP-1	283	283	1.0000
MPO	73	88	0.1969
IL-6	1.3500	1.4900	0.6376
IL-1	1742	1934	0.3514
lp-PLA2	130	125	0.6187

MMP: matrix-metalloproteases; TNF: tumor necrosis factor; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; MCP: monocyte chemoattractant protein; MPO: myeloperoxidase; IL: interleukin; lp-PLA2: lipoprotein-associated phospholipase; TBR: target to background ratio; ^{18}F FDG-PET-CT: ^{18}F Fluorodeoxyglucose positron emission tomography/computed tomography

**Figure 1.** Correlation between RANTES and target to background ratio (TBR) values, rho 0.249 ($P = 0.008$)

We found a significant correlation between higher ^{18}F FDG uptake (TBR ≥ 1.9) [Table 3] and the plasmatic levels of chemokine RANTES ($P = 0.03$). The correlation between RANTES and TBR values was of rho 0.249 ($P = 0.008$) [Figure 1].

There were higher levels of ICAM-1 ($P = 0.03$) in MES+ patients [Table 4]. The correlation with the number of MES was of rho = 0.21 ($P = 0.03$).

The predictive values of RANTES and ICAM-1 are shown on Table 5.

MMP-2 levels were more important in patients with lower TBR values ($P = 0.02$) and MMP-3 and P-selectin in those who were MES- (respectively $P = 0.001$ and $P = 0.009$) [Tables 3 and 4]. There was an inverse correlation between number of MES and MMP-3 with rho = -0.319 ($P = 0.0008$) and number of MES and P-selectin with rho = -0.26 ($P = 0.006$).

Table 4. Inflammatory plasmatic biomarkers according to presence or absence of MES

	MES+ (n = 19), pg/mL	MES- (n = 88), pg/mL	P value
MMP-9	257,003	230,582	0.9938
MMP-8	11,885	9698	0.4884
MMP-3	13,022	24,087	0.001
MMP-2	319,239	344,595	0.06
TNF- α	5.8	5.3	0.6712
ICAM-1	304,084	272,250	0.03
VCAM-1	1,250,217	963,976	0.2967
P-selectin	69,370	100,415	0.009
E-selectin	35,766	39,562	0.22
RANTES	40,975	47,131	0.3653
MCP-1	265	270	0.6836
MPO	83	85	0.7081
IL-6	1.3500	1.3500	0.9022
IL-1	1968	1794	0.2006
lp-PLA2	124	139	0.7652
TBR	2.3	1.8	0.01

MMP: matrix-metalloproteases; TNF: tumor necrosis factor; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; MCP: monocyte chemoattractant protein; MPO: myeloperoxidase; IL: interleukin; lp-PLA2: lipoprotein-associated phospholipase; TBR: target to background ratio; MES: microembolic signal

Table 5. Sensitivity and specificity of biomarkers to predict high TBR values of ^{18}F FDG or presence of MES

	Sensitivity (%)	Specificity (%)	AUC	Criterion	P value
RANTES	82	42	0.614	> 27,835 pg/mL	0.03
ICAM-1	90	42	0.615	> 239,642 pg/mL	0.015

AUC: area under the curve; ICAM: intercellular adhesion molecule; TBR: target to background ratio; MES: microembolic signal; ^{18}F FDG: ^{18}F Fluorodeoxyglucose

DISCUSSION

The clinical role of plasmatic biomarkers in the setting of carotid atherosclerosis has been extensively studied in these recent years^[17-19]. This not only for the assessment of the embolic risk but also for the choice of the best type of carotid intervention^[20-22].

The present study has examined the relationship between plasmatic mediators of inflammation, ^{18}F FDG uptake in carotid plaques and the presence of MES. We found a significant correlation between higher ^{18}F FDG uptake and the plasmatic levels chemokine RANTES [Table 3 and Figure 1]. Furthermore in MES+ patients higher levels of ICAM-1 were present.

In the dal-PLAQUE study, baseline ^{18}F FDG uptake positively correlated with blood MPO and IL-6^[23,24]. Interestingly, hs-CRP, P-selectin, E-selectin, ICAM-1, MMP-3 and MMP-9 did not correlate with TBR values^[23,24]. In an earlier report by Rudd *et al.*^[9], ^{18}F FDG uptake was significantly associated with serum MMP-9 levels. We did not find a correlation of PET-CT to MMP-9 in our study. One possible explanation is that MMP-9 may be influenced by the burden of ischemic brain lesions and does not only reflect inflammation within the carotid plaque. Supporting this hypothesis, we found a higher level of MMP-9 in stroke in contrast to TIA patients with a trend towards significance [Table 2].

Chemokines coordinate communication between circulating inflammatory cells and endothelium^[25,26]. We found that circulating RANTES correlated positively to ^{18}F FDG uptake in the carotid plaque, but there was no significant difference between symptomatic and asymptomatic patients. These findings are inline to those of Zaremba *et al.*^[27] who found no differences in RANTES levels between the sera of stroke patients and those of

controls. Furthermore, in the present study the analysis of the plasmatic levels of RANTES between patients with and without a lesion on MRI showed no statistical significant difference. Also correlation to TBR persisted even in patients without a stroke lesion. RANTES may therefore be a marker of inflammation in atherosclerosis and be less influenced by ischemic damage in stroke [Table 5]. In the present study, we found higher levels of ICAM-1 in MES+ patients, but not in patients with higher TBR values. These results possibly reflect different plaque components; in fact ¹⁸FDG-PET-CT mainly depicts the inflammatory state of the whole carotid plaque whereas presence of MES reflects surface abnormalities such as ulceration and/or plaque thrombus^[7,10]. Although a significant correlation between presence of symptoms and MES could be demonstrated in our cohort [Table 1], no difference was found between symptomatic and asymptomatic patients with respect to ICAM-1 levels, again giving more strength to the link between biomarkers and thrombotically active plaques *per se* [Table 5]. Cellular adhesion molecules, including ICAM-1, VCAM-1 and E-selectin promote recruitment of inflammatory cells into the arterial wall where they interact with lipid particles leading subsequently to plaque formation^[28]. In the Atherosclerosis Risk In Communities (ARIC) study, the relationship of ICAM-1 and E-selectin with coronary heart disease and carotid artery atherosclerosis was independent of other known risk factors^[29]. Cellular adhesion molecules have also been implicated in the destabilisation of atherosclerotic plaques. In a recent study including human carotid endarterectomy (CEA) specimens from asymptomatic ($n = 30$) and symptomatic ($n = 30$) patients, expression of VCAM-1 on the endothelium of CEA specimens from symptomatic patients was 2.4-fold greater than that from asymptomatic patients ($P < 0.01$)^[30]. In another study performed upon 40 patients undergoing carotid endarterectomy it was possible to determine the influence of surgery on the levels of adhesion molecules. A statistically significant decrease of the ICAM-1 levels 1 h and 6 h after the endarterectomy compared to levels before the operation was found suggesting that decrease of ICAM-1 could be a possible marker of endothelial de-activation after plaque removal^[31]. Only very few studies investigated the relationship between biomarkers and presence of MES^[32,33]. One study including 104 controls and 118 patients found increased values of CXCL16 in stroke and in MES+ patients^[33]. Other studies reported the following biomarker candidates for MES: P-selectin, fibrinogen, high neutrophil count, reduced ratio of CD4+CD25, high regulatory T cells and the C allele of TNF receptor superfamily member^[32]. At present ICAM-1 has never been reported in association with MES. However, as this biomarker may be involved in the process of plaque destabilization, its relationship to MES is nevertheless very likely. Interestingly in our cohort MMP-2 levels were significantly more important in patients with lower TBR values and MMP-3 and P-selectin in those who were MES-. There was also a trend of MMP-2 levels to be higher in MES- patients. MMPs are a class of proteases involved in extracellular matrix degradation, which appear to play a key role in the process of vascular remodeling during the course of vascular disease^[34,35]. Numerous studies suggest that MMPs and in particular MMP-3, MMP-7, MMP-9 and MMP-12, may be involved in the process of plaque destabilization^[36,37]. However there are conflicting results in particular regarding MMP-3. The correlation between MMP-3 blood levels and carotid atherosclerotic disease has been reported in several studies. Lien *et al.*^[38] showed in a study including 433 patients that MMP-3 was significantly associated with the presence of higher carotid plaques scores reflecting more unstable plaques. On the other hand, experimental studies show that MMP-3 is required for efficient neointima formation after carotid ligation and for smooth cell migration, supporting the fact that MMP-3 acts on plaque stability^[39]. The relationship between MMP-2 and stable plaques has been already described by Sluijter *et al.*^[40] who showed in a study including 150 subjects that there was an increased activity of MMP-2 in association with the presence of smooth muscle cells and a fibrous phenotype. This finding suggested that MMP-2 may be considered as a marker of a stable plaque. The correlation between P-selectin and stable plaques has been less well documented. In the study reported by Yin *et al.*^[32], P-selectin was increased in MES+ patients. On the opposite, in our cohort there was a significant increase of P-selectin levels in MES- patients with an inverse correlation between the number of MES and the plasmatic levels of P-selectin.

To conclude, in the present study ICAM-1 was associated with the presence of thrombotically active atherosclerotic plaques, while RANTES mainly correlated with the inflammatory process. MMP-2, MMP-3

and P-selectin levels were more important in patients with stable plaques. Further studies combining ¹⁸FDG-PET-CT and MES detection are needed in order to confirm our results.

DECLARATIONS

Authors' contributions

Contributed to manuscript redaction: Mueller H

Contributed to patients inclusion: Fisch L, Bonvin C

Helped to define the the appropriate biomarkers: Lalive P, Pagano S, Vuilleumier N

Contributed to the PET-CT protocole and to the interpretation of results: Ratib O, Willy JP, Lovblad K

Contributed to the study design and to manuscript redaction: Sztajzel R

Availability of data and materials

All data were collected by our research study nurse and are available.

Financial support and sponsorship

The study was granted by the reasearch Center of the University Hospital of Geneve, sustained by the National Swiss Foundation.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study was approved by Human Central Ethics commission of University Hospital Geneva and all patients gave their written consent.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. *Stroke* 2000;31:774-81.
2. Spagnoli LG, Mauriello A, Sangiorgi G, Fratoni S, Bonanno E, Schwartz RS, Piepgras DG, Pistolese R, Ippoliti A, Holmes DR Jr. Extracranial thrombotically active carotid plaque as a risk factor for ischemic stroke. *JAMA* 2004;292:1845-52.
3. Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012;32:2045-51.
4. Jander S, Sitzer M, Schumann R, Schroeter M, Siebler M, Steinmetz H, Stoll G. Inflammation in high-grade carotid stenosis: a possible role for macrophages and T cells in plaque destabilization. *Stroke* 1998;29:1625-30.
5. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. *J Am Coll Cardiol* 2006;47:C13-8.
6. Schwartz SM, Galis ZS, Rosenfeld ME, Falk E. Plaque rupture in humans and mice. *Arterioscler Thromb Vasc Biol* 2007;27:705-13.
7. Rudd JH. Imaging atherosclerotic plaque inflammation with [18F]-Fluorodeoxyglucose positron emission tomography. *Circulation* 2002;105:2708-11.
8. Marnane M, Merwick A, Sheehan OC, Hannon N, Foran P, Grant T, Dolan E, Moroney J, Murphy S, O'Rourke K, O'Malley K, O'Donohoe M, McDonnell C, Noone I, Barry M, Crowe M, Kavanagh E, O'Connell M, Kelly PJ. Carotid plaque inflammation on 18F-fluorodeoxyglucose positron emission tomography predicts early stroke recurrence. *Ann Neurol* 2012;71:709-18.
9. Rudd JH, Myers KS, Bansilal S, Machac J, Woodward M, Fuster V, Farkouh ME, Fayad ZA. Relationships among regional arterial inflammation, calcification, risk factors, and biomarkers: a prospective fluorodeoxyglucose positron-emission tomography/computed tomography imaging study. *Circ Cardiovasc Imaging* 2009;2:107-15.
10. Sitzer M, Müller W, Siebler M, Hort W, Kniemeyer HW, Jäncke L, Steinmetz H. Plaque ulceration and lumen thrombus are the main sources of cerebral microemboli in high-grade internal carotid artery stenosis. *Stroke* 1995;26:1231-3.
11. King A, Markus HS. Doppler embolic signals in cerebrovascular disease and prediction of stroke risk: a systematic review and meta-

- analysis. *Stroke* 2009;40:3711-7.
12. Markus HS, King A, Shipley M, Topakian R, Cullinane M, Reihill S, Bornstein NM, Schaafsma A. Asymptomatic embolisation for prediction of stroke in the Asymptomatic Carotid Emboli Study (ACES): a prospective observational study. *Lancet Neurol* 2010;9:663-71.
 13. Müller HF, Viaccoz A, Fisch L, Bonvin C, Lovblad KO, Ratib O, Lalive P, Pagano S, Vuilleumier N, Willi JP, Sztajzel R. 18FDG-PET-CT: an imaging biomarker of high-risk carotid plaques. Correlation to symptoms and microembolic signals. *Stroke* 2014;45:3561-6.
 14. Naylor AR, Rothwell PM, Bell PR. Overview of the principal results and secondary analyses from the European and North American randomised trials of endarterectomy for symptomatic carotid stenosis. *Eur J Vasc Endovasc Surg* 2003;26:115-29.
 15. Ringelstein RB, Droste DW, Babikian VI, Evans DH, Grosset DG, Kaps M, Markus HS, Russell D, Siebler M. Consensus on microembolus detection by TCD: international consensus group on microembolus detection. *Stroke* 1998;29:725-9.
 16. Choi Y, Saqqur M, Asil T, Jin A, Stewart E, Stephenson C, Ibrahim M, Roy J, Boulanger JM, Coutts S, Khan F, Demchuk AM. A combined power M-mode and single gate transcranial doppler ultrasound microemboli signal criteria for improving emboli detection and reliability. *J Neuroimaging* 2010;20:359-67.
 17. Koutouzis M, Rallidis LS, Peros G. Serum interleukin-6 is elevated in symptomatic carotid bifurcation disease. *Acta Neurol Scand* 2009;119:119-25.
 18. Papas TT, Maltezos CK, Papanas N. High-sensitivity CRP is correlated with neurologic symptoms and plaque instability in patients with severe stenosis of the carotid bifurcation. *Vase Endovascular Surg* 2008;42:249-55.
 19. Elkind MS, Luna JM, Moon YP, Liu KM, Spitalnik SL, Paik MC, Sacco RL. High-sensitivity C-reactive protein predicts mortality but not stroke: the Northern Manhattan Study. *Neurology* 2009;73:1300-7.
 20. Avgerinos ED, Kadoglou NP, Moulakakis KG, Giannakopoulos TG, Liapis CD. Current role of biomarkers in carotid disease: a systematic review. *Int J Stroke* 2011;6:337-45.
 21. Heider P, Poppert H, Wolf O, Liebig T, Pelisek J, Schuster T, Eckstein HH. Fibrinogen and high-sensitive C-reactive protein as serologic predictors for perioperative cerebral microembolic lesions after carotid endarterectomy. *J Vasc Surg* 2007;46:449-54.
 22. Versaci F, Reimers B, Prati F, Gaspardone A, Del Giudice C, Pacchioni A, Mauriello A, Cortese C, Nardi P, De Fazio A, Chiariello GA, Proietti I, Chiariello L. Prediction of cardiovascular events by inflammatory markers in patients undergoing carotid stenting. *Mayo Clin Proc* 2012;87:50-8.
 23. Duivenvoorden R, Mani V, Woodward M, Kallend D, Suchankova G, Fuster V, Rudd JH, Tawakol A, Farkouh ME, Fayad ZA. Relationship of serum inflammatory biomarkers with plaque inflammation assessed by FDG PET/CT: the dal-PLAQUE study. *JACC Cardiovasc Imaging* 2013;6:1087-94.
 24. Mani V, Woodward M, Samber D, Bucerius J, Tawakol A, Kallend D, Rudd JH, Abt M, Fayad ZA. Predictors of change in carotid atherosclerotic plaque inflammation and burden as measured by 18-FDG-PET and MRI, respectively, in the dal-PLAQUE study. *Int J Cardiovasc Imaging* 2014;30:571-82.
 25. Barlic J, Murphy PM. Chemokine regulation of atherosclerosis. *J Leukoc Biol* 2007;82:226-36.
 26. von Hundelshausen P, Weber KSC, Huo Y, Proudfoot AE, Nelson PJ, Ley K, Weber C. RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. *Circulation* 2001;103:1772-7.
 27. Zaremba J, Ilkowski J, Losy J. Serial measurements of levels of the chemokines CCL2, CCL3 and CCL5 in serum of patients with acute ischaemic stroke. *Folia Neuropathol* 2006;44:282-9.
 28. Ammirati E, Moroni F, Norata GD, Magnoni M, Camici PG. Markers of inflammation associated with plaque progression and instability in patients with carotid atherosclerosis. *Mediators Inflamm* 2015;2015:718329.
 29. Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM Jr, Boerwinkle E. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. *Circulation* 1997;96:4219-25.
 30. Weinkauff CC, Concha-Moore K, Lindner JR, Marinelli ER, Hadinger KP, Bhattacharjee S, Berman SS, Goshima K, Leon LR Jr, Matsunaga TO, Unger E. Endothelial vascular cell adhesion molecule 1 is a marker for high-risk carotid plaques and target for ultrasound molecular imaging. *J Vasc Surg* 2018; doi: 10.1016/j.jvs.2017.10.088.
 31. Palenkić H, Bačun T, Čosić A, Lovričević I, DeSyo D, Drenjančević I. Serum levels of ICAM-1, VCAM-1 and E-selectin in early postoperative period and three months after eversion carotid endarterectomy. *Med Glas (Zenica)* 2014;11:313-9.
 32. Yin R, Ma A, Pan X, Yang S. Biomarkers of cerebral microembolic signals. *Clin Chim Acta* 2017;475:164-8.
 33. Ma A, Yang S, Wang Y, Wang X, Pan X. Increase of serum CXCL16 level correlates well to microembolic signals in acute stroke patients with carotid artery stenosis. *Clin Chim Acta* 2016;460:67-71.
 34. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res* 2002;90:251-62.
 35. Ramos-Fernandez M, Bellolio MF, Stead LG. Matrix metalloproteinase-9 as a marker for acute ischemic stroke: a systematic review. *J Stroke Cerebrovasc Dis* 2011;20:47-54.
 36. Loftus IM, Naylor AR, Bell PR, Thompson MM. Plasma MMP-9 - a marker of carotid plaque instability. *Eur J Vasc Endovasc Surg* 2001;21:17-21.
 37. Johnson JL, Jenkins NP, Huang WC, Di Gregoli K, Sala-Newby GB, Scholtes VP, Moll FL, Pasterkamp G, Newby AC. Relationship of MMP-14 and TIMP-3 expression with macrophage activation and human atherosclerotic plaque vulnerability. *Mediators Inflamm* 2014;2014:276457.

38. Lien LM, Hsieh YC, Bai CH, Chen WH, Chiu HC, Hsieh FI, Shyu KG, Chiou HY, Hsu CY. Association of blood active matrix metalloproteinase-3 with carotid plaque score from a community population in Taiwan. *Atherosclerosis* 2010;212:595-600.
39. Johnson JL, Dwivedi A, Somerville M, George SJ, Newby AC. Matrix metalloproteinase (MMP)-3 activates MMP-9 mediated vascular smooth muscle cell migration and neointima formation in mice. *Arterioscler Thromb Vasc Biol* 2011;31:e35-44.
40. Sluijter JP, Pulskens WP, Schoneveld AH, Velema E, Strijder CF, Moll F, de Vries JP, Verheijen J, Hanemaaijer R, de Kleijn DP, Pasterkamp G. Matrix metalloproteinase 2 is associated with stable and matrix metalloproteinases 8 and 9 with vulnerable carotid atherosclerotic lesions: a study in human endarterectomy specimen pointing to a role for different extracellular matrix metalloproteinase inducer glycosylation forms. *Stroke* 2006;37:235-9.

Review

Open Access



Percutaneous coronary intervention in the elderly: current updates and trends

Mohammed J. Arisha¹, Dina A. Ibrahim², Ahmed A. Abouarab³, Mohamed Rahouma³, Mohamed K. Kamel³, Massimo Baudo³, Kritika Mehta³, Mario F. L. Gaudino³

¹Division of Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, AL 35233, USA.

²Department of Medicine, Brown University, Providence, RI 01912, USA.

³Department of Cardiothoracic Surgery, Weill Cornell/New York Presbyterian Hospital, New York, NY 10065, USA.

Correspondence to: Dr. Mario F. L. Gaudino, Department of Cardiothoracic Surgery, Weill Cornell Medicine, 525 East 68th St., Suite M 404, New York, NY 10065, USA. E-mail: mfg9004@med.cornell.edu

How to cite this article: Arisha MJ, Ibrahim DA, Abouarab AA, Rahouma M, Kamel MK, Baudo M, Mehta K, Gaudino MFL. Percutaneous coronary intervention in the elderly: current updates and trends. *Vessel Plus* 2018;2:14. <http://dx.doi.org/10.20517/2574-1209.2018.29>

Received: 8 May 2018 **Accepted:** 5 Jul 2018 **Published:** 9 Jul 2018

Science Editors: Aaron S. Dumont, Alexander D. Verin **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Ischemic heart disease is the leading cause of death with acute coronary syndrome accounting for more than 30% of causes of mortality in the elderly population. The rate of growth of the older segment of the population has increased exponentially and will become more pronounced in the future. Historically, there has been a paucity of clinical trials investigating the challenges and outcomes of more invasive treatment strategies such as percutaneous coronary intervention (PCI) for that very segment of the population. However, the safety, efficacy, and outcomes of PCI in the older population have started to receive more attention, leading to some changes in their trends. There are several factors that make interventional cardiologists more resistant to direct the elderly to PCI. Most of these challenging factors, such as the complexity of coronary lesions, frailty, hematological and vascular changes, are discussed in this review. In addition, more advanced technologies have been introduced to PCI platform such as second- and third-generations stents, several alternative approaches have been adopted like transradial approach and the usage of bivalirudin instead of heparin and GP IIb/IIIa inhibitor, and several imaging modalities have been optimized to assess patients' outcome and prognosis more accurately. Several recent studies have shown better results when these strategies are adopted. The most recent recommendations regarding performing PCI in the elderly are also discussed in this review.

Keywords: Percutaneous coronary intervention, coronary artery disease, acute coronary syndrome, coronary stents, angioplasty, elderly, old age patients, frail patients, high risk patients



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



INTRODUCTION

Ischemic heart disease is one of the major challenges that encounter healthcare providers all over the world. It is considered the leading cause of death with acute coronary syndrome (ACS) accounting for more than 30% of causes of mortality in the elderly population (aged 65 years or older)^[1]. The elderly group of population has grown substantially. For many reasons, the rate of growth has increased exponentially and will become more pronounced in the future, especially in the developed world. In 1970, the population aged 65 years and older constituted only 9.8% of the total population in the United States, however, in 2012 it increased to 13.7% and it is expected to exceed 20% by the year 2030. Octogenarians and older populations constituted a smaller segment of only 3.7% comparing to their younger counterparts in 2012 but it is also expected to jump up to 3.9% and 5.4% by 2020 and 2030, respectively^[2]. Despite the recent advancements that have been achieved in both clinical and interventional cardiology realms, the management of coronary artery disease (CAD) in the elderly is still a major concern, both for cardiac interventionists and surgeons. Historically, older patients often receive conservative management rather than invasive procedures and there is a paucity of clinical trials investigating the challenges and outcomes of more invasive treatment strategies for that very segment of the population. Therefore, this relative under-representation of elderly in clinical trials and the consequent lack of knowledge made many cardiology interventionists more reluctant to perform percutaneous coronary intervention (PCI) for very elderly patients which hinders their optimal evidence-based therapy^[3]. Recently, safety and outcomes of PCI in the older population has started to receive more attention, therefore, changes in its trends have to be studied thoroughly. In this review, we discuss age and its impact on older patients' stratification and prognosis, the most relevant challenges that make PCI more difficult in this group of patients, recent changes in trends of PCI in the elderly, and the latest guidelines and recommendations.

AGE AND PCI

There is no specific age beyond which PCI cannot be performed, however, with increasing age less invasive therapy is usually preferred. In the literature, even a few centenarians underwent successful PCI procedures^[4,5]. The oldest reported case was a 106-year-old lady who presented with inferior wall ST-segment elevation acute myocardial infarction (STEMI)^[5]. It is also difficult to assign a clear-cut age threshold to classify patients based on their ages as risky vs. non-risky patients. However, according to the data from the Global Registry of Acute Coronary Events (GRACE), patients aged 75 years or more had more cardiovascular risk factors such as history of congestive heart failure (CHF), myocardial infarction (MI), hypertension, atrial fibrillation, diabetes mellitus, and stroke comparing to younger patients^[3]. Also, patients aged 75 years and older were considered a special group in the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) guidelines^[6,7]. Thus, in this review, we define risky old patients as patients aged 75 or more.

Age of patients presenting with ACS has a significant prognostic value and it is considered the second most important predictor of mortality after Killip class as it has been shown that the in-hospital mortality risk of a patient with ACS increases by 1.7 fold for each 10 years and by 2 folds for each Killip class deterioration^[8]. Risk stratification plays a crucial and decisive rule during the initial management of ACS patients, as it helps to determine the appropriate site of care and the intensity of therapy. Age among other patients' demographic characteristics profoundly affects this stratification as well as the initial estimate of death and/or other cardiac events even before performing any physical examination or reviewing electrocardiograms (ECG) and laboratory results^[6]. This is why, age is usually a vital criterion in several scoring systems that are used to estimate the in-hospital, 30 days, and even 1-year mortality rates of patients presenting with ACS and risk of complications as well. Among these scoring systems, the GRACE risk score^[9,10], the thrombolysis in myocardial infarction (TIMI) risk score^[11,12], and the "platelet IIb/IIIa in unstable angina: receptor suppression using integrilin therapy" (PURSUIT) risk score^[13]. According to the GRACE score, age of more than 75 adds a mortality risk score of 73. For TIMI score, age of more than 65 adds a 5% risk at 14 days of: all-cause mortality, new or recurrent MI, or severe recurrent ischemia requiring urgent revascularization.

As far as the PURSUIT score, age of 70 or more adds a mortality only risk of 4 points and a risk of mortality and infarction of 11 points.

PCI CHALLENGES IN OLDER POPULATION

Providing proper management and rehabilitation for older patients could be very challenging. Certainly, this becomes more sophisticated if a more invasive procedure such as PCI is required. Factors that make interventional cardiologists more resistant to perform PCI for an elderly patient can be either general factors related to the patient's general status such as frailty, co-morbidities, functionalities of their cardiovascular and other systems or local factors related to coronary lesions such as the complexity of these lesions. Here, we discuss the most relevant factors in more details.

FRAILITY AND MULTI-MORBIDITY

Frailty is often defined as gradual insufficiency and regress of multiple body systems that eventually lead to an ultimate state of low reserve, functional/ cognitive decline, and inability to cope with different stressors. It is also considered by geriatricians to be a clinical syndrome that makes patients vulnerable to a variety of adverse outcomes^[14]. Frailty becomes more apparent with aging, and unfortunately, even with the best healthcare and interventions provided to the elderly in order to support, improve, and maintain their quality of life, frailty is usually inevitable at a certain point of their age^[15]. Based on the analysis of data from 4735 out of 5888 participants of the Cardiovascular Health Study (CHS), the mean ages of non-frail, intermediate state, and frail patients were 71.5, 73.4, and 77.2 years, respectively. The same study demonstrated a higher prevalence of cardiac risk factors such as CHF, history of angina, MI, peripheral vascular disease (PVD), and carotid stenosis in frail patients^[16]. Some inflammatory markers such as C-reactive protein (CRP) and some clotting factors like factor VIII and fibrinogen were found to be in higher levels in frail patients compared to non-frail ones, suggesting that the high prevalence of some PCI adverse outcomes such as thrombotic complications in the aging frail population can be explained by an inflammatory process yet to be understood^[17]. With aging, a variety of cardiac and non-cardiac morbidities usually exist concurrently with the patients' coronary problems which makes it even more difficult for them to suit such procedures and to overcome any ominous adverse event. In the United States, the prevalence of cardiac diseases, hypertension, stroke, chronic obstructive pulmonary disease (COPD), kidney diseases, arthritis, and a lot of cancers is higher among the population aged 75 and older more than any other age group^[18].

POLYPHARMACY

With the increased prevalence of different morbidities among older patients, being on multiple medications at the same time is an expected consequence. Polypharmacy is more pronounced in the geriatric population and it makes patients more prone to many cardiac events which makes deciding PCI for them more unlikely. Data collected from 384 old frail patients participated in the Geriatric Evaluation and Management (GEM) Drug Study revealed that more than 40% of the participants were on 5 to 8 different medications and more than 37% had even more than 8 medications at the time of their discharge^[19]. In a prospective cohort study on old aged men with a mean age of 77 years, polypharmacy was associated with poor cardiovascular events such as MI and stroke with a statistically significant hazard ratio of 1.09 (95% CI: 1.06-1.12)^[20]. In addition to that, many factors associated with advanced age such as the decrease in renal function, low glomerular filtration rate, decreased liver mass and blood flow can alter many drugs pharmacokinetics and reduce their hepatic and renal elimination predisposing patients to more adverse events^[21]. Another challenge that could be faced during dealing with any elderly who needs PCI, is adjusting the dose of their cardiac medications as changes in water-fat balance in their body composite affect drugs distribution and dosing to a significant extent. Older patients have a lower total body water that leads to a lower volume of distribution and a higher serum level of water soluble medications such as digoxin that necessitate reduction of its loading

dose. In contrary, relative increase in total body fat resulted from reduction of adipose-free body mass in elderly people leads to increased volume of distribution of fat-soluble medications such as lidocaine and prolongation of their half-life^[21]. Another issue is that the recommended use of dual antiplatelet therapy (DAPT) in the elderly PCI patients has many additional complications which may influence the choice of the stent and the mode of management of these patients, including; higher risk of bleeding, need for concomitant warfarin therapy for atrial fibrillation, the increased likelihood of having future non-cardiac surgery, and the increased risk of falls^[13]. When added to therapy, anti-coagulants dosing is also altered with advanced age. It has been shown that old age is usually associated with a lower warfarin maintenance dose with patients aged 80 to 89 years usually requiring only half of a total weekly dose (TWD) of warfarin compared to patients aged between 20 and 59 years^[22].

HEMATOLOGICAL AND VASCULAR CHANGES

A lot of changes that occur with aging may cause the elderly to paradoxically experience hemorrhagic or thrombotic complications after PCI. Age is a significant independent predictor of major bleeding in ACS patients who had PCI and it is associated with higher in-hospital mortality rates^[23]. Case fatality has been shown to be more than 18% in patients who experienced any major bleeding following PCI while 5% in patients without major bleeding^[23]. Interestingly, thrombotic complications such as stent thrombosis and restenosis occurred more frequently with advanced age as well. In a previous study, 47% of all patients aged 75 years and older who had PCI with stenting experienced a 50% or more restenosis at the stent site or adjacent to it compared to only 28% in younger patients. Also, older patients experienced more diffuse restenosis (1 cm or more in length) than their younger counterparts^[24]. Increased risk of bleeding in elderly can be explained by several hematological alterations such as higher level of tissue plasminogen activator (tPA)^[25], lower platelets aggregation^[26], and the presence of more advanced and complicated vascular disease with more local changes, more atherosclerosis and hypertension^[23]. In contrary, older people have a higher blood viscosity, higher activity of several coagulation factors, and a lower fibrinolytic activity as it has been proven that plasminogen activator inhibitor (PAI-1) level increases with age^[27,28]. These changes cause a prothrombotic state in older patients that potentially increases risk of post-PCI thrombotic complications as well. In addition, aging is associated with impairment of vascular structure and endothelial function caused by several interacting histological and molecular alterations such as increased collagen content, smooth muscle changes, and altered composition of the extracellular matrix of the arterial wall. This can lead to a gradual decrease in elastic fibers, arterial wall rigidity, and increased risk of atherosclerosis and arterial thrombosis^[28]. Many PCI-related vascular complications such as large hematomas in femoral regions, pseudoaneurysms, and arteriovenous fistulas have been also associated with advanced age^[29]. Also, the gradual impairment of endothelial cells function that occur with aging leads to lower production of nitric oxide and prostacyclin which play an important role in promoting vasodilatation as well as preventing platelets aggregation^[28,30].

CORONARY LESIONS COMPLEXITY

Older patients usually have more complex and advanced coronary lesions which make PCI procedures more difficult with a higher risk of complications and a lower chance of procedure success. Batchelor *et al.*^[31] compared the angiographic characteristics of 7472 octogenarian patients with 102,236 younger others undergoing PCI. Older patients had more left main coronary artery (LMCA) and proximal left anterior descending (LAD) lesions than patients aged 79 and younger, 7.3% vs. 5.7% ($P < 0.01$) and 24% vs. 20% ($P < 0.01$), respectively. In a different sample of patients, the angiographic characteristics based on the modified ACC/AHA criteria revealed that 863 out of 2551 (33.8%) patients aged less than 75 years had coronary lesions of type B1 or less, in contrast to only 37 out of 137 (27%) patients aged 75 years or more ($P = 0.002$). On the other hand, lesion types B2 and C were more prevalent in older than younger patients, 72% vs. 65% ($P = 0.002$), respectively^[24]. Older patients had a higher number of affected vessels as well, as 55% of patients aged 75 or more had 3 diseased coronary

vessels compared to only 24% of younger patients ($P = 0.0001$). Also, 22% of older patients *vs.* 38% of their younger counterpart had only 1 diseased vessel ($P = 0.0001$)^[24]. The Synergy between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery (SYNTAX) score has been used to predict clinical outcomes in patients undergoing PCI especially those with LMCA lesions and/or multivessel coronary disease based on their lesions complexity^[32]. More recently, this scoring system has been integrated with some independent clinical variables such as the patient's age, creatinine serum level, and left ventricular ejection fraction (LVEF) to obtain the clinical SYNTAX score (CSS)^[33]. Both scoring systems have been shown to be valid in risk stratification and early mortality prediction among older patients with ACS undergoing PCI. While the SYNTAX score did not predict long-term clinical outcomes, the CSS was useful in predicting the 1-year major adverse cardiac and cerebrovascular events, reflecting the potentially significant impact of the patient's clinical and demographic factors such as their ages on their clinical outcomes^[34]. Previous studies have also shown that age is significantly associated with increased coronary artery calcium score^[35,36]. Calcification of the coronary system is associated with coronary artery disease (CAD) and coronary artery calcium content is highly associated with increased cardiovascular events^[36].

PCI ADVERSE EVENTS

As a result of the previously mentioned factors, PCI outcome is expected to be worse in the older patients comparing to the general population. Indeed, the most devastating outcome would be death. Although studies have demonstrated reasonable short and long-term PCI outcomes in the elderly, the in-hospital, 30 days, and even 1 to 5 years follow-up all-cause mortality rates are still higher^[37-39]. Aside from death, there is a higher chance that older frail patients experience a variety of complications that can occur consequently as a result of this procedure and affect patients' clinical outcome and quality of life than other younger patients^[40]. Many cardiac complications have been described such as cardiogenic shock, acute MI, acute ventricular septal rupture (VSR), iatrogenic coronary dissection, coronary perforation, and stent thrombosis. Other non-cardiac complications have also been reported such as hemorrhage, acute kidney injury, stroke, and access site complications like femoral or radial dissection and/or hematoma^[41,42]. Major bleeding is one of the complications associated with unfavorable clinical outcome. Pooled data from 5 different trials that participated in the RESOLUTE study program and included 5130 patients undergoing PCI with the resolute zotarolimus-eluting stent showed that rates of some complications such as MI and repeat revascularization in 1675 patients aged 70 years or older (33%) were similar to those of younger participants, however, significant bleeding events occurred more frequently among older population. In-hospital and 1-month follow-up bleeding complications occurred in 1.3% and 1.6% of patients aged 70 years or older, and 0.3% and 0.5% of younger participants ($P = 0.009$ and 0.014), respectively. Death occurred in 26% of old patients who experienced bleeding events with a median time of 21 days between the bleeding event and time of death^[38]. Another study showed an increase of 2.4% in over-all rates of bleeding events among patients undergoing PCI in their octogenarian years than younger patients^[37]. Many different bleeding complications have been reported to be associated with PCI such as, access site bleeding, pericardial bleeding that can lead to tamponade, retroperitoneal bleeding, and gastrointestinal bleeding as well^[37]. Beside age of the patient, many other variables have been proven to be an independent predictor of unfavorable outcome in the elderly undergoing PCI. Reduced cardiac function with left ventricular ejection fraction (LVEF) lower than 40%, Killip class of 3 or worse, cardiogenic shock, and hypotension with systolic blood pressure (SBP) lower than 100 mmHg have all been identified as independent predictors of an increased risk of 1-year mortality^[43]. Also, the activity of daily living (ADL) of old age patients after PCI can be used to predict mortality. ADL assessment by Barthel index (BI) at the time of admission and discharge has been investigated by Higuchi *et al.*^[44] to predict 1-year mortality in very old patients undergoing PCI for ACS. They have shown that lower BI at the discharge of the patient can be a predictor of higher mortality in patients aged 85 years and older with each 5 unites decrease in BI being associated with 1.1 fold increase in 1-year mortality risk.

Table 1. Comparison of the predictive value of the CHA2DS2VASc score for stroke and atrial fibrillation in elderly patients based on gender

Elderly > 75 years old	Baseline score according to age and gender only	Score after adding a single additional risk factor	Target high risk score for atrial fibrillation	Stroke risk (%) at baseline score	Stroke risk (%) after adding a single additional risk factor	Maximum possible score
Male	2.0	3.0-4.0	≥ 3.0	2.2	3.2-4.0	8.0
Female	3.0	4.0-5.0	≥ 3.0	3.2	4.0-6.7	9.0

Aside from this, the CHA2DS2VASc score^[45] is a recognized predictor of the risk of having cerebrovascular episodes. Patients aged 75 or more are assigned 2 on the scale, making the elderly patients more likely to end up with higher scores. Adding the factor of gender, elderly females have a risk of 3 at baseline without adding the risk attributed to the other comorbidities on the scale and the PCI procedure. Interestingly, CHA2DS2VASc score of 2 or more was also found to be predictive of atrial fibrillation after cardiac procedures^[46]. Table 1 summarizes the significance of CHA2DS2VASc score in predicting stroke and atrial fibrillation in the elderly population.

PCI TEMPORAL TRENDS

Historically, there have been a lot of variations in the reported data regarding procedure outcomes and mortality rates among the older population undergoing PCI. Some of the papers published in the late eighties through nineties showed the success rate of percutaneous transluminal coronary angioplasty (PTCA) in the elderly to be approximately 82%-84% and highly variable mortality rates^[47-49], however, Both success and mortality rates have varied a lot in other papers published in the same era. Kern *et al.*^[50] reported in 1988 a clinical success rate of a 67% in a group of 21 patients who had undergone PTCA in their octogenarian years. After that, a clinical success rate of 57% was reported in 43 patients aged 75 years and older by other investigators^[51]. In contrary, Jeroudi *et al.*^[52] demonstrated PTCA angiographic and clinical success in 50 and 49 patients, respectively out of 54 octogenarian patients (93% and 91%, respectively). Procedure success has differed considerably between septuagenarians, octogenarians and older participants in the same group of old aged patients as it was 85%, 73%, and as low as 50% for patients aged 70 to 74, 75 to 79, and 80 years and older^[49]. Also, procedural mortality rate varied and reached up to 19% during the same era^[50,53]. Many cardiac and non-cardiac complications were reported and occurred in more than third of the participants in some of the previous studies^[47,50]. Although PCI has been proven to be feasible in the older population, the previous rates of success, procedural mortality, and consequent complications were unacceptable to many healthcare providers which created a high level of prudence and caution before deciding to perform PCI on such patients. Over years, several advanced technologies have been introduced, useful cardiac imaging modalities have been more available, less invasive approaches and protocols have been investigated to be adopted, and the operator techniques have been improved. As a result, a less conservative trend in performing more invasive procedures on older patients has gradually appeared. In a cohort of 31,758 patients who had undergone PCI between 2000 and 2007, the incidence of PCI in patients aged 75 and older has increased from 56/100,000 in 2000 to 216/100,000 in 2007^[54]. PCI share of older patients has increased even in the very old segment such as the nonagenarians. Among 26,696 PCI performed over 11 years, only 177 were performed on nonagenarians, however, the prevalence of PCI in this very subgroup of patients was 0.17% in 2004 and increased to 1.22% in 2014^[41]. Recent studies have also shown some changes in PCI mortality and complications trends. Generally, the success rate has improved, mortality and complications risk started to approach those of younger population. In a very recent study that was published in 2018, the outcomes of PCI in octogenarians and younger patients using second-generation cobalt-chromium everolimus-eluting stents were practically the same^[42]. Angiographic success was 98.4% in octogenarians and 98% in younger participants ($P = 0.85$). A lot of both in-hospital and 1-year follow-up post PCI-complications were also comparable between the 2 groups. In-hospital major bleeding events and cerebrovascular accidents have not occurred in either of the 2 groups, however, acute kidney injury occurred more frequently in octogenarians,

3.7% vs. 1.5% ($P = 0.58$). One-year follow-up myocardial infarction occurred in 1.9% and 1.5% ($P = 1.00$) in octogenarians and younger patients, respectively. Interestingly, some complications occurred less frequently with the octogenarian group than its younger counterpart such as in-hospital subacute stent thrombosis and 1-year follow-up cerebrovascular accidents, 0% vs. 1.5% ($P = 1.00$) and 1.9% vs. 2.3% ($P = 1.00$), respectively. Although recent studies have also shown some variations in their results, in 2017 many papers have reported relatively high PCI success rates in the elderly that ranged approximately from 75% to 95%^[55-57]. Many of these recent papers have reported better mortality and some complications trends than before^[42,55,58,59]. Some of them even reported similar rates of major adverse events in both young adults and elderly in more critical situations such as patients with atrial fibrillation (A-fib) undergoing PCI. Lahtela et al.^[60] conducted a *post-hoc* analysis of 925 A-fib patients' data from the atrial fibrillation undergoing coronary artery stenting (AFCAS) registry and showed comparable incidence of in-hospital and 1-month major adverse cardiac and cerebrovascular events (MACCE) in octogenarian patients and younger ones^[61]. It is worth mentioning that some recent studies still demonstrate a quite high adverse outcomes rates including in-hospital mortality that reached up to 20%^[62,63].

PROCEDURAL ASPECTS IN ELDERLY

Many aspects regarding PCI procedure in the elderly can be modified, adjusted, and tailored in order to make this very segment of the population more suitable for such a procedure and to render the interventional cardiologists more comfortable to decide to perform these procedures in older patients. Among these aspects are the length of procedure, the volume of contrast agent, the access site, the nature of the intervention, the type of the stent, and the length of hospital stay.

ACCESS SITE

Generally, transfemoral approach has been the traditional standard of care for many years, however, previous studies have shown that the newer transradial route is superior to the transfemoral one as the former has been associated with better results and lower rates of complications and it has been increasingly used instead of the femoral access in the general population^[64,65]. In terms of the use of this approach in the elderly undergoing PCI, the net benefit is still not totally clear. The differences between both approaches in older patients have been reported in some studies and they demonstrated a high percentage of old patients' PCI in whom transradial approaches have been performed. The transradial access has been used in up to almost 80% of old aged patients of some cohorts^[66,67]. In a 1:1 propensity score analysis of data from 1098 patients aged 75 years and older who underwent PCI with either transfemoral or transradial approach, lower rates of in-hospital and 1-year follow-up major adverse clinical events, in-hospital MI, access site complications, and major bleeding were associated with the use of transfemoral access^[66]. Other adverse clinical outcomes occurred less frequently with the transradial approach such as in-hospital death and target vessel revascularization but there were no significant statistical differences between the two groups. The same study demonstrated comparable rates of non-access site related major bleeding events with both approaches^[66]. In contrary, some recent studies have also shown a very low usage of the transradial approach with the elderly. Among 1945 octogenarian patients of the Korea Acute Myocardial Infarction Registry, 1609 participants (82.7%) underwent PCI using the transfemoral approach and only 336 (17.3%) with the transradial approach. Nevertheless, using the transradial access has been found to be a predictor of a lower in-hospital mortality in the same group of patients^[68]. In the same study, intra-aortic balloon pump (IABP) had to be used in 103 (6.4%) patients in the transfemoral group vs. only 5 (1.5%) patients of the transradial group. Access site can also affect other variables like the time and volume of the contrast agent and the length of hospital stay, as the transradial approach was shown to be associated with a shorter hospital stay and a lower dose of contrast dye comparing to transfemoral one among older patients^[67]. However, several other studies showed comparable contrast volume and procedure time with both approaches^[41,66]. In terms of procedural success, several studies demonstrated almost similar PCI success in elderly using

both approaches^[41,68], however, many investigators believe that older population can benefit more from the transradial approach and it should be used more often with the elderly undergoing PCI.

TYPE OF STENT

As it was discussed above, the coronary lesions in the elderly tend to be more complex and extensive which may render them suitable only for plain old balloon angioplasty (POBA) due to a failure of stent delivery, or inability to stent lesions in distal or small diameter vessels^[43]. On the other hand, stenting technology has revolutionized during the last era. Since their first successful clinical application in 2002, drug-eluting stents (DES) have been utilized more frequently comparing to bare metal stents (BMS) as lower rates of stent restenosis, major adverse cardiac events, and revascularization of target lesions have all been associated with the use of DES^[69]. The second-generation DES even have a better stent design than the first-generation ones with a thinner strut and more biocompatible polymers which lead to a higher efficacy and lower complications^[70,71]. However, the use DAPT for at least 1 year to prevent stent thrombosis associated with DES raises concerns regarding increased risk of bleeding especially in populations with an already high risk of bleeding such as the elderly^[70]. Although some studies suggested reducing the DAPT to 3-6 months without an increase in the risk of many adverse clinical events^[72]. Recent data still suggesting under-utilization of DES in elderly patients undergoing PCI with stenting^[70]. The characteristics and clinical outcomes of 1564 high bleeding risk old patients aged 75 years or older who participated in the LEADERS FREE trial and underwent PCI with the deployment of either polymer-free DES or similar BMS and only 1 month of DAPT were analyzed^[73,74]. They showed a high yet similar bleeding rate in the 2 groups. However, rates of mortality, stent thrombosis, MI, and target lesion revascularization were lower in patients underwent stenting with DES reflecting superior safety and efficacy benefits compared to BMS^[74]. In addition to that, major bleeding rates did not differ significantly between octogenarian patients who received PCI with BMS and only 1-month mandatory DAPT and others with DES and a 1-year course of DAPT in the XIMA trial^[75]. Also, compared to the first-generation DES, the use of second-generation DES has been associated with better outcomes in the older population, as the latter has been associated with a lower risk of MI in the following year among patients aged 70 years or older with a hazard ratio of 0.40 (95% CI: 0.19-0.82); $P = 0.012$ ^[58]. Most recently, the SENIOR trial demonstrated lower rates of the 1-year all-cause mortality, MI, stroke, and revascularization in elderly patients who underwent PCI and received third-generation DES with bioabsorbable polymer and a short-term DAPT compared to those who received BMS^[76]. In the same trial, the duration of DAPT was decided before patients' random assignment to the two different types of stents and it was recommended to be 1 month for stable patients and 6 months for unstable ones, however, the bleeding complications were comparable in both study arms.

BLEEDING AVOIDANCE STRATEGIES

With peri-procedural bleeding being one of the most concerning topics regarding PCI in elderly patients^[23], several approaches and strategies have been developing aiming at reducing the amount of blood loss and improving the safety and efficacy of these procedures in populations of high risk. Bleeding avoidance strategies (BAS) include the usage of vascular closure devices (VCD), transradial approach instead of the transfemoral, and bivalirudin instead of heparin and GP IIb/IIIa inhibitor^[77]. Previous data showed that BAS have been associated with lower risk of Peri-PCI bleeding, nevertheless, these strategies are underutilized among patients with higher risk of bleeding suggesting what we call "risk-treatment paradox"^[77,78]. Khambatta *et al.*^[79] evaluated the data of 124,606 patients with different ages who underwent PCI over a period over 4 years to study the effect of BAS on rates of bleeding and other variables in different age groups. They have demonstrated a lower incidence of bleeding with the utilization of BAS with an adjusted odds ratio of 0.982 (95% CI: 0.980-0.984) compared to those without BAS usage in all age groups even patients older than 80 years. BAS was also associated with lower in-hospital mortality with an adjusted odds ratio of 0.993 (95% CI: 0.992-0.994). Interestingly, although the overall usage of BAS has been improving in all age groups over the whole study period, their utilization was still less frequent in old age patients^[79].

TIME OF INTERVENTION

There are some conflicting data regarding the perfect timing of PCI in patients presenting with STEMI, however, several previous studies have shown that shorter door-to-balloon time (DTBT) is associated with better outcomes and many investigators believe that attempts to avoid any DTBT delay should be adopted regardless the patient's baseline risks^[80,81]. Optimally, reperfusion should be attained within the recommended 90-minute window, however, many patients still undergo PCI beyond this time limit^[82]. DTBT has been shown to be longer among older populations compared to their younger counterparts in several recent studies^[57,62,79]. The median DTBT in a cohort of 2972 consecutive patients who underwent primary PCI for STEMI was 70, 76, and 80 min for patients aged < 75, 75 to 84, and ≥ 85 years, respectively ($P < 0.001$)^[57]. Being an old age patient *per se*, has been shown to be an independent predictor for the door-to-balloon delay^[83]. Other predictors have been described such as non-daytime presentation, the absence of typical chest pain, the need for hospital transfer, female sex, and non-white race^[83]. Elderly patients with ACS often present with non-specific and atypical symptoms like nausea, vomiting, diaphoresis, and dyspnea. Chest pain occurs only in approximately 40% of patients older than 85 years^[84]. Also, the higher prevalence of left bundle branch block (LBBB) in older population patients makes the electrocardiographic diagnosis of STEMI more difficult^[84]. These unusual presentations of ACS and time-wasting factors may cause some sort of delayed diagnosis and management which consequently leads to worse outcomes in elderly undergoing PCI that must be investigated thoroughly in the near future.

AUXILIARY CARDIAC IMAGING UTILIZATION

Over the last years, we have witnessed dramatic advancements in the field of cardiac imaging. Several imaging modalities such as transesophageal echocardiography (TEE), cardiac computed tomography (CT), cardiac magnetic resonance imaging (CMR), and intravascular ultrasonography (IVUS) have been evolving and their usefulness in the diagnosis and assessment of a variety of clinical and/or surgical situations has been studied^[85,86]. We believe that the optimal utilization of several cardiac imaging modalities can provide an additive benefit to the patients undergoing PCI, especially among older populations. Moreover, the integration of clinical and imaging data can assess patients' prognosis and predict their clinical outcomes. It also can define and classify several procedural complications and guide healthcare providers to decide whether to adopt a conservative management strategy or to proceed with more aggressive options^[87]. For instance, intra-operative TEE has been used to guide several procedures in the catheterization theater and it provided useful assessments to some age-related PCI complications such as iatrogenic aortic intramural hematomas and helped to assess the patient's outcome and to decide the best management^[87]. Furthermore, with the latest developments of three-dimensional TEE, more accurate intra-operative images of stent scaffolds can be obtained which enabled some investigators to more confidently diagnose and assess some PCI complications like LMCA stent protrusion and migration^[88,89]. Thus, and as we mentioned above, with the older populations usually having more frequent LMCA lesions than younger patients, they can benefit from this modality. Also, IVUS and optical coherent tomography (OCT) have been shown to be useful in the qualitative assessment and preparation of LMCA lesions and in stent sizing and optimization as well^[90,91]. In addition to that, post-PCI risk stratification has been proven to be helpful in evaluating STEMI patients' prognosis. The use of CMR during the hyperacute phase of STEMI after primary PCI has been shown to be safe and feasible^[92]. Although the value of most of these imaging modalities in the elderly undergoing PCI as a separate high-risk group has not been investigated in large scales studies, many of them may provide useful contributions during the management of these patients in the future.

DIFFERENT INDICATIONS OF PCI IN ELDERLY

It has been shown that PCI was indicated for older population in a wide spectrum of different clinical situations with variable disease severities, from primary PCI in unstable old patients with STEMI and urgent PCI for those presenting with non-STEMI and unstable angina pectoris to elective PCI for old patients with stable CAD. Many previous studies have demonstrated that older patients who underwent PCI for unstable

ACS constituted the bigger portion of the total elderly underwent PCI. Among 102 CAD patients aged 85 years and older, PCI was indicated in an ACS setting in 72.6% of them and only 24.5% of PCIs were performed for patients with stable angina^[93]. In another cohort, 93% of 177 PCI performed on nonagenarians were indicated in ACS settings and only 7% were elective PCI^[41]. It is worth mentioning that clinical presentation and PCI indication are considered significant determinants of post-PCI clinical outcomes. As it has been shown that short and long-term clinical outcomes are usually superior in older patients who underwent PCI for stable CAD compared to unstable patients of the same age^[93,94]. Among 102 PCI performed for a variety of indications in very old patients, aged ≥ 85 years, there were 4 in-hospital deaths, all of them were patients presented to PCI due to acute STEMI. However, there were no deaths among very old patients from the same age group in whom PCI was indicated for stable coronary syndromes, post-STEMI, and other indications^[93]. Teplitsky *et al.*^[95] reported a zero percent cumulative mortality rate at 6-month after elective PCI performed for nonagenarian patients with stable CAD. The 6-month cumulative mortality rate in patients underwent emergent PCI for clinically unstable ACS was 23% in the same study.

PCI AMONG OTHER REVASCULARIZATION AND REPERFUSION STRATEGIES IN ELDERLY

Generally, PCI is the most commonly used reperfusion strategy among all age groups^[96]. In terms of revascularization in older patients with CAD, a variety of strategies have been utilized, from conservative management with no revascularization at all to the most invasive surgical revascularization. However, the decision to choose the best reperfusion strategy for this high-risk group of patients has never been simple. Peiyuan *et al.*^[97] have compared the clinical outcomes of 3 groups of 3082 STEMI patients aged 75 years and older. Reperfusion by PCI was performed in 1000 patients, fibrinolysis was administered to 160 patients, and the third group of 1922 patients did not have reperfusion therapy. PCI group had a significant lower mortality rates than fibrinolysis and no reperfusion groups of 7.7%, 15%, and 19.9%, respectively, $P < 0.001$. Several adverse outcomes such as recurrent MI and MI-related complications like heart failure and cardiac arrest occurred less frequently in PCI group. Other previous studies from different populations have demonstrated better clinical outcomes in patients underwent PCI compared to those whom received fibrinolysis in all age groups including the elderly^[98,99]. A meta-analysis of 22 randomized trials that included 6763 patients, also showed higher death and adverse outcomes rates of patients whom received fibrinolysis compared to primary PCI group among all ages except patients aged 50 years and younger^[99]. Another strategy that involves combining fibrinolysis to urgent PCI can be potentially beneficial for elderly patients with ACS. The pre-hospital administration of a reduced-dose fibrinolytic agent before urgent PCI, termed FAST-PCI, showed better 30-day mortality rates than primary PCI alone, 4.2% vs. 18.1%, respectively, $P < 0.01$ in STEMI patients aged 75 years and older without an increase in rates of major bleeding events, stroke, or reinfarction^[100]. Despite that many health care providers are still hesitant to direct old patients toward PCI, it has been shown that with aging, the frequency of PCI increases and that of coronary artery bypass grafting (CABG) decreases. Nicolini *et al.*^[101] compared the clinical outcomes and adverse events between PCI and CABG in a cohort of patients aged 80 years and older with multivessel disease or LMCA lesions. PCI was performed in 947 patients, while 441 underwent CABG. It is worth mentioning that, all nonagenarian patients included in the PCI group. Although the 1-month mortality rates of both study arms were comparable, many adverse outcomes were more frequent among patients who underwent PCI in the follow-up period such as cardiac mortality, MI, and target vessel revascularization. In another larger cohort of 10,141 patients aged 85 years or older with ACS and multivessel CAD, CABG was more frequently performed compared to PCI, and it was associated with better survival and freedom from composite morbidity at 3 years follow-up^[102]. Thus, it appears that CABG is still the best strategy of revascularization in patients with multivessel disease or LMCA lesions even in elderly.

RECOMMENDATIONS

Several foundations and societies such as the ACCF, AHA, European Society of Cardiology (ESC), and the Society for Cardiovascular Angiography and Interventions (SCAI) have tried to adopt some guidelines and

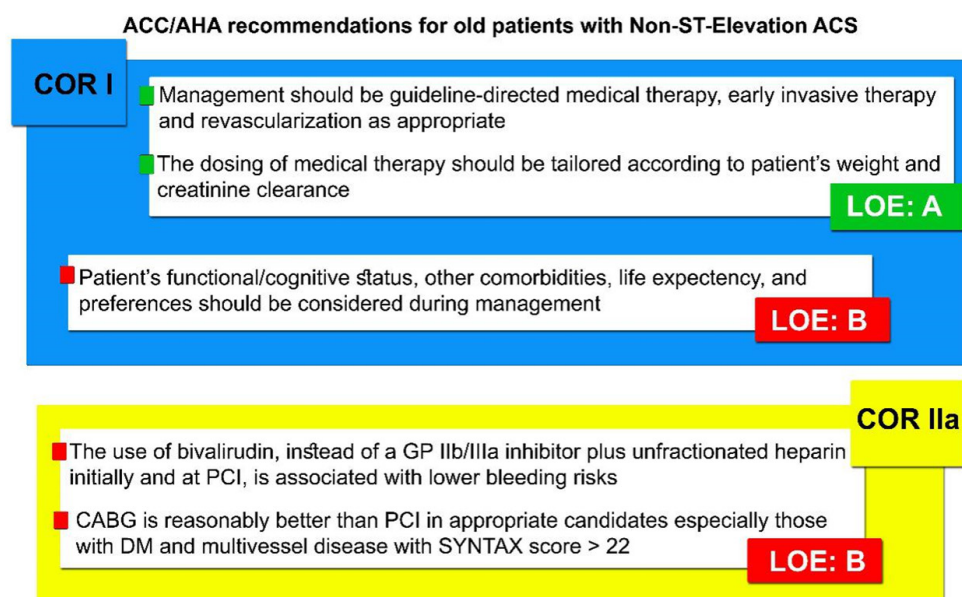


Figure 1. Summary of the 2014 AHA/ACC recommendations for the management of old patients (≥ 75 years of age) with non-ST-elevation acute coronary syndromes. ACC: American College of Cardiology; AHA: American Heart Association; ACS: acute coronary syndrome; CABG: coronary artery bypass graft; COR: class of recommendation; LOE: level of evidence; DM: diabetes mellitus; PCI: percutaneous coronary intervention

recommendations based on the latest available clinical trials and observational studies in order to make the decision of performing PCI on older patients clearer and more evidence-based. The management of older population with CAD should be patient-centered and the decision whether to direct the patient toward reperfusion therapy or to adopt a less invasive and more conservative management should not be taken solely based on the patient's age. On the other hand, the patient's preferences, life expectancy, all his other co-morbidities, and functional status should be considered before denying or recommending PCI. A report from the ACCF, AHA, and SCAI stated that the PCI clinical benefits in younger and older population are comparable. However, the increased risk of some adverse outcomes in elderly like bleeding events and stroke should be taken into consideration^[103]. The latest ESC guidelines for management of patients presenting with acute STEMI have also emphasized maintaining a high level of suspicion when dealing with any elderly presenting with atypical symptoms to avoid any delay in the diagnosis and reperfusion therapy^[104]. Primary PCI should not have an upper age limit and any patient can be qualified for PCI based on his individual circumstances. The transradial approach was also recommended whenever it is possible in these patients. In addition, dosing of thrombolytic therapy should be adjusted carefully according to the patients' kidney function, other medications, and comorbidities. The ACCF and AHA have also recommended the use of bivalirudin, instead of a GP IIb/IIIa inhibitor plus unfractionated heparin, both initially and at PCI in elderly presenting with non-ST segment elevation ACS, as the former is associated with lower bleeding risks. However, the dosing of all the medical therapy must be modified according to the patients' body weight and creatinine clearance^[6]. They have also stated that CABG can be preferred over PCI for appropriate candidates, especially those with diabetes mellitus and multivessel disease with SYNTAX score of more than 22 [Figure 1].

CONCLUSION

There are several factors that render PCI a more challenging procedure among the elderly such as more complex coronary lesions, co-morbidities, frailty, and hematological alterations. Historically, PCI clinical outcomes have been demonstrated to be worse among older populations compared to their younger counterparts. Moreover, the participation of the elderly in the clinical trials that investigated different aspects of PCI has been markedly under-represented which created a vague state of decision making capability that

consequently could hinder the optimal treatment for many old patients. Recently, more advanced technologies have been introduced to interventional cardiology platforms, a variety of medical therapy options have been available, less invasive PCI approaches have been adopted, more advanced cardiac imaging modalities have been improving, and more attention to elderly undergoing PCI has been given. Although that PCI mortality rates are still practically higher among the elderly, many recent studies demonstrated that PCI is safe and effective for this segment of the population and some adverse clinical outcomes became similar to those occurring in younger patients. Thus, the decision to perform PCI should not merely rely on the patient's age. Instead, many other considerations should be taken into account such as the patient's functional and/or cognitive status, preferences, co-morbidities, current medications, and life expectancy. In addition, more elderly must be a part of the future clinical trials and the safety and efficacy of all the available as well as the emerging less invasive PCI strategies have to be investigated more thoroughly in order to provide a clearer knowledge regarding the optimal utilization of PCI among the elderly.

DECLARATIONS

Authors' contributions

Design: Arisha MJ, Rahouma M, Baudo M

Organization of the manuscript: Rahouma M, Baudo M

Literature search: Arisha MJ, Ibrahim DA, Abouarab AA, Mehta K

Manuscript writing: Arisha MJ, Ibrahim DA, Abouarab AA, Kamel MK

Figures arrangement: Arisha MJ, Kamel MK

References arrangement: Mehta K

Read and approved the final manuscript: Ibrahim DA, Rahouma M, Baudo M, Gaudino MFL

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Alexander KP, Newby LK, Cannon CP, Armstrong PW, Gibler WB, Rich MW, Van de Werf F, White HD, Weaver WD, Naylor MD. Acute coronary care in the elderly, part I: non-ST-segment-elevation acute coronary syndromes: a scientific statement for healthcare professionals from the American Heart Association Council on Clinical Cardiology: in collaboration with the Society of Geriatric Cardiology. *Circulation* 2007;115:2549-69.
2. Ortman JM, Velkoff VA, Hogan H. An aging nation: the older population in the United States. United States Census Bureau, Economics and Statistics Administration, US Department of Commerce; 2014.
3. Avezum A, Makdisse M, Spencer F, Gore JM, Fox KA, Montalescot G, Eagle KA, White K, Mehta RH, Knobel E. Impact of age on

- management and outcome of acute coronary syndrome: observations from the Global Registry of Acute Coronary Events (GRACE). *Am Heart J* 2005;149:67-73.
4. Aksoy S, Velibey Y, Koroglu B, Cagdas M, Guzelburc O, Cam N, Eren M. Successful primary percutaneous coronary intervention in a centenarian patient with acute myocardial infarction: online article-case report. *Cardiovasc J Afr* 2014;25:8-10.
 5. Cloutier JM, Zieroth S, Elbarouni B. Primary percutaneous coronary intervention as treatment for ST-elevation myocardial infarction in a centenarian: choosing carefully. *Can J Cardiol* 2017;33:1066-e1.
 6. Amsterdam EA, Wenger NK, Brindis RG, Casey DE, Ganiats TG, Holmes DR, Jaffe AS, Jneid H, Kelly RF, Kontos MC. 2014 AHA/ACC guideline for the management of patients with non-ST-elevation acute coronary syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014;64:e139-228.
 7. Levine GN, Bates ER, Bittl JA, Brindis RG, Fihn SD, Fleisher LA, Granger CB, Lange RA, Mack MJ, Mauri L. 2016 ACC/AHA guideline focused update on duration of dual antiplatelet therapy in patients with coronary artery disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Thorac Cardiovasc Surg* 2016;152:1243-75.
 8. Granger CB, Goldberg RJ, Dabbous O, Pieper KS, Eagle KA, Cannon CP, Van de Werf F, Avezum A, Goodman SG, Flather MD. Predictors of hospital mortality in the global registry of acute coronary events. *Arch Intern Med* 2003;163:2345-53.
 9. GRACE Investigators. Rationale and design of the GRACE (Global Registry of Acute Coronary Events) Project: a multinational registry of patients hospitalized with acute coronary syndromes. *Am Heart J* 2001;141:190-9.
 10. Abu-Assi E, Ferreira-González I, Ribera A, Marsal JR, Cascant P, Heras M, Bueno H, Sánchez PL, Arós F, Marrugat J. Do GRACE (Global Registry of Acute Coronary events) risk scores still maintain their performance for predicting mortality in the era of contemporary management of acute coronary syndromes? *Am Heart J* 2010;160:826-34.
 11. Antman EM, Cohen M, Bernink PJ, McCabe CH, Horacek T, Papuchis G, Mautner B, Corbalan R, Radley D, Braunwald E. The TIMI risk score for unstable angina/non-ST elevation MI: a method for prognostication and therapeutic decision making. *JAMA* 2000;284:835-42.
 12. Antman EM, McCabe CH, Gurfinkel EP, Turpie AG, Bernink PJ, Salein D, de Luna AB, Fox K, Lablanche JM, Radley D. Enoxaparin prevents death and cardiac ischemic events in unstable angina/non-Q-wave myocardial infarction: results of the Thrombolysis In Myocardial Infarction (TIMI) 11B trial. *Circulation* 1999;100:1593-601.
 13. Boersma E, Pieper KS, Steyerberg EW, Wilcox RG, Chang WC, Lee KL, Akkerhuis KM, Harrington RA, Deckers JW, Armstrong PW. Predictors of outcome in patients with acute coronary syndromes without persistent ST-segment elevation: results from an international trial of 9461 patients. *Circulation* 2000;101:2557-67.
 14. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001;56:M146-57.
 15. Guinan P. Frailty and old age. *Linacre Q* 2016;83:131-3.
 16. Newman AB, Gottdiener JS, McBurnie MA, Hirsch CH, Kop WJ, Tracy R, Walston JD, Fried LP. Associations of subclinical cardiovascular disease with frailty. *J Gerontol A Biol Sci Med Sci* 2001;56:M158-66.
 17. Walston J, McBurnie MA, Newman A, Tracy RP, Kop WJ, Hirsch CH, Gottdiener J, Fried LP. Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study. *Arch Intern Med* 2002;162:2333-41.
 18. Blackwell DL, Lucas JW, Clarke TC. Summary health statistics for U.S. adults: national health interview survey, 2012. *Vital Health Stat* 2014;(260):1-161.
 19. Hajjar ER, Hanlon JT, Sloane RJ, Lindblad CI, Pieper CF, Ruby CM, Branch LC, Schmader KE. Unnecessary drug use in frail older people at hospital discharge. *J Am Geriatr Soc* 2005;53:1518-23.
 20. Beer C, Hyde Z, Almeida OP, Norman P, Hankey GJ, Yeap BB, Flicker L. Quality use of medicines and health outcomes among a cohort of community dwelling older men: an observational study. *Br J Clin Pharmacol* 2011;71:592-9.
 21. Mangoni AA, Jackson SH. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. *Br J Clin Pharmacol* 2004;57:6-14.
 22. Khoury G, Sheikh-Taha M. Effect of age and sex on warfarin dosing. *Clin Pharmacol Adv Appl* 2014;6:103.
 23. Moscucci M, Fox KA, Cannon CP, Klein W, López-Sendón J, Montalescot G, White K, Goldberg RJ. Predictors of major bleeding in acute coronary syndromes: the Global Registry of Acute Coronary Events (GRACE). *Eur Heart J* 2003;24:1815-23.
 24. De Gregorio J, Kobayashi Y, Albiero R, Reimers B, Di Mario C, Finci L, Colombo A. Coronary artery stenting in the elderly: short-term outcome and long-term angiographic and clinical follow-up. *J Am Coll Cardiol* 1998;32:577-83.
 25. Tofler GH, Massaro J, Levy D, Mittleman M, Sutherland P, Lipinska I, Muller JE, D'Agostino RB. Relation of the prothrombotic state to increasing age (from the Framingham Offspring Study). *Am J Cardiol* 2005;96:1280-3.
 26. Knight CJ, Panesar M, Wright C, Clarke D, Butowski PS, Patel D, Patrinely A, Fox K, Goodall AH. Altered platelet function detected by flow cytometry: effects of coronary artery disease and age. *Arterioscler Thromb Vasc Biol* 1997;17:2044-53.
 27. Becker RC. Thrombotic preparedness in aging: a translatable construct for thrombophilias? *J Thromb Thrombolysis* 2007;24:323-5.
 28. Franchini M. Hemostasis and aging. *Crit Rev Oncol Hematol* 2006;60:144-51.
 29. Popma JJ, Satler LF, Pichard A, Kent KM, Campbell A, Chuang YC, Clark C, Merritt AJ, Bucher TA, Leon MB. Vascular complications after balloon and new device angioplasty. *Circulation* 1993;88:1569-78.
 30. Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, Salvetti A. Age-related reduction of NO availability and oxidative stress in humans. *Hypertension* 2001;38:274-9.

31. Batchelor WB, Anstrom KJ, Muhlbaier LH, Grosswald R, Weintraub WS, O'Neill WW, Peterson ED. Contemporary outcome trends in the elderly undergoing percutaneous coronary interventions: results in 7,472 octogenarians. *J Am Coll Cardiol* 2000;36:723-30.
32. Valgimigli M, Serruys PW, Tsuchida K, Vaina S, Morel MA, van den Brand MJ, Colombo A, Morice MC, Dawkins K, de Bruyne B. Cyphering the complexity of coronary artery disease using the syntax score to predict clinical outcome in patients with three-vessel lumen obstruction undergoing percutaneous coronary intervention. *Am J Cardiol* 2007;99:1072-81.
33. Garg S, Sarno G, Garcia-Garcia HM, Girasis C, Wykrzykowska J, Dawkins KD, Serruys PW; ARTS-II Investigators. A new tool for the risk stratification of patients with complex coronary artery disease: the Clinical SYNTAX Score. *Circ Cardiovasc Interv* 2010;3:317-26.
34. Scherff F, Vassalli G, Sürder D, Mantovani A, Corbacelli C, Pasotti E, Klersy C, Auricchio A, Moccetti T, Pedrazzini GB. The SYNTAX score predicts early mortality risk in the elderly with acute coronary syndrome having primary PCI. *J Invasive Cardiol* 2011;23:505-10.
35. Newman AB, Naydeck BL, Sutton-Tyrrell K, Feldman A, Edmundowicz D, Kuller LH. Coronary artery calcification in older adults to age 99: prevalence and risk factors. *Circulation* 2001;104:2679-84.
36. Raggi P, Callister TQ, Coöl B, He ZX, Lippolis NJ, Russo DJ, Zelinger A, Mahmarian JJ. Identification of patients at increased risk of first unheralded acute myocardial infarction by electron-beam computed tomography. *Circulation* 2000;101:850-5.
37. Bromage DI, Jones DA, Rathod KS, Grout C, Iqbal MB, Lim P, Jain A, Kalra SS, Crake T, Astroulakis Z. Outcome of 1051 octogenarian patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention: observational cohort from the London Heart Attack Group. *J Am Heart Assoc* 2016;5:e003027.
38. Belardi J, Manoharan G, Albertal M, Widimský P, Neumann FJ, Silber S, Leon MB, Saito S. The influence of age on clinical outcomes in patients treated with the resolute zotarolimus-eluting stent. *Catheter Cardiovasc Interv* 2016;87:253-61.
39. Antonsen L, Jensen LO, Terkelsen CJ, Tilsted HH, Junker A, Maeng M, Hansen KN, Lassen JF, Thuesen L, Thayssen P. Outcomes after primary percutaneous coronary intervention in octogenarians and nonagenarians with ST-segment elevation myocardial infarction: from the Western Denmark heart registry. *Catheter Cardiovasc Interv* 2013;81:912-9.
40. Hamonangan R, Wijaya IP, Setiati S, Harimurti K. Impact of frailty on the first 30 days of major cardiac events in elderly patients with coronary artery disease undergoing elective percutaneous coronary intervention. *Acta Medica Indones* 2016;48:91-8.
41. Spagnoli V, de Hemptinne Q, Nosair M, Gosselin G. Percutaneous coronary intervention in nonagenarians: prevalence, indications, vascular approach and mortality at 3 months. *J Cardiovasc Dis Diagn* 2016;4:239.
42. Kitabata H, Kubo T, Mori K, Yamamoto Y, Kashiwagi M, Arita Y, Tanimoto T, Akasaka T. Safety and efficacy outcomes of second-generation everolimus-eluting stents in octogenarians compared to non-octogenarians. *Cardiovasc Revascularization Med Mol Interv* 2018;19:12-6.
43. Caretta G, Passamonti E, Pedroni PN, Fadin BM, Galeazzi GL, Pirelli S. Outcomes and predictors of mortality among octogenarians and older with ST-segment elevation myocardial infarction treated with primary coronary angioplasty. *Clin Cardiol* 2014;37:523-9.
44. Higuchi S, Kabeya Y, Matsushita K, Taguchi H, Ishiguro H, Kohshoh H, Yoshino H. Barthel index as a predictor of 1-year mortality in very elderly patients who underwent percutaneous coronary intervention for acute coronary syndrome: better activities of daily living, longer life. *Clin Cardiol* 2016;39:83-9.
45. Lip GY, Nieuwlaar R, Pisters R, Lane DA, Crijns HJ. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the euro heart survey on atrial fibrillation. *Chest* 2010;137:263-72.
46. Sareh S, Toppen W, Mukdad L, Satou N, Shemin R, Buch E, Benharash P. CHADS2 score predicts atrial fibrillation following cardiac surgery. *J Surg Res* 2014;190:407-12.
47. Santana JO, Haft JJ, LaMarche NS, Goldstein JE. Coronary angioplasty in patients eight years of age or older. *Am Heart J* 1992;124:13-8.
48. Bonnier H, de Vries C, Michels R, El Gamal M. Initial and long-term results of coronary angioplasty and coronary bypass surgery in patients of 75 or older. *Heart* 1993;70:122-5.
49. Tan KH, Sulke N, Taub N, Karani S, Sowton E. Percutaneous transluminal coronary angioplasty in patients 70 years of age or older: 12 years' experience. *Heart* 1995;74:310-7.
50. Kern MJ, Deligonul U, Galan K, Zelman R, Gabliani G, Bell ST, Bodet J, Naunheim K, Vandormael M. Percutaneous transluminal coronary angioplasty in octogenarians. *Am J Cardiol* 1988;61:457-8.
51. Imburgia M, King TR, Soffer AD, Rich MW, Krone RJ, Salimi A. Early results and long-term outcome of percutaneous transluminal coronary angioplasty in patients age 75 years or older. *Am J Cardiol* 1989;63:1127-9.
52. Jeroudi MO, Kleiman NS, Minor ST, Hess KR, Lewis JM, Winters WL, Raizner AE. Percutaneous transluminal coronary angioplasty in octogenarians. *Ann Intern Med* 1990;113:423-8.
53. Rich JJ, Crispino CM, Saporito JJ, Domat I, Cooper WM. Percutaneous transluminal coronary angioplasty in patients 80 years of age and older. *Am J Cardiol* 1990;65:675-6.
54. Johnman C, Oldroyd KG, Mackay DF, Slack R, Pell ACH, Flapan AD, Jennings KP, Eteiba H, Irving J, Pell JP. Percutaneous coronary intervention in the elderly: changes in case-mix and periprocedural outcomes in 31,758 patients treated between 2000 and 2007. *Circ Cardiovasc Interv* 2010;3:341-5.
55. Bogana Shanmugam V, Wong DT, Rashid H, Cameron JD, Malaiapan Y, Psaltis PJ. Bleeding outcomes after non-emergency percutaneous coronary intervention in the very elderly. *J Geriatr Cardiol* 2017;14:624-31.
56. Sim WL, Mutha V, Ul-Haq MA, Sasongko V, Van-Gaal W. Clinical characteristics and outcomes of octogenarians presenting with ST elevation myocardial infarction in the Australian population. *World J Cardiol* 2017;9:437-41.
57. Yudi MB, Hamilton G, Farouque O, Andrianopoulos N, Duffy SJ, Lefkovits J, Brennan A, Fernando D, Hiew C, Freeman M, Reid CM, Dakis R, Ajani AE, Clark DJ; Melbourne Interventional Group. Trends and impact of door-to-balloon time on clinical outcomes in patients aged <75, 75 to 84, and ≥85 years with ST-elevation myocardial infarction. *Am J Cardiol* 2017;120:1245-53.

58. Wańha W, Kawecki D, Roleder T, Morawiec B, Gładysz S, Kowalówka A, Jadczyk T, Adamus B, Pawłowski T, Smolka G, Kaźmierski M, Ochala A, Nowalany-Kozielska E, Wojakowski W. Second-generation drug-eluting stents in the elderly patients with acute coronary syndrome: the in-hospital and 12-month follow-up of the all-comer registry. *Aging Clin Exp Res* 2017;29:885-93.
59. Gayed M, Yadak N, Qamhia W, Daralammouri Y, Ohlow MA. Comorbidities and complications in nonagenarians undergoing coronary angiography and intervention. *Int Heart J* 2017;58:180-4.
60. Lahtela HM, Bah A, Kiviniemi T, Nammias W, Schlitt A, Rubboli A, Karjalainen PP, Proietti M, Hartikainen JEK, Lip GYH, Airaksinen KEJ. Outcome of octogenarians with atrial fibrillation undergoing percutaneous coronary intervention: insights from the AFCAS registry. *Clin Cardiol* 2017;40:1264-70.
61. Rubboli A, Schlitt A, Kiviniemi T, Biancari F, Karjalainen PP, Valencia J, Laine M, Kirchhof P, Niemelä M, Vikman S, Lip GYH, Airaksinen KEJ; AFCAS Study Group. One-year outcome of patients with atrial fibrillation undergoing coronary artery stenting: an analysis of the AFCAS registry. *Clin Cardiol* 2014;37:357-64.
62. Sharma R, Hiebert B, Cheung D, Jassal DS, Minhas K. Primary coronary intervention in octogenarians and nonagenarians with ST-segment elevation myocardial infarction: a Canadian single-center perspective. *Angiology* 2017; doi: 10.1177/0003319717746520.
63. Sappa R, Grillo MT, Cinquetti M, Prati G, Spedicato L, Nucifora G, Perkan A, Zanuttini D, Sinagra G, Proclemer A. Short and long-term outcome in very old patients with ST-elevation myocardial infarction after primary percutaneous coronary intervention. *Int J Cardiol* 2017;249:112-8.
64. Bhat FA, Chahal KH, Raina H, Trambho NA, Rather HA. Transradial versus transfemoral approach for coronary angiography and angioplasty - a prospective, randomized comparison. *BMC Cardiovasc Disord* 2017;17:23.
65. Anjum I, Khan MA, Aadil M, Faraz A, Farooqui M, Hashmi A. Transradial vs. transfemoral approach in cardiac catheterization: a literature review. *Cureus* 2017;9:e1309.
66. He PY, Yang YJ, Qiao SB, Xu B, Yao M, Wu YJ, Yuan JQ, Chen J, Liu HB, Dai J, Tang XR, Wang Y, Li W, Gao RL. A comparison of transradial and transfemoral approaches for percutaneous coronary intervention in elderly patients based on a propensity score analysis. *Angiology* 2015;66:448-55.
67. Tammam K, Ikari Y, Yoshimachi F, Saito F, Hassan W. Impact of transradial coronary intervention on bleeding complications in octogenarians. *Cardiovasc Interv Ther* 2017;32:18-23.
68. Lee HW, Cha KS, Ahn J, Choi JC, Oh JH, Choi JH, Lee HC, Yun E, Jang HY, Choi JH, Hong TJ, Jeong MH, Ahn Y, Chae SC, Kim YJ; Korea Acute Myocardial Infarction Registry Investigators. Comparison of transradial and transfemoral coronary intervention in octogenarians with acute myocardial infarction. *Int J Cardiol* 2016;202:419-24.
69. Moses JW, Leon MB, Popma JJ, Fitzgerald PJ, Holmes DR, O'Shaughnessy C, Caputo RP, Kereiakes DJ, Williams DO, Teirstein PS, Jaeger JL, Kuntz RE; SIRIUS Investigators. Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. *N Engl J Med* 2003;349:1315-23.
70. Tian W, Mahmoudi M, Lhermusier T, Kiramijyan S, Chen F, Torguson R, Suddath WO, Satler LF, Pichard AD, Waksman R. The influence of advancing age on implantation of drug-eluting stents. *Catheter Cardiovasc Interv* 2016;88:516-21.
71. Navarese EP, Tandjung K, Claessen B, Andreotti F, Kowalewski M, Kandzari DE, Kereiakes DJ, Waksman R, Mauri L, Meredith IT, Finn AV, Kim HS, Kubica J, Suryapranata H, Aprami TM, Di Pasquale G, von Birgelen C, Kedhi E. Safety and efficacy outcomes of first and second generation durable polymer drug eluting stents and biodegradable polymer biolimus eluting stents in clinical practice: comprehensive network meta-analysis. *BMJ* 2013;347:f6530.
72. El-Hayek G, Messerli F, Bangalore S, Hong MK, Herzog E, Benjo A, Tamis-Holland JE. Meta-analysis of randomized clinical trials comparing short-term versus long-term dual antiplatelet therapy following drug-eluting stents. *Am J Cardiol* 2014;114:236-42.
73. Urban P, Meredith IT, Abizaid A, Pocock SJ, Carrié D, Naber C, Lipiecki J, Richardt G, Iñiguez A, Brunel P, Valdes-Chavarri M, Garot P, Talwar S, Berland J, Abdellaoui M, Eberli F, Oldroyd K, Zambahari R, Gregson J, Greene S, Stoll HP, Morice MC; LEADERS FREE Investigators. Polymer-free drug-coated coronary stents in patients at high bleeding risk. *N Engl J Med* 2015;373:2038-47.
74. Morice MC, Talwar S, Gaemperli O, Richardt G, Eberli F, Meredith I, Zaman A, Fajadet J, Copt S, Greene S, Urban P. Drug-coated versus bare-metal stents for elderly patients: a predefined sub-study of the LEADERS FREE trial. *Int J Cardiol* 2017;243:110-5.
75. de Belder A, de la Torre Hernandez JM, Lopez-Palop R, O'Kane P, Hernandez Hernandez F, Strange J, Gimeno F, Cotton J, Diaz Fernandez JF, Carrillo Saez P, Thomas M, Pinar E, Curzen N, Baz JA, Cooter N, Lozano I, Skipper N, Robinson D, Hildick-Smith D; XIMA Investigators. A prospective randomized trial of everolimus-eluting stents versus bare-metal stents in octogenarians: the XIMA Trial (Xience or Vision Stents for the Management of Angina in the Elderly). *J Am Coll Cardiol* 2014;63:1371-5.
76. Varenne O, Cook S, Sideris G, Kedev S, Cuisset T, Carrié D, Hovasse T, Garot P, El Mahmoud R, Spaulding C, Helft G, Diaz Fernandez JF, Brugaletta S, Pinar-Bermudez E, Mauri Ferre J, Commeau P, Teiger E, Bogaerts K, Sabate M, Morice MC, Sinnaeve PR; SENIOR investigators. Drug-eluting stents in elderly patients with coronary artery disease (SENIOR): a randomised single-blind trial. *Lancet Lond Engl* 2018;391:41-50.
77. Daugherty SL, Thompson LE, Kim S, Rao SV, Subherwal S, Tsai TT, Messenger JC, Masoudi FA. Patterns of use and comparative effectiveness of bleeding avoidance strategies in men and women following percutaneous coronary interventions: an observational study from the National Cardiovascular Data Registry. *J Am Coll Cardiol* 2013;61:2070-8.
78. Marso SP, Amin AP, House JA, Kennedy KF, Spertus JA, Rao SV, Cohen DJ, Messenger JC, Rumsfeld JS; National Cardiovascular Data Registry. Association between use of bleeding avoidance strategies and risk of periprocedural bleeding among patients undergoing percutaneous coronary intervention. *JAMA* 2010;303:2156-64.
79. Khambatta S, Othman H, Seth M, Lalonde T, Rosman HS, Gurm HS, Mehta RH; Blue Cross Blue Shield of Michigan Cardiovascular Consortium (BMC2) Investigators. Association of bleeding avoidance strategies with age-related bleeding and in-hospital mortality in

- patients undergoing percutaneous coronary interventions. *Cardiovasc Revascularization Med Mol Interv* 2016;17:233-40.
80. McNamara RL, Wang Y, Herrin J, Curtis JP, Bradley EH, Magid DJ, Peterson ED, Blaney M, Frederick PD, Krumholz HM; NRM Investigators. Effect of door-to-balloon time on mortality in patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol* 2006;47:2180-6.
 81. Yudi MB, Ramchand J, Farouque O, Andrianopoulos N, Chan W, Duffy SJ, Lefkovits J, Brennan A, Spencer R, Fernando D, Hiew C, Freeman M, Reid CM, Ajani AE, Clark DJ; Melbourne Interventional Group. Impact of door-to-balloon time on long-term mortality in high- and low-risk patients with ST-elevation myocardial infarction. *Int J Cardiol* 2016;224:72-8.
 82. Swaminathan RV, Wang TY, Kaltenbach LA, Kim LK, Minutello RM, Bergman G, Wong SC, Feldman DN. Nonsystem reasons for delay in door-to-balloon time and associated in-hospital mortality: a report from the National Cardiovascular Data Registry. *J Am Coll Cardiol* 2013;61:1688-95.
 83. Angeja BG, Gibson CM, Chin R, Frederick PD, Every NR, Ross AM, Stone GW, Barron HV; Participants in the National Registry of Myocardial Infarction 2-3. Predictors of door-to-balloon delay in primary angioplasty. *Am J Cardiol* 2002;89:1156-61.
 84. Engberding N, Wenger NK. Acute coronary syndromes in the elderly. *F1000Research* 2017;6:1791.
 85. Kim HY, Choi JH. How to utilize coronary computed tomography angiography in the treatment of coronary artery disease. *J Cardiovasc Ultrasound* 2015;23:204-8.
 86. Karacsonyi J, Alaswad K, Jaffer FA, Yeh RW, Patel M, Bahadorani J, Karatasakis A, Danek BA, Doing A, Grantham JA, Karmaliotis D, Moses JW, Kirtane A, Parikh M, Ali Z, Lombardi WL, Kandzari DE, Lembo N, Garcia S, Wyman MR, Alame A, Nguyen-Trong PKJ, Resendes E, Kalsaria P, Rangan BV, Ungi I, Thompson CA, Banerjee S, Brilakis ES. Use of intravascular imaging during chronic total occlusion percutaneous coronary intervention: insights from a contemporary multicenter registry. *J Am Heart Assoc* 2016;5:e003890.
 87. Danzi GB, Campanile A, Sozzi FB, Bonanomi C. Retrograde dissection during percutaneous coronary intervention: sealing of the entry site by covered stent implantation. *BMJ Case Rep* 2012;2012:bcr0320126014.
 88. Chen HC, Lee WC, Fu M. "Rail track picture": diagnosis of the protruding of left main coronary stent by transthoracic echocardiography especially with three-dimensional images. *Eur Heart J Cardiovasc Imaging* 2014;15:946.
 89. Arisha MJ, Hsiung MC, Ahmad A, Nanda NC, Elkaryoni A, Mohamed AH, Yin WH. Incremental benefit of three-dimensional transesophageal echocardiography in the assessment of left main coronary artery stent protrusion. *Echocardiogr Mt Kisco N* 2017;34:915-8.
 90. De Maria GL, Banning AP. Use of intravascular ultrasound imaging in percutaneous coronary intervention to treat left main coronary artery disease. *Interv Cardiol Lond Engl* 2017;12:8-12.
 91. Terashima M, Kaneda H, Suzuki T. The role of optical coherence tomography in coronary intervention. *Korean J Intern Med* 2012;27:1-12.
 92. Larose E, Côté J, Rodés-Cabau J, Noël B, Barbeau G, Bordeleau E, Miró S, Brochu B, Delarochellière R, Bertrand OF. Contrast-enhanced cardiovascular magnetic resonance in the hyperacute phase of ST-elevation myocardial infarction. *Int J Cardiovasc Imaging* 2009;25:519-27.
 93. Oqueli E, Dick R. Percutaneous coronary intervention in very elderly patients. In-hospital mortality and clinical outcome. *Heart Lung Circ* 2011;20:622-8.
 94. Parikh R, Chennareddy S, Debari V, Hamdan A, Konlian D, Shamoan F, Bikkina M. Percutaneous coronary interventions in nonagenarians: in-hospital mortality and outcome at one year follow-up. *Clin Cardiol* 2009;32:E16-21.
 95. Teplitsky I, Assali A, Lev E, Brosh D, Vaknin-Assa H, Kornowski R. Results of percutaneous coronary interventions in patients > or =90 years of age. *Catheter Cardiovasc Interv* 2007;70:937-43.
 96. Spoon DB, Psaltis PJ, Singh M, Holmes DR, Gersh BJ, Rihal CS, Lennon RJ, Moussa ID, Simari RD, Gulati R. Trends in cause of death after percutaneous coronary intervention. *Circulation* 2014;129:1286-94.
 97. Peiyuan H, Jingang Y, Haiyan X, Xiaojin G, Ying X, Yuan W, Wei L, Yang W, Xinran T, Ruohua Y, Chen J, Lei S, Xuan Z, Rui F, Yunqing Y, Qiuting D, Hui S, Xinxin Y, Runlin G, Yuejin Y; CAMI Registry study group. The comparison of the outcomes between primary PCI, fibrinolysis, and no reperfusion in patients ≥ 75 years old with ST-segment elevation myocardial infarction: results from the Chinese Acute Myocardial Infarction (CAMI) Registry. *PLoS One* 2016;11:e0165672.
 98. Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes (GUSTO IIb) Angioplasty Substudy Investigators. A clinical trial comparing primary coronary angioplasty with tissue plasminogen activator for acute myocardial infarction. *N Engl J Med* 1997;336:1621-8.
 99. de Boer SPM, Westerhout CM, Simes RJ, Granger CB, Zijlstra F, Boersma E; Primary Coronary Angioplasty Versus Thrombolysis-2 (PCAT-2) Trialists Collaborators Group. Mortality and morbidity reduction by primary percutaneous coronary intervention is independent of the patient's age. *JACC Cardiovasc Interv* 2010;3:324-31.
 100. Solhpour A, Chang KW, Balan P, Cai C, Sdringola S, Denktas AE, Smalling RW, Anderson HV. Comparison of outcomes for patients ≥75 years of age treated with pre-hospital reduced-dose fibrinolysis followed by percutaneous coronary intervention versus percutaneous coronary intervention alone for treatment of ST-elevation myocardial infarction. *Am J Cardiol* 2014;113:60-3.
 101. Nicolini F, Contini GA, Fortuna D, Pacini D, Gabbieri D, Vignali L, Campo G, Manari A, Zussa C, Guastaroba P, De Palma R, Gherli T. Coronary artery surgery versus percutaneous coronary intervention in octogenarians: long-term results. *Ann Thorac Surg* 2015;99:567-74.
 102. Sheridan BC, Stearns SC, Rossi JS, D'Arcy LP, Federspiel JJ, Carey TS. Three-year outcomes of multivessel revascularization in very elderly acute coronary syndrome patients. *Ann Thorac Surg* 2010;89:1889-94; discussion 1894-5.
 103. Levine GN, Bates ER, Blankenship JC, Bailey SR, Bittl JA, Cercek B, Chambers CE, Ellis SG, Guyton RA, Hollenberg SM, Khot UN,

- Lange RA, Mauri L, Mehran R, Moussa ID, Mukherjee D, Nallamothu BK, Ting HH; American College of Cardiology Foundation, American Heart Association Task Force on Practice Guidelines, Society for Cardiovascular Angiography and Interventions. 2011 ACCF/AHA/SCAI Guideline for percutaneous coronary intervention. A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. *J Am Coll Cardiol* 2011;58:e44-122.
104. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimský P; ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2018;39:119-77.

Review

Open Access



Histone deacetylases in vascular permeability and remodeling associated with acute lung injury

Laszlo Kovacs^{1,†}, Anita Kovacs-Kasa^{2,†}, Alexander D. Verin^{2,3}, David Fulton^{1,2}, Rudolf Lucas^{1,2,3}, Yunchao Su^{1,2,3,4}

¹Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA.

²Vascular Biology Center, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA.

³Department of Medicine, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA.

⁴Research Service, Charlie Norwood Veterans Affairs Medical Center, Augusta, GA 30912, USA.

[†]Authors contributed equally.

Correspondence to: Dr. Yunchao Su, Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta University, 1120 15th Street, Augusta, GA 30912, USA. E-mail: ysu@augusta.edu

How to cite this article: Kovacs L, Kovacs-Kasa A, Verin AD, Fulton D, Lucas R, Su Y. Histone deacetylases in vascular permeability and remodeling associated with acute lung injury. *Vessel Plus* 2018;2:15. <http://dx.doi.org/10.20517/2574-1209.2018.06>

Received: 1 Mar 2018 **First Decision:** 20 Jun 2018 **Revised:** 28 Jun 2018 **Accepted:** 30 Jun 2018 **Published:** 10 Jul 2018

Science Editor: Evgenia V. Gerasimovskaya **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

Acute lung injury (ALI) is a severe progressive disorder that arises from a wide range of causes such as toxins or inflammation, resulting in significant morbidity and mortality. There are no effective therapeutic options apart from mechanical ventilation strategies. While the mechanisms that govern the clinically relevant process of increased endothelial cell (EC) permeability and remodeling associated with ALI are under intense investigation, our knowledge of the processes that determine barrier enhancement or preservation are far from completion. Recently, epigenetic mechanisms have emerged as a major regulator of enduring changes in cell behavior and the therapeutic potential of inhibiting histone deacetylases (HDACs) for the treatment of cardiovascular and inflammatory diseases has gained remarkable attention. Although HDACs have been shown to play an important role in regulating EC barrier function, the involved HDAC subtypes and mechanisms remain undefined. Further investigation of the HDAC signaling may provide therapeutic approaches for the prevention and treatment of ALI.

Keywords: Acute lung injury, endothelial barrier function, histone deacetylases

INTRODUCTION

Acute lung injury (ALI) is a significant source of morbidity and mortality with over 200,000 incidences per year in the US^[1,2]. ALI arises from a wide range of causes such as toxins or inflammation resulting in



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



significant morbidity and frequently in death^[3]. To date no pharmacological therapies have been proven to improve ALI in clinical trials^[4]. Approaches remain non-specific and rely on supportive care and control of initial causes^[5]. A major pathophysiological alteration of ALI is the dysfunction of the pulmonary vascular endothelial barrier resulting in pulmonary infiltrates, hypoxemia and pulmonary edema^[6,7]. Endothelial cells (ECs) line the lumen of the blood vessels serving every organ system and provide a semi-selective barrier between the blood and the interstitial space^[8]. In ALI, the EC barrier is compromised leading to elevated vascular permeability^[9]. A lack of understanding the pathologic mechanisms involved in ALI remains an obstacle to new and effective therapies that reduces the vascular leakage in ALI.

Recently, epigenetic mechanisms have gained great therapeutic potential in the treatment of inflammatory and autoimmune diseases^[10,11]. Previous studies have demonstrated that inflammatory gene expression is regulated by the precise balance between histone-modulating enzymes; histone acetyltransferases (HATs) and histone deacetylases (HDACs)^[12,13]. In addition to modulating histone protein, it has been shown that HDACs can also deacetylate many non-histone proteins to regulate cellular functions such as cytoskeletal acetylation and polymerization, and signal transduction^[14-16]. Acetylation of non-histone proteins might crosstalk with other posttranslational modifications (PTMs) to control cellular signaling^[17]. HDACs are classes of enzymes that catalyze the removal of the acetyl groups from specific lysine residues of the histone or non-histone proteins^[18]. The inhibition of HDACs has been shown to play an important role in EC barrier protection^[19,20]. In this review, we intend to summarize the current knowledge focusing on the potential effect of HDAC inhibition on the pathogenesis of the ALI.

CHARACTERIZATION OF HDACS

There are 18 characterized members of HDAC superfamily in human which can be divided into two families and four classes according to their function and homology to yeast proteins^[21] [Table 1]. The classical family includes the classes I, II, and IV HDACs which are zinc-dependent enzymes and perform metal ion-mediated hydrolysis of acetamide bond in their acetylated substrates while silent information regulator 2 (Sir2)-related protein (Sirtuin) family contains the class III HDACs requiring NAD⁺ as a cofactor for enzymatic activity^[22,23]. Mechanisms for HDAC regulation occurs at multiple levels involving protein complex formation, PTMs such as phosphorylation, SUMOylation, subcellular localization, availability of metabolic cofactors and proteolytic processing^[24].

The class I HDACs (HDAC1, 2, 3, and 8) have sequence similarity to the yeast transcriptional regulator reduced potassium dependency 3 (RPD3) protein. They are ubiquitously expressed and mainly localized within the cellular nucleus^[25]. HDAC1 and HDAC2 contain nuclear localization signal (NLS), but not nuclear export signal (NES), therefore they are restricted to the nucleus^[26]. HDAC3 are able to shuttle between the nucleus and the cytoplasm which is regulated by competing nuclear import and nuclear export signals^[27]. HDAC8 shows relatively low expression and can be found in the nucleus and in the cytoplasm^[28]. Class I HDACs are ~350-500 amino acids long. They consist of the conserved deacetylase domain (DAC) with short amino- and carboxy-terminal tails, latter can be modified by PTMs including phosphorylation or SUMOylation in order to regulate the enzymatic activity^[24]. HDACs 1-3 form multiprotein complexes with transcription factors and co-repressors to fulfil their deacetylase activities. HDAC8 does not form complexes and is fully active in single molecule^[29,30].

The class II HDACs are protein orthologous to the yeast histone deacetylase-A 1 (HDA1) protein and can be divided into two subclasses such as class IIa (HDAC4, 5, 7 and 9) and class IIb (HDAC6 and 10)^[25]. Class II HDACs show tissue-specific expression pattern and functions^[31]. They are able to shuttle between nucleus and cytoplasm, however, HDAC6 and 10 are predominantly localized in the cytoplasm^[32]. Class II HDACs are considerably larger molecules than class I HDACs, and they have approximately 700-1200 amino acid

Table 1. Characterization of HDACs

Class	HDAC isoform	Size (amino acids)	Subcellular localization	Expression pattern
I	HDAC1	482	Nucleus	Ubiquitous
	HDAC2	488	Nucleus	Ubiquitous
	HDAC3	428	Mainly nucleus	Ubiquitous
	HDAC8	377	Nucleus/cytoplasm	Ubiquitous
IIa	HDAC4	1084	Nucleus/cytoplasm	Heart, skeletal muscle, brain
	HDAC5	1122	Nucleus/cytoplasm	Heart, skeletal muscle, brain
	HDAC7	952	Nucleus/cytoplasm	Heart, placenta, pancreas, skeletal muscle
	HDAC9	1011	Nucleus/cytoplasm	Skeletal muscle, brain
IIb	HDAC6	1215	Mainly cytoplasm	Heart, liver, kidney, pancreas
	HDAC10	669	Mainly cytoplasm	Liver, spleen, kidney
III	SIRT1	747	Nucleus	Ubiquitous
	SIRT2	352	Cytoplasm	Ubiquitous
	SIRT3	399	Mitochondria	Ubiquitous
	SIRT4	314	Mitochondria	Ubiquitous
	SIRT5	310	Mitochondria	Ubiquitous
	SIRT6	355	Nucleus	Ubiquitous
	SIRT7	400	Nucleolus	Ubiquitous
IV	HDAC11	347	Mainly nucleus	Brain, heart, skeletal muscle, kidney and testis

HDACs: histone deacetylases

residues. The class IIa HDACs have the deacetylase domain on the C-terminal and possess a long N-terminal tail containing conserved multiple binding domains and regulatory sites which play a crucial role in the regulation of the nucleocytoplasmic trafficking^[29,33]. The catalytic domain of class IIb HDACs is localized on the N-terminus of the protein. In addition, the HDAC6 has a secondary DAC domain and an ubiquitin binding site on the C-terminal^[34]. HDAC10 is closely related to HDAC6 and has a putative second catalytic domain and two putative Rb binding domains on the C-terminal of the enzyme^[35].

The HDAC11 shares sequence similarity to both RPD3 and HDA1 proteins and falls into the Class IV as a unique member. It is the smallest HDAC isoform consisting 347 amino acid residues and are located predominantly in the nucleus. HDAC11 has tissue-specific distribution and can be found in the brain, heart, skeletal muscle kidney and testis. It contains a catalytic domain at the N-terminus and short N- and C-terminal extensions^[36].

The class III HDACs have seven members (SIRT1, 2, 3, 4, 5, 6 and 7) and possess homology to the yeast Sir2 protein^[37]. They are ubiquitously expressed and show different subcellular localization. SIRT1, 6 and 7 are mainly localized to the nucleus, the SIRT2 can be found in cytoplasm, while the SIRT3, 4 and 5 are mitochondrial proteins^[38]. They do not comprise zinc in the catalytic site and uses NAD⁺ as a cofactor in their catalytic reactions. The 275 amino acid long catalytic domain is highly conserved among the sirtuins flanking with variable length of amino- and carboxy-terminal extensions^[39]. They can serve as a NAD⁺-dependent lysine deacetylase or as a mono-ADP-ribosyltransferase. In the deacetylation reaction, nicotinamide, 2'-O-acetyl-ADP-ribose and deacetylated product are generated with the hydrolysis of one NAD⁺ molecule^[40]. During the ADP-ribosylation reaction, ADP-ribose from the NAD⁺ is transferred to the acetylated substrate and nicotinamide is released^[41]. Both reactions depend on the ratio of NAD⁺/NADH. Therefore the cellular metabolism can be a potential regulatory mechanism of SIRTs.

BIOLOGICAL FUNCTION OF HDACS

Lysine acetylation/deacetylation of histone and non-histone proteins are major reversible PTMs that are dynamically maintained by two enzymes families, HAT and HDAC^[42]. Changes in histone acetylation

modify chromatin structure and the activity of transcription factors that serve as an important mechanism for the regulation of gene expression^[43]. Deacetylation of histones by HDACs contributes to the compaction of the chromatin structure correlating with gene silencing^[44]. Genome-wide mapping revealed that HDACs are also associated with active genes and positively correlated with gene transcription, indicating that they do not only remove the acetyl group in active genes but also reset the chromatin for activation of gene transcription^[45]. HDACs deacetylate transcriptional factors mostly repressing the gene expression. However, HDACs can also activate transcription by either reducing the transcription of transcriptional repressor proteins or by deacetylating and activating the transcription activators or by deacetylating and inhibiting the transcription repressors^[21]. In addition to regulation of gene expression, activities of HDACs have been shown to indirectly regulate other PTMs including phosphorylation, ubiquitination, methylation, SUMOylation, NEDDylation, biotinylation, *etc.*^[17]. Lysine acetylation can interfere with other lysine modifications, conversely, removal of the acetyl group can promote other lysine modifications that control vital cellular functions such as mRNA integrity, translation, enzymatic activity as well as protein stability, function, localization and interactions^[46]. For example, there is a direct competition between acetylation and ubiquitination, namely acetylation blocks ubiquitination and proteasome-mediated degradation of target proteins. Similarly, the deacetylation facilitates protein degradation^[47]. Members of the HDAC superfamily have been shown to be involved in many physiological processes such as cell migration, proliferation and survival, cell differentiation, cell cycle, signal transduction, aging, DNA repair and apoptosis^[48,49]. Growing body of evidence show that malfunction of the HDACs play a critical role in many human disorders including cancer, neurodegenerative diseases, metabolic and immunological disorders, inflammatory, cardiac and pulmonary diseases^[50,51]. HDAC inhibition has been reported to display antitumor and anti-inflammation properties^[52-55]. It has also been shown that HDACs play an important role in various inflammatory lung diseases including ALI, COPD, and asthma^[56-58].

HDACS IN ALI

Several reports have indicated that HDAC inhibitors possess beneficial effect in ALI animal models. The primary causes of ALI are the endothelial barrier dysfunction and inflammation^[59,60]. It has been shown that HDAC6-specific inhibitor tubacin ameliorates pulmonary edema in the LPS-induced ALI^[19]. Pharmacological inhibition of HDAC6 suppresses the thrombin-induced endothelial barrier dysfunction through increased acetylation of α -tubulin and microtubules stabilization^[19]. In addition, Yu *et al.*^[61] found that selective inhibition of HDAC6 by tubastatin A (Tub A) blocks TNF- α -induced lung endothelial permeability and prevents endotoxin-induced pulmonary edema. Pretreatment with Tub A enhanced α -tubulin acetylation and decreased the TNF- α -induced microtubule disassembly, endothelial cell contraction and actin stress fiber formation as well as reduced the phosphorylation of the myosin light chain attenuating the lung endothelial cell hyperpermeability caused by the cytokine^[61]. In addition, HDAC6 inhibition by Tub A increases β -catenin acetylation and consequently its membrane translocation leading to increased stabilization of adherens junctions in endothelial cells^[61]. Moreover, inhibition of HDAC6 prevents endotoxin-induced deacetylation of α -tubulin and β -catenin in lung tissues and attenuates lung edema formation in mouse model of endotoxemia^[61]. The same group demonstrated that selective inhibitors of HDAC6 such as CAY10603 and Tub A prevented the TNF- α -induced caspase 3 activation and endothelial barrier dysfunction via maintaining cell-cell junction integrity^[62]. In addition, inhibition of HDAC6 by CAY10603 alleviated the endotoxin-induced lung vascular permeability and caspase-3 activation as well as reduced the lung edema formation^[62]. Joshi *et al.*^[20] revealed the mediating role of HDACs in LPS-induced endothelial hyperpermeability and ALI. Inhibition of various HDACs by pan-HDAC inhibitors including panobinostat or trichostatin (TSA) attenuates LPS-induced decrease in transendothelial electrical resistance (TER), Hsp90 activation and chaperone function as well as diminishes the RhoA activity and signaling. Moreover, pre-treatment with HDAC3-selective inhibitor RGFP-966 or with HDAC6-selective inhibitor Tub

A, or combined inhibition of HDAC3 and -6 prevents the LPS-mediated endothelial barrier dysfunction^[20]. More importantly, combined pharmacological inhibition of HDAC3 and -6 protected against LPS-stimulated inflammation, capillary permeability, and structural abnormalities in murine model of ALI^[20].

HDAC inhibition has been shown to ameliorate the inflammatory responses associated with ALI. Ni *et al.*^[63] reported that broad HDAC inhibitor, butyrate markedly diminishes the pulmonary inflammation in LPS-induced ALI in mice. LPS administration induces histopathological changes in murine lungs, increases the production of TNF- α , IL-1 β and NO, as well as increases MPO activity and NF- κ B p65 expression that are significantly attenuated by butyrate pretreatment^[63]. The lung edema is markedly reduced by butyrate, indicating its protective effect on the LPS-induced ALI^[63]. Zhang *et al.*^[64] further confirmed the beneficial effect of butyrate on lung injury. They found that two structurally unrelated HDAC inhibitors, sodium butyrate (SB) and TSA diminished the sepsis-induced lung edema and leukocyte infiltration in lung tissue^[64]. In addition, SB and TSA decrease the expression of ICAM-1 and E-selectin in lung tissue and reduces plasma levels of IL-6, indicating that these HDAC inhibitors attenuate sepsis-induced inflammatory lung injury^[64]. Other group has also demonstrated that early administration of broad-spectrum HDAC inhibitor valproic acid (VPA) significantly reduces the levels of IL-6 and tumor necrosis factor in bronchoalveolar lavage (BAL) fluid and in plasma as well as improves survival in murine ALI model in gram-negative pneumonia^[65]. They also showed that VPA reduces the production of proinflammatory cytokines and the neutrophil influx into the pulmonary parenchyma and alleviates the host systemic and pulmonary inflammatory responses^[66]. In addition, the HDAC inhibitor VPA decreases the MPO activity reducing the lung injury induced by the bacterial infection and improves the histopathologic changes related to ALI^[66]. VPA has also been reported to prevent ALI induced by ischemia-reperfusion (I/R) in rat lungs. I/R significantly increases the lung edema, pulmonary arterial pressure, lung inflammation and the concentrations of the inflammatory mediators [TNF- α , cytokine-induced neutrophil chemoattractant-1 (CINC-1)] in bronchoalveolar lavage fluid (BALF). Pretreatment with VPA diminishes the I/R-caused alterations via increased heme oxygenase-1 (HO-1) activity that is an essential protective regulator in lung injury^[67]. Lu *et al.*^[68] demonstrated that HDAC inhibitors, suberanilohydroxamic acid (SAHA) and its analogue 4-(dimethylamino)-N-[7-(hydroxyamino)-7-oxoheptyl] benzamide significantly decreases early neutrophilic inflammation in murine model of ALI. They showed that these inhibitors are able to block the leukotriene A4 hydrolase (LTA4H) activity and to prevent the leukotriene B4 (LTB4) biosynthesis suppressing the LPS-induced neutrophils migration and infiltration into the murine lungs as well as reduces the production of inflammatory mediator (e.g., TNF- α , IL-1 β , and IL-6) induced by LPS^[68].

HDAC6 plays an important role in the cigarette smoke extract (CSE)-induced lung endothelial barrier disruption^[69,70]. Cigarette smoke (CS) activates HDAC6 that results in the deacetylation of α -tubulin and microtubule destabilization, leading to the impairment of lung endothelial barrier function and the exacerbation of LPS- or *P. aeruginosa*-induced elevation in lung vascular endothelial permeability^[70]. Down-regulation of HDAC6 by tubacin or by siRNAs to HDAC6 significantly reduces the CSE-induced elevation in the endothelial permeability *in vitro*. In addition, inhibition of HDAC6 attenuates the lung inflammation and lung edema induced by CSE in LPS- or *P. aeruginosa*-induced ALI animal model, indicating the involvement of HDAC6 in the CS exacerbation of LPS- or *P. aeruginosa*-induced ALI^[70].

CONCLUSION

In summary, convincing evidence support that members of the HDAC family play a critical role in the development of ALI [Figure 1]. Manipulating the HDAC signaling pathways using multidisciplinary approaches would provide novel therapeutic strategies for the protection of endothelial barrier function and for the intervention of ALI in inflammatory lung diseases.

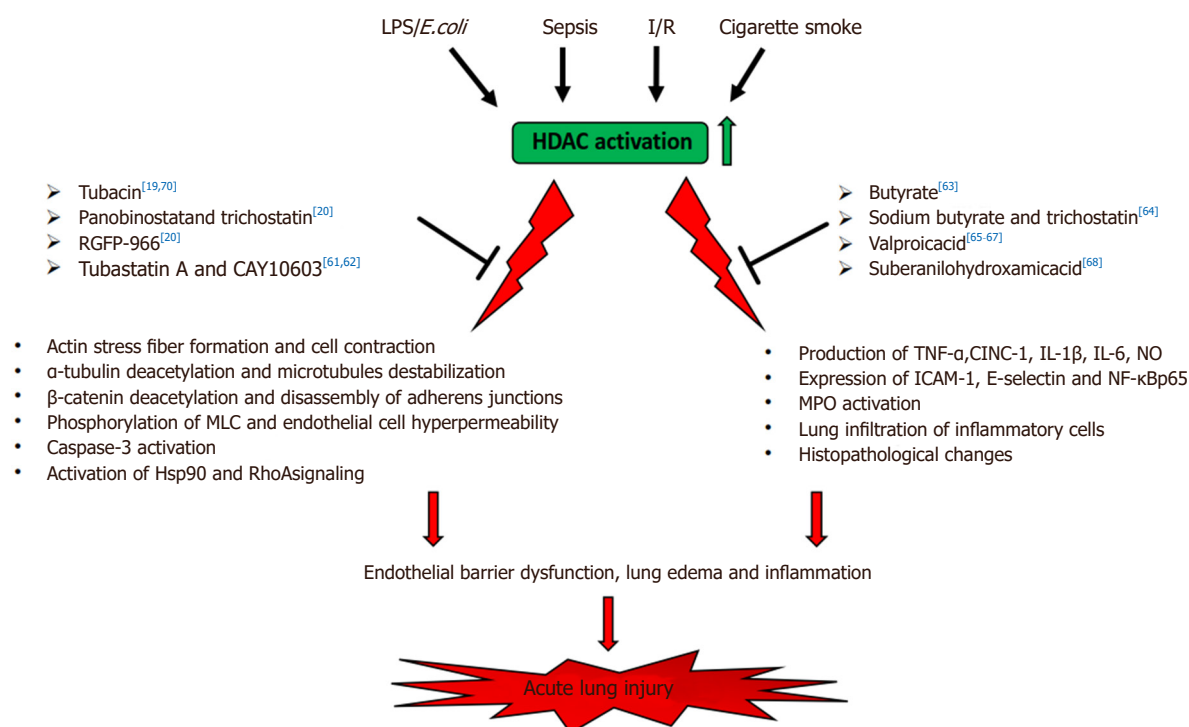


Figure 1. Schematic illustration of role of HDACs in acute lung injury (ALI). HDAC inhibitors: Tubacin (N1-[4-[(2R,4R,6S)-4-[[[(4,5-diphenyl-2-oxazolyl)thio]methyl]-6-[4-(hydroxymethyl)phenyl]-1,3-dioxan-2-yl]phenyl]-N8-hydroxy-octanediamide)^[19,70]; Panobinostat (NVP-LBH589)^[20]; Trichostatin A (TSA, 7-[4-(dimethylamino)phenyl]-N-hydroxy-4,6R-dimethyl-7-oxo-2E,4E-heptadienamide)^[20,64]; RGFP-966 ((2E)-N-(2-Amino-4-fluorophenyl)-3-[(2E)-1-(3-phenyl-2-propen-1-yl)-1H-pyrazol-4-yl]-2-propenamide)^[20]; Tubastatin A (N-hydroxy-4-[(1,2,3,4-tetrahydro-2-methyl-5H-pyrido[4,3-b]indol-5-yl)methyl]-benzamide)^[61,62]; CAY10603 (N-[4-[3-[[[7-(hydroxyamino)-7-oxoheptyl]amino]carbonyl]-5-isoxazolyl]phenyl]-1,1-dimethylethyl ester, carbamic acid)^[61,62]; Sodium Butyrate (butanoic acid sodium salt)^[63,64]; Valproic acid (VPA, 2-Propylpentanoic acid)^[65-67]; Vorinostat (Suberoylanilide Hydroxamic Acid, SAHA)^[68]. LPS: lipopolysaccharides; *E.coli*: *Escherichia coli*; I/R: ischemia-reperfusion; MLC: myosin light chain; TNF- α : tumor necrosis factor- α ; CINC-1: cytokine-induced neutrophil chemoattractant-1; IL-1 β : interleukin 1 β ; IL-6: interleukin 6; NO: nitric oxide; ICAM-1: intercellular adhesion molecule 1; NF- κ B p65: nuclear factor kappa-B p65 subunit; MPO: myeloperoxidase

DECLARATIONS

Authors' contributions

Concept/design: Kovacs L, Kovacs-Kasa A, Su Y

Draft: Kovacs L, Kovacs-Kasa A

Manuscript editing and review: all authors

Availability of data and materials

Not applicable.

Financial support and sponsorship

This work was supported by NIH/NHLBI R01 HL134934 (YS), VA Merit Review Award BX002035 (YS), Flight Attendants Medical Research Institute grant 140083_CIA (YS), AHA Career Development Award 18CDA34110225 (LK) and AHA Postdoctoral Fellowship 18POST33990193 (AKK).

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

- Johnson ER, Matthay MA. Acute lung injury: epidemiology, pathogenesis, and treatment. *J Aerosol Med Pulm Drug Deliv* 2010;23:243-52.
- Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD. Incidence and outcomes of acute lung injury. *N Engl J Med* 2005;353:1685-93.
- Butt Y, Kurdowska A, Allen TC. Acute lung injury: a clinical and molecular review. *Arch Pathol Lab Med* 2016;140:345-50.
- Parekh D, Dancer RC, Thickett DR. Acute lung injury. *Clin Med (Lond)* 2011;11:615-8.
- Gonzales JN, Lucas R, Verin AD. The acute respiratory distress syndrome: mechanisms and perspective therapeutic approaches. *Austin J Vasc Med* 2015;2:1009.
- Maniatis NA, Kotanidou A, Catravas JD, Orfanos SE. Endothelial pathomechanisms in acute lung injury. *Vascul Pharmacol* 2008;49:119-33.
- Lucas R, Verin AD, Black SM, Catravas JD. Regulators of endothelial and epithelial barrier integrity and function in acute lung injury. *Biochem Pharmacol* 2009;77:1763-72.
- Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiol Rev* 2004;84:869-901.
- Kasa A, Csontos C, Verin AD. Cytoskeletal mechanisms regulating vascular endothelial barrier function in response to acute lung injury. *Tissue Barriers* 2015;3:e974448.
- Jeffries MA, Sawalha AH. Autoimmune disease in the epigenetic era: how has epigenetics changed our understanding of disease and how can we expect the field to evolve? *Expert Rev Clin Immunol* 2015;11:45-58.
- Greer JM, McCombe PA. The role of epigenetic mechanisms and processes in autoimmune disorders. *Biologics* 2012;6:307-27.
- Struhl K. Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev* 1998;12:599-606.
- Kuo MH, Allis CD. Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays* 1998;20:615-26.
- Glozak MA, Seto E. Histone deacetylases and cancer. *Oncogene* 2007;26:5420-32.
- Halili MA, Andrews MR, Sweet MJ, Fairlie DP. Histone deacetylase inhibitors in inflammatory disease. *Curr Top Med Chem* 2009;9:309-19.
- Ito K, Barnes PJ, Adcock IM. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol* 2000;20:6891-903.
- Yang XJ, Seto E. Lysine acetylation: codified crosstalk with other posttranslational modifications. *Mol Cell* 2008;31:449-61.
- Peng L, Yuan Z, Seto E. Histone deacetylase activity assay. *Methods Mol Biol* 2015;1288:95-108.
- Saito S, Lasky JA, Guo W, Nguyen H, Mai A, Danchuk S, Sullivan DE, Shan B. Pharmacological inhibition of HDAC6 attenuates endothelial barrier dysfunction induced by thrombin. *Biochem Biophys Res Commun* 2011;408:630-4.
- Joshi AD, Barabutis N, Birmas C, Dimitropoulou C, Thangjam G, Cherian-Shaw M, Dennison J, Catravas JD. Histone deacetylase inhibitors prevent pulmonary endothelial hyperpermeability and acute lung injury by regulating heat shock protein 90 function. *Am J Physiol Lung Cell Mol Physiol* 2015;309:L1410-9.
- Seto E, Yoshida M. Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harb Perspect Biol* 2014;6:a018713.
- de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J* 2003;370:737-49.
- Dali-Youcef N, Lagouge M, Froelich S, Koehl C, Schoonjans K, Auwerx J. Sirtuins: the 'magnificent seven', function, metabolism and longevity. *Ann Med* 2007;39:335-45.
- Sengupta N, Seto E. Regulation of histone deacetylase activities. *J Cell Biochem* 2004;93:57-67.
- Bjerling P, Silverstein RA, Thon G, Caudy A, Grewal S, Ekwall K. Functional divergence between histone deacetylases in fission yeast by distinct cellular localization and in vivo specificity. *Mol Cell Biol* 2002;22:2170-81.
- Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discov* 2002;1:287-99.
- Yang WM, Tsai SC, Wen YD, Fejer G, Seto E. Functional domains of histone deacetylase-3. *J Biol Chem* 2002;277:9447-54.
- Van den Wyngaert I, de Vries W, Kremer A, Neefs J, Verhasselt P, Luyten WH, Kass SU. Cloning and characterization of human histone deacetylase 8. *FEBS Lett* 2000;478:77-83.
- Yang XJ, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. *Nat Rev Mol Cell Biol* 2008;9:206-18.
- Watson PJ, Millard CJ, Riley AM, Robertson NS, Wright LC, Godage HY, Cowley SM, Jamieson AG, Potter BV, Schwabe JW. Insights into the activation mechanism of class I HDAC complexes by inositol phosphates. *Nat Commun* 2016;7:11262.
- Verdin E, Dequiedt F, Kasler HG. Class II histone deacetylases: versatile regulators. *Trends Genet* 2003;19:286-93.

32. Bertos NR, Wang AH, Yang XJ. Class II histone deacetylases: structure, function, and regulation. *Biochem Cell Biol* 2001;79:243-52.
33. Parra M, Verdin E. Regulatory signal transduction pathways for class IIa histone deacetylases. *Curr Opin Pharmacol* 2010;10:454-60.
34. Valenzuela-Fernandez A, Cabrero JR, Serrador JM, Sanchez-Madrid F. HDAC6: a key regulator of cytoskeleton, cell migration and cell-cell interactions. *Trends Cell Biol* 2008;18:291-7.
35. Guardiola AR, Yao TP. Molecular cloning and characterization of a novel histone deacetylase HDAC10. *J Biol Chem* 2002;277:3350-6.
36. Gao L, Cueto MA, Asselbergs F, Atadja P. Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. *J Biol Chem* 2002;277:25748-55.
37. Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochem Biophys Res Commun* 2000;273:793-8.
38. Haigis MC, Guarente LP. Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. *Genes Dev* 2006;20:2913-21.
39. Min J, Landry J, Sternglanz R, Xu RM. Crystal structure of a SIR2 homolog-NAD complex. *Cell* 2001;105:269-79.
40. Denu JM. The Sir 2 family of protein deacetylases. *Curr Opin Chem Biol* 2005;9:431-40.
41. Frye RA. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. *Biochem Biophys Res Commun* 1999;260:273-9.
42. Drazic A, Myklebust LM, Ree R, Arnesen T. The world of protein acetylation. *Biochim Biophys Acta* 2016;1864:1372-401.
43. Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293:1074-80.
44. Ruthenburg AJ, Li H, Patel DJ, Allis CD. Multivalent engagement of chromatin modifications by linked binding modules. *Nat Rev Mol Cell Biol* 2007;8:983-94.
45. Wang Z, Zang C, Cui K, Schones DE, Barski A, Peng W, Zhao K. Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* 2009;138:1019-31.
46. Spange S, Wagner T, Heinzel T, Kramer OH. Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *Int J Biochem Cell Biol* 2009;41:185-98.
47. Caron C, Boyault C, Khochbin S. Regulatory cross-talk between lysine acetylation and ubiquitination: role in the control of protein stability. *Bioessays* 2005;27:408-15.
48. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat Rev Genet* 2009;10:32-42.
49. Bassett SA, Barnett MP. The role of dietary histone deacetylases (HDACs) inhibitors in health and disease. *Nutrients* 2014;6:4273-301.
50. Lawless MW, Norris S, O'Byrne KJ, Gray SG. Targeting histone deacetylases for the treatment of disease. *J Cell Mol Med* 2009;13:826-52.
51. Tang J, Yan H, Zhuang S. Histone deacetylases as targets for treatment of multiple diseases. *Clin Sci (Lond)* 2013;124:651-62.
52. Pan LN, Lu J, Huang B. HDAC inhibitors: a potential new category of anti-tumor agents. *Cell Mol Immunol* 2007;4:337-43.
53. Walkinshaw DR, Yang XJ. Histone deacetylase inhibitors as novel anticancer therapeutics. *Curr Oncol* 2008;15:237-43.
54. Adcock IM. HDAC inhibitors as anti-inflammatory agents. *Br J Pharmacol* 2007;150:829-31.
55. Leoni F, Zaliani A, Bertolini G, Porro G, Pagani P, Pozzi P, Dona G, Fossati G, Sozzani S, Azam T, Bufler P, Fantuzzi G, Goncharov I, Kim SH, Pomerantz BJ, Reznikov LL, Siegmund B, Dinarello CA, Mascagni P. The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. *Proc Natl Acad Sci U S A* 2002;99:2995-3000.
56. Ito K, Ito M, Elliott WM, Cosio B, Caramori G, Kon OM, Barczyk A, Hayashi S, Adcock IM, Hogg JC, Barnes PJ. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N Engl J Med* 2005;352:1967-76.
57. Ito K, Charron CE, Adcock IM. Impact of protein acetylation in inflammatory lung diseases. *Pharmacol Ther* 2007;116:249-65.
58. Adcock IM, Ito K, Barnes PJ. Histone deacetylation: an important mechanism in inflammatory lung diseases. *COPD* 2005;2:445-55.
59. Chong DL, Sriskandan S. Pro-inflammatory mechanisms in sepsis. *Contrib Microbiol* 2011;17:86-107.
60. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood* 2003;101:3765-77.
61. Yu J, Ma Z, Shetty S, Ma M, Fu J. Selective HDAC6 inhibition prevents TNF-alpha-induced lung endothelial cell barrier disruption and endotoxin-induced pulmonary edema. *Am J Physiol Lung Cell Mol Physiol* 2016;311:L39-47.
62. Yu J, Ma M, Ma Z, Fu J. HDAC6 inhibition prevents TNF-alpha-induced caspase 3 activation in lung endothelial cell and maintains cell-cell junctions. *Oncotarget* 2016;7:54714-22.
63. Ni YF, Wang J, Yan XL, Tian F, Zhao JB, Wang YJ, Jiang T. Histone deacetylase inhibitor, butyrate, attenuates lipopolysaccharide-induced acute lung injury in mice. *Respir Res* 2010;11:33.
64. Zhang L, Jin S, Wang C, Jiang R, Wan J. Histone deacetylase inhibitors attenuate acute lung injury during cecal ligation and puncture-induced polymicrobial sepsis. *World J Surg* 2010;34:1676-83.
65. Kasotakis G, Galvan MD, Osathanugrah P, Dharia N, Bufo L, Breed Z, Mizgerd JP, Remick DG. Timing of valproic acid in acute lung injury: prevention is the best therapy? *J Surg Res* 2017;220:206-12.
66. Kasotakis G, Galvan M, King E, Sarkar B, Stucchi A, Mizgerd JP, Burke PA, Remick D. Valproic acid mitigates the inflammatory response and prevents acute respiratory distress syndrome in a murine model of *Escherichia coli* pneumonia at the expense of bacterial clearance. *J Trauma Acute Care Surg* 2017;82:758-65.
67. Wu SY, Tang SE, Ko FC, Wu GC, Huang KL, Chu SJ. Valproic acid attenuates acute lung injury induced by ischemia-reperfusion in rats. *Anesthesiology* 2015;122:1327-37.
68. Lu W, Yao X, Ouyang P, Dong N, Wu D, Jiang X, Wu Z, Zhang C, Xu Z, Tang Y, Zou S, Liu M, Li J, Zeng M, Lin P, Cheng F, Huang J. Drug repurposing of histone deacetylase inhibitors that alleviate neutrophilic inflammation in acute lung injury and idiopathic

- pulmonary fibrosis via inhibiting leukotriene A4 hydrolase and blocking LTB4 biosynthesis. *J Med Chem* 2017;60:1817-28.
69. Lu Q, Sakhatskyy P, Grinnell K, Newton J, Ortiz M, Wang Y, Sanchez-Esteban J, Harrington EO, Rounds S. Cigarette smoke causes lung vascular barrier dysfunction via oxidative stress-mediated inhibition of RhoA and focal adhesion kinase. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L847-57.
70. Borgas D, Chambers E, Newton J, Ko J, Rivera S, Rounds S, Lu Q. Cigarette smoke disrupted lung endothelial barrier integrity and increased susceptibility to acute lung injury via histone deacetylase 6. *Am J Respir Cell Mol Biol* 2016;54:683-96.

Original Article

Open Access



Immunohistochemistry of the circadian clock in mouse and human vascular tissues

Ciprian B. Anea¹, Ana M. Merloiu¹, David J. R. Fulton¹, Vijay Patel², R. Dan Rudic¹

¹Department of Pharmacology & Toxicology, Medical College of Georgia at Augusta University, Augusta, GA 30912, USA.

²Department of Surgery, Medical College of Georgia at Augusta University, Augusta, GA 30912, USA.

Correspondence to: Dr. R. Dan Rudic, Department of Pharmacology & Toxicology, Medical College of Georgia at Augusta University, Augusta, GA 30912, USA. E-mail: rrudic@augusta.edu

How to cite this article: Anea CB, Merloiu AM, Fulton DJR, Patel V, Rudic RD. Immunohistochemistry of the circadian clock in mouse and human vascular tissues. *Vessel Plus* 2018;2:16. <http://dx.doi.org/10.20517/2574-1209.2018.46>

Received: 14 Jun 2018 **First Decision:** 3 Jul 2018 **Revised:** 10 Jul 2018 **Accepted:** 11 Jul 2018 **Published:** 20 Jul 2018

Science Editor: Alexander D. Verin **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Aim: The circadian clock is a molecular network that controls the body physiological rhythms. In blood vessels, the circadian clock components modulate vascular remodeling, blood pressure, and signaling. The goal in this study was to determine the pattern of expression of circadian clock proteins in the endothelium, smooth muscle, and adventitia of the vasculature of human and mouse tissues.

Methods: Immunohistochemistry was performed in frozen sections of mouse aorta, common carotid artery, femoral artery, lung, and heart at 12 AM and 12 PM for Bmal1, Clock, Npas2, Per and other clock components. Studies of expression were also assessed in human saphenous vein both by immunoblotting and immunohistochemistry.

Results: In this study, we identified the expression of Bmal1, Clock, Npas, Per1, Cry1, and accessory clock components by immunohistochemical staining in the endothelium, smooth muscle and adventitia of the mouse vasculature with differing temporal and cellular profiles depending on vasculature and tissue analyzed. The human saphenous vein also exhibited expression of clock genes that exhibited an oscillatory pattern in Bmal1 and Cry by immunoblotting.

Conclusion: These studies show that circadian clock components display differences in expression and localization throughout the cardiovascular system, which may confer nuances of circadian clock signaling in a cell-specific manner.

Keywords: Circadian blood vessel, vascular endothelium, smooth muscle, Clock, Bmal1, aorta, human, mouse, Per, Cry



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



INTRODUCTION

This circadian clock is a signaling mechanism that controls 24 h rhythmic oscillations. Heterodimeric interactions of clock transcription factors including Clock, Npas2 with Bmal1 bind E-box response elements of respective genes and drive transcription of downstream negative clock regulators or putative output genes that regulate physiologic function. In the circadian clock loop, the Bmal1-Clock heterodimer serves to cause transcription of Period and Cry genes which are then translated to proteins, form heterodimers themselves, and cycle from the cytoplasm back into to the nucleus to inhibit Bmal1 and Clock. This molecular mechanism is expressed through the body, generating cyclic 24 h physiological rhythms. In blood vessels, the circadian clock is also oscillating^[1-3]. Since blood vessels are comprised of three distinct cellular layers, the endothelial cell layer, smooth muscle cell layer, and the adventitial layer, circadian clocks may exert cell specific and cell-coordinated functional actions intrinsic within the vasculature. Evidence of vascular cell-specific functions of the circadian clock has first been demonstrated in global knockout models. Global disruption of Bmal1 or Period genes impairs endothelial function and detrimentally influences the adaptation of the vasculature both acutely and chronically^[4-9]. While the circadian clock is a resilient or robust signaling pathway whereby its disruption is not lethal due to redundancy, intercellular, and intracellular coupling^[10,11], overexpression of clock components has been shown to offer protection against deleterious vascular phenotypes^[12,13]. In cell specific knockout models of the intrinsic vasculature, smooth muscle disruption of Bmal1 results in alterations in rhythmic blood pressure^[14], disruption of the circadian clock in the endothelium worsens the thrombogenic response and also affects blood pressure^[15], and vascular transplantation of Bmal1-KO mice into WT mice induces arteriosclerotic response^[16]. In the current study, we sought to examine the cellular expression of the circadian clock in the mouse and human vascular tissue.

METHODS

Animals

All animal studies were performed according to protocols approved by the Medical College of Georgia Institutional Committee for Use and Care of Laboratory Animals at Augusta University. Normal wild type C57BL6 mice were used in all experiments.

Human blood vessels

Segments of intact human saphenous vein were obtained as discarded tissue from other surgical procedures. The procurement of these tissues conforms to the principles outlined in the Declaration of Helsinki and was approved by the human assurances committee of the Augusta University.

Materials

Tissue sections were probed using the following Antibodies: polyclonal for Bmal1 (Affinty Bioreagents), monoclonal for Clock (Santa Cruz), monoclonal for Npas2 (Abnova), polyclonal for Per1 (Affinty Bioreagents), polyclonal for Cry1 (Novus Biologicals), polyclonal for Rev-erb α (Cell Signaling Technology), polyclonal for Rora (Cell Signaling Technology), monoclonal for Ck1-e (Bectin Dickinson), and polyclonal antibody for Epas (Novus Biologicals), to determine protein expression and localization within the blood vessel.

Western blotting

Excess tissue from human saphenous veins from patients undergoing coronary arterial bypass surgeries (CABG) were transferred to a dish and kept in EBM2 media (Lonza) in the incubator at 37 °C for further processing at serial time points. Vessels were pooled to permit detection of specific proteins, pulverized on liquid nitrogen, and then immersed into protein lysis buffer.

Immunohistochemistry

Vascular tissue samples were dissected from regular wild type C57Bl6 mice, and rapidly embedded for frozen cross-sectioning. Sections were cut at 5 μ m and mounted onto glass slides. Afterwards, Clock components were immunohistochemically detected. Briefly, the indirect avidin biotin-horseradish peroxidase visualization method was used (ABC Standard and Elite, Vector Red, Vector Laboratories, Burlingame, USA). Samples were incubated with the detection primary antibody at the manufacturer's recommended concentration.

RESULTS

To determine the cellular and temporal expression of the vascular clock, we harvested tissues from wild-type mice at 12 AM and 12 PM and conducted immunohistochemical analysis of different circadian clock components. At 12 AM, the common carotid artery, aorta, and femoral artery and vein exhibited Bmal1 expression that was largely delineated in the outer adventitial region of the blood vessel [Figure 1A]. Smooth muscle cell expression and endothelial expression were virtually absent. Similarly, at 12 AM, heart and lung exhibited low expression of Bmal1. At 12:00 PM, adventitial Bmal1 expression was reduced, while smooth muscle and endothelial Bmal1 was increased in carotid, aorta, and femoral artery and vein. In heart, there was increased Bmal1 staining that was also evident in lung. Clock staining exhibited enhanced endothelial positivity at 12 AM and was virtually absent adventitial staining in contrast to Bmal1, but did exhibit increased overall tissue expression at 12 PM similar to Bmal1 [Figure 1B]. Cry 1 exhibited little adventitial staining in carotid arteries at either time point, but medial (smooth muscle) staining was increased at 12 PM, as it was also observed in aorta [Figure 1C]. In femoral artery and vein, in contrast to Bmal1 and Clock, Cry exhibited robust expression at 12 PM in the adventitia. In both heart and lung, Cry1 positive cells were robustly increased at 12 PM, with a punctate nuclear stain. In carotid artery and aorta at 12 AM, casein kinase expression was robust, but restricted to the adventitia, and became diffuse in the media at 12 PM [Figure 1D]. Femoral artery, vein, heart and lung followed expression patterns that aligned with the other circadian clock components. Npas2 exhibited a distinctive adventitial staining in all three vessel beds examined, different from the other clock components that occurred at 12 PM, and exhibited punctate nuclear staining in heart tissue and more diffuse staining in lung [Figure 1E]. Ror exhibited medial staining in the blood vessels but most distinctive was in lung tissue, where epithelial cells of bronchioles were highly positive, unique from other clock components [Figure 1F]. Per1 exhibited a strong medial expression in all vascular tissues [Figure 1G, first 3 panels], and also a diffuse expression in heart and lung [Figure 1G, last 2 panels].

To complement the study, we next assessed circadian clock expression in the human saphenous vein [Figure 2]. Bmal1 and Clock expression were increased throughout the media at 12 PM relative to 12 AM and exhibited strong endothelium staining. Npas2 staining was relatively absent in the saphenous vein, while Per1 exhibited increased expression at 12 AM relative to 12 PM. Cry1 did not exhibit any temporal difference in expression but was expressed throughout the media. Rev-erb α expression was distributed throughout the media and did not exhibit a temporal expression pattern while Rora and Epas were increased in the media at 12 PM. Casein kinase was not robustly expressed in the saphenous but was increased at 12 PM. We then examined saphenous vein expression of the positive limb component Bmal1 and negative limb component Cry and examined expression by western blot [Figure 3]. Bmal1 trough occurred at 6 PM, while Cry peaked at 6 PM, and Bmal1 peaked at 15:00 (3 PM) while Cry1 was at nadir at 18:00, consistent with the antiphase nature of the positive and negative limb components.

DISCUSSION

The circadian clock is a cell synchronized signaling pathway that serves to control timing. Within the cardiovascular system, heart^[17,18], vascular^[2,19,20], lung^[21,22], and even kidney circadian clocks^[23,24] are

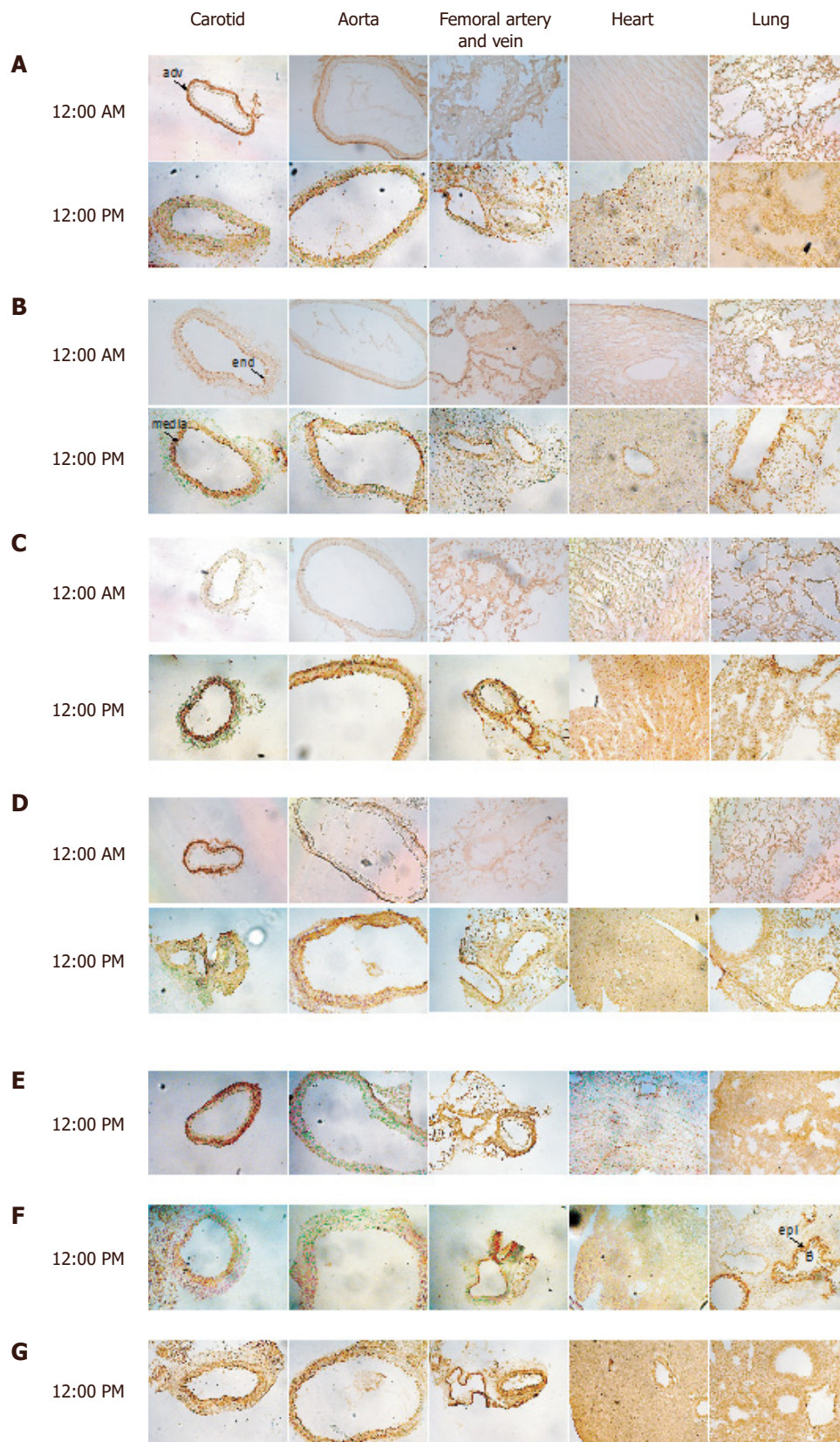


Figure 1. Circadian clock expression in murine cardiovascular tissues. Antibodies to Bmal1 (A), Clock (B), Cry1 (C), Casein kinase (D), Npas2 (E), Rora (F), and Per1 (G) were incubated with frozen carotid artery, aorta, femoral artery/vein, heart, and lung isolated at indicated times of the day to assess localization expression

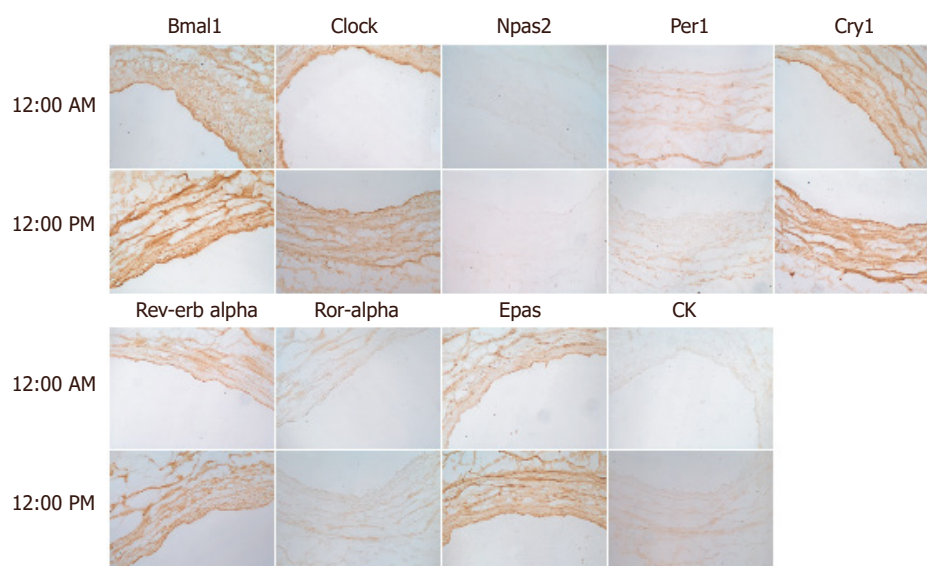


Figure 2. Circadian clock expression in human saphenous vein. Human saphenous veins that remained from coronary artery bypass were immediately procured post-operatively, and incubated in an oxygenated 37 °C incubator. At indicated times, sections of the saphenous vein were frozen in OCT, then sectioned, and incubated with indicated antibodies to the circadian clock

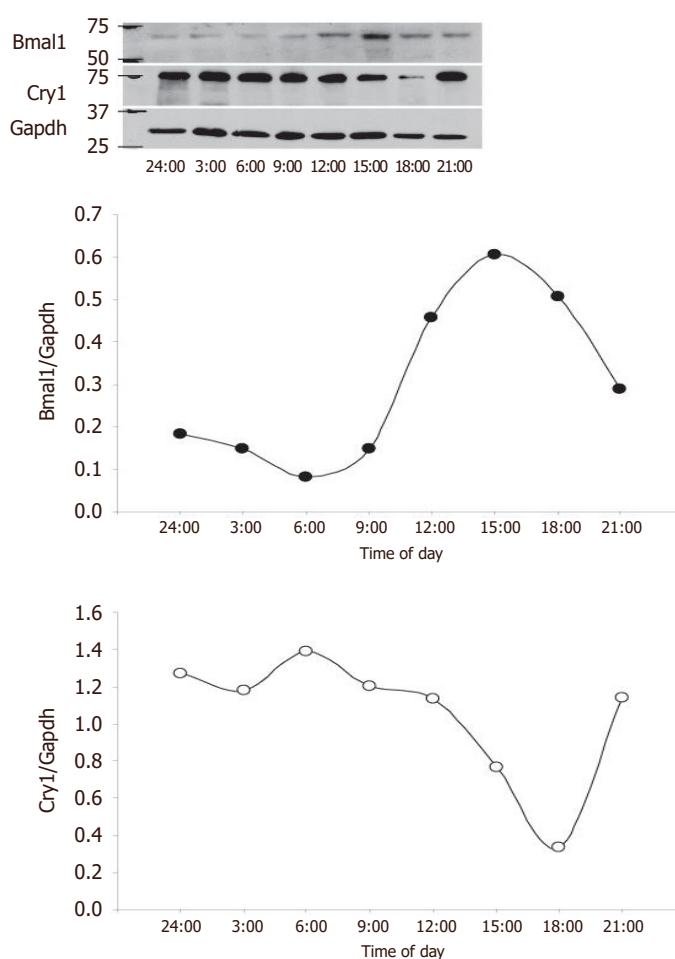


Figure 3. Bmal1 and Cry expression in the human saphenous vein. Human saphenous vein was incubated at 37 °C, and sections were harvested at 3 h intervals from midnight (24:00) to 21:00 (9 PM) for a 21-h time span. At indicated times, saphenous veins were flash frozen and subsequently protein lysates isolated for western blotting (top panel) that was densitometrically quantified (bottom panels)

functional and rhythmic. Because organs are comprised of a heterogeneous population of cells, there are likely cell-specific circadian profiles and expression patterns distinct to the various cell types. Indeed it is known that at the level of the organ, oscillations are different. Central clock oscillation in the SCN is known to oscillate in a different phase than peripheral tissues^[25], whereby the SCN is phase advanced to the kidney, which is phase advanced to aorta which is then phase advanced relative to liver^[26]. The arterial system is composed of three layers; there is an adventitial layer (fibroblasts, pericytes, macrophages)^[27], smooth muscle cell layer (media), and an endothelial cell layer (endothelium). The current study examined clock expression in mouse vascular tissue and the human saphenous vein. The approach we elected to use was immunohistochemistry to study clock expression. One key advantage of immunohistological techniques is the ability to differentiate cellular localization. We in particular were interested in endothelial vs. smooth muscle, vs. the adventitia. To some extent, immunohistochemistry can also provide a rough estimate of nuclear staining or extranuclear staining, but generally distinguishing between subcellular compartments is limited by IHC. In terms of using a fluorescent secondary vs. a chromogenic secondary antibody, both are useful, and generally there can be some background fluorescence in tissues with regard to lamina in the vasculature, so we chose the chromogenic substrate reaction. In mouse, *Bmal1* expression revealed a very strong expression pattern at midnight in the adventitia, in the common carotid artery and aorta and this adventitial staining was decreased at noon. Similarly, casein kinase and *Npas2* were also highly expressed in the adventitia. Conversely, media and endothelium staining for *Bmal1* was stronger at noon, suggesting that the vascular cell layers are uniquely controlled, which may reflect specific timing of the individual cell-type but may also relate to coordination of paracrine signaling from cell-layer to cell layer. In the femoral artery, adventitial staining was most striking for *Npas2* and *Cry1*. Clock the heterodimeric partner to *Bmal1* was not as strongly expressed, but followed a similar temporal profile to *Bmal1* in the carotid and aorta in the media. In the heart, all clock components exhibited nominal staining at 12 AM, but expression was robustly increased at 12 PM. In lung, the bronchiole epithelial cells were highly positive for *Rora*. In the human saphenous vein, *Bmal1* exhibited stronger expression at 12 PM vs. 12 AM, while *Per1* was in antiphase to *Bmal1*. Similarly, by western blotting, *Bmal1* was antiphase to *Cry1*. Interestingly, we have previously found that endothelial mechanisms such as eNOS and Akt follow or mirror circadian clock expression in particular in regions of altered blood flow^[28], while others have demonstrated that eNOS follows the clock in aging^[29]. In the human veins, the clock was also expressed, and although blood pressure is lower in the venous system than arterial, there is also evidence of a circadian rhythm in blood pressure in the venous system^[30]. Another potential significance of the circadian clock in the venous system is that it may also relate to disorders such as orthostatic hypotension. Orthostatic hypotension has a prominent circadian component, which may relate to autonomic input dysfunction^[31] on both the arterial and venous system, and interestingly has emerged as characteristic in Parkinson's disease^[32]. Thus the circadian system may be exerting different functions in the cells within the arterial system and venous system, all which still is largely unknown. Our studies show that circadian clock components display differences in expression and localization throughout the cardiovascular system, which may confer nuances of circadian clock signaling in a cell-specific manner. The bloodstream is a key conduit that relays biomechanical (hypertension) and biochemical information (hypercholesterolemia) from environmental change or disturbance (jet lag, shift work, sleep dysfunction) to the vasculature, while there may also be direct acting clock dampeners such as aging that act on endothelial cells directly to worsen clock function [Figure 4]. These signals may impair function of the clock in ECs to impair other EC's or to impair SMCs, though it is still not clear if EC clocks communicate with SMC clocks and if there is even EC to EC cell communication. Understanding oscillations of the clock in the cellular milieu of the vasculature will be crucial in delineating how clocks can influence pathology of hypertension and atherosclerosis and ultimately permit the development of improved therapeutic approaches that include timing and clocks into maximizing efficacy and treatment.

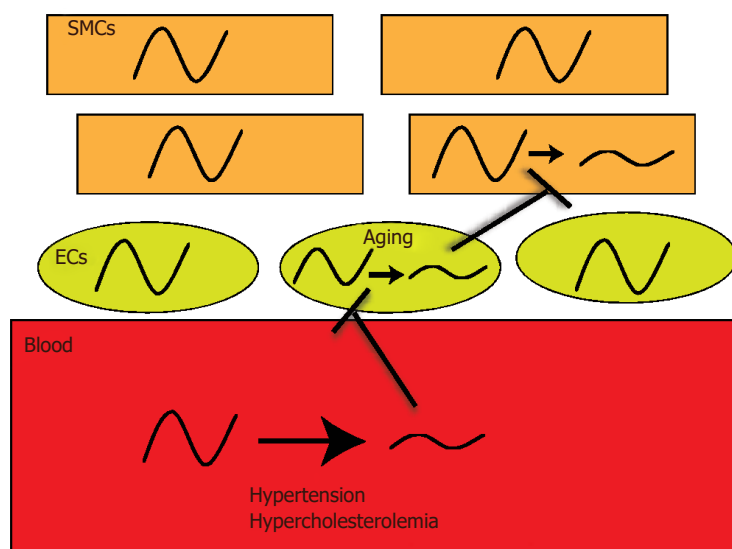


Figure 4. Dysfunctional oscillations in blood, ECs, and SMCs: a potential path to cardiovascular disease. The vasculature has an indirect interaction to the external environment via the bloodstream which is the relay between brain-secreted signals and of course just eating, for example. Changes in biomechanics and biochemistry of blood could impact underlying endothelial and smooth muscle clocks. Aging and other factors could even impact the ECs and SMCs directly. SMCs: smooth muscle cells; ECs: endothelial cells

DECLARATIONS

Authors' contributions

Conducted experiments, and analyzed data: Anea CB, Merloiu AM

Provided reagents and support: Fulton DJR, Patel V, Rudic RD

Designed experiments and wrote the paper: Anea CB, Rudic RD

Availability of data and materials

All data and materials and mice are publicly available.

Financial support and sponsorship

This work was supported by the National Institutes of Health (R01AG054651) and American Heart Association (17GRNT33700216).

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

All human samples (deidentified) were obtained after protocol approval by the Human Assurance Committee of MCG at Augusta University.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

- Davidson AJ, London B, Block GD, Menaker M. Cardiovascular tissues contain independent circadian clocks. *Clin Exp Hypertens* 2005;27:307-11.

2. McNamara P, Seo SB, Rudic RD, Sehgal A, Chakravarti D, Fitzgerald GA. Regulation of CLOCK and MOP4 by nuclear hormone receptors in the vasculature: a humoral mechanism to reset a peripheral clock. *Cell* 2001;105:877-89.
3. Rudic RD, McNamara P, Reilly D, Grosser T, Curtis AM, Price TS, Panda S, Hogenesch JB, Fitzgerald GA. Bioinformatic analysis of circadian gene oscillation in mouse aorta. *Circulation* 2005;112:2716-24.
4. Anea CB, Zhang M, Stepp DW, Simkins GB, Reed G, Fulton DJ, Rudic RD. Vascular disease in mice with a dysfunctional circadian clock. *Circulation* 2009;119:1510-7.
5. Viswambharan H, Carvas JM, Antic V, Marecic A, Jud C, Zaugg CE, Ming XF, Montani JP, Albrecht U, Yang Z. Mutation of the circadian clock gene *Per2* alters vascular endothelial function. *Circulation* 2007;115:2188-95.
6. Somanath PR, Podrez EA, Chen J, Ma YI, Marchant K, Antoch M, Byzova TV. Deficiency in core circadian protein *Bmal1* is associated with a prothrombotic and vascular phenotype. *J Cell Physiol* 2011;226:132-40.
7. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, Fitzgerald GA. Circadian variation of blood pressure and the vascular response to asynchronous stress. *Proc Natl Acad Sci U S A* 2007;104:3450-5.
8. Wang CY, Wen MS, Wang HW, Hsieh IC, Li Y, Liu PY, Lin FC, Liao JK. Increased vascular senescence and impaired endothelial progenitor cell function mediated by mutation of circadian gene *Per2*. *Circulation* 2008;118:2166-73.
9. Pan X, Jiang X-C, Hussain MM. Impaired cholesterol metabolism and enhanced atherosclerosis in clock mutant mice. *Circulation* 2013;128:1758-69.
10. Hogenesch JB, Herzog ED. Intracellular and intercellular processes determine robustness of the circadian clock. *FEBS Lett* 2011;585:1427-34.
11. Liu AC, Welsh DK, Ko CH, Tran HG, Zhang EE, Priest AA, Buhr ED, Singer O, Meeker K, Verma IM, Doyle III FJ, Takahashi JS, Kay SA. Intercellular coupling confers robustness against mutations in the SCN circadian clock network. *Cell* 2007;129:605-16.
12. Qin B, Deng Y. Overexpression of circadian clock protein cryptochrome (CRY) 1 alleviates sleep deprivation-induced vascular inflammation in a mouse model. *Immunol Lett* 2015;163:76-83.
13. Yang L, Chu Y, Wang L, Wang Y, Zhao X, He W, Zhang P, Yang X, Liu X, Tian L, Li B, Dong S, Gao C. Overexpression of CRY1 protects against the development of atherosclerosis via the TLR/NF-kappaB pathway. *Int Immunopharmacol* 2015;28:525-30.
14. Xie Z, Su W, Liu S, Zhao G, Esser K, Schroder EA, Lefta M, Stauss HM, Guo Z, Gong MC. Smooth-muscle BMAL1 participates in blood pressure circadian rhythm regulation. *J Clin Invest* 2015;125:324-36.
15. Westgate EJ, Cheng Y, Reilly DF, Price TS, Walisser JA, Bradfield CA, Fitzgerald GA. Genetic components of the circadian clock regulate thrombogenesis in vivo. *Circulation* 2008;117:2087-95.
16. Cheng B, Anea CB, Yao L, Chen F, Patel V, Merloiu A, Pati P, Caldwell RW, Fulton DJ, Rudic RD. Tissue-intrinsic dysfunction of circadian clock confers transplant arteriosclerosis. *Proc Natl Acad Sci U S A* 2011;108:17147-52.
17. Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ. Extensive and divergent circadian gene expression in liver and heart. *Nature* 2002;417:78-83.
18. Young ME, Razeghi P, Taegtmeier H. Clock genes in the heart - characterization and attenuation with hypertrophy. *Circ Res* 2001;88:1142-50.
19. Anea CB, Zhang MX, Stepp DW, Simkins GB, Reed G, Fulton DJ, Rudic RD. Vascular disease in mice with a dysfunctional circadian clock. *Circulation* 2009;119:1510-7.
20. Davidson AJ, London B, Block GD, Menaker M. Cardiovascular tissues contain independent circadian clocks. *Clin Exp Hypertens* 2005;27:307-11.
21. Gibbs JE, Beesley S, Plumb J, Singh D, Farrow S, Ray DW, Loudon AS. Circadian timing in the lung; a specific role for bronchiolar epithelial cells. *Endocrinology* 2009;150:268-76.
22. Hwang JW, Sundar IK, Yao HW, Sellix MT, Rahman I. Circadian clock function is disrupted by environmental tobacco/cigarette smoke, leading to lung inflammation and injury via a SIRT1-BMAL1 pathway. *FASEB J* 2014;28:176-94.
23. Zuber AM, Centeno G, Pradervand S, Nikolaeva S, Maquelin L, Cardinaux L, Bonny O, Firsov D. Molecular clock is involved in predictive circadian adjustment of renal function. *Proc Natl Acad Sci U S A* 2009;106:16523-8.
24. Gumz ML, Stow LR, Lynch IJ, Greenlee MM, Rudin A, Cain BD, Weaver DR, Wingo CS. The circadian clock protein Period 1 regulates expression of the renal epithelial sodium channel in mice. *J Clin Invest* 2009;119:2423-34.
25. Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Slepka SM, Hong HK, Oh WJ, Yoo OJ, Menaker M, Takahashi JS. PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proc Natl Acad Sci U S A* 2004;101:5339-46.
26. Rudic RD, McNamara P, Reilly D, Grosser T, Curtis AM, Price TS, Panda S, Hogenesch JB, Fitzgerald GA. Bioinformatic analysis of circadian gene oscillation in mouse aorta. *Circulation* 2005;112:2716-24.
27. Majesky MW, Dong XR, Hoglund V, Mahoney WM, Daum G. The adventitia: a dynamic interface containing resident progenitor cells. *Arterioscler Thromb Vasc Biol* 2011;31:1530-9.
28. Shang X, Pati P, Anea CB, Fulton DJ, Rudic RD. Differential regulation of BMAL1, CLOCK, and endothelial signaling in the aortic arch and ligated common carotid artery. *J Vasc Res* 2016;53:269-78.
29. Kunieda T, Minamino T, Miura K, Katsuno T, Tateno K, Miyauchi H, Kaneko S, Bradfield CA, Fitzgerald GA, Komuro I. Reduced nitric oxide causes age-associated impairment of circadian rhythmicity. *Circ Res* 2008;102:607-14.
30. Engel BT, Talan MI. Diurnal variations in central venous pressure. *Acta Physiol Scand* 1991;141:273-8.
31. Mann S, Altman DG, Raftery EB, Bannister R. Circadian variation of blood pressure in autonomic failure. *Circulation* 1983;68:477-83.
32. Berganzo K, Diez-Arrola B, Tijero B, Somme J, Lezcano E, Llorens V, Ugarriza I, Ciordia R, Gomez-Esteban JC, Zarranz JJ. Nocturnal hypertension and dysautonomia in patients with Parkinson's disease: are they related? *J Neurol* 2013;260:1752-6.

Review

Open Access



Who is the next for aortic valve implantation? Present and future indications

Giuseppe Verolino, Alessia Delli Veneri, Myriam Carpenito, Francesco Piccirillo, Leonardo Aurino, Annunziata Nusca

Department of Cardiac Sciences, Campus Bio-Medico University of Rome, Rome 00128, Italy.

Correspondence to: Dr. Annunziata Nusca, Unit of Cardiovascular Science, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 200, Rome 00128, Italy. E-mail: a.nusca@unicampus.it

How to cite this article: Verolino G, Delli Veneri A, Carpenito M, Piccirillo F, Aurino L, Nusca A. Who is the next for aortic valve implantation? Present and future indications. *Vessel Plus* 2018;2:17. <http://dx.doi.org/10.20517/2574-1209.2018.32>

Received: 14 May 2018 **First Decision:** 5 Jul 2018 **Revised:** 14 Jul 2018 **Accepted:** 19 Jul 2018 **Published:** 2 Aug 2018

Science Editor: Cristiano Spadaccio, Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

Aortic valve stenosis (AS) represents the most prevalent valvular defect worldwide. It is a progressive disease with a long latency interval and a poor prognosis after symptoms present. According to current European Society of Cardiology guidelines, transcatheter aortic valve implantation (TAVI) is recommended in all patients with severe symptomatic AS and a predicted survival longer than one year, who are not suitable for surgical valve replacement. Despite these recommendations, several studies over the past few years suggest extending these indications towards lower risk AS populations. Otherwise, current available operative risk scores such as Society of Thoracic Surgeons score and EuroSCORE, may offer an incomplete risk assessment; in this setting, the Heart Team plays a crucial role in defining the most appropriate therapeutic strategy in patients with AS. In this review, we aim to discuss the current and future indications for TAVI, analyzing available literature according to patients' profile risk (high/mid/low risk) and other specific conditions (valve-in-valve, bicuspid valve and pure aortic regurgitation).

Keywords: Aortic valve implantation, aortic stenosis, structural valve intervention, bicuspid valve, valve-in-valve

INTRODUCTION

Aortic valve stenosis (AS) is the most prevalent valvular defect worldwide. It mainly affects elderly patients; it is a progressive disease with a long latency phase that, however, has a poor prognosis after symptoms of dyspnoea, angina or syncope occur. Despite significant strides in medical therapy for several other cardiovascular pathological conditions, little progress has been made in medical therapy for AS. Observational studies demonstrated a mortality rate of 75% in patients with AS within 3 years of the symptom onset unless



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



the outflow obstruction is removed by aortic valve intervention^[1]. Surgical aortic valve replacement (SAVR) has been the gold standard treatment for a long time; however, with ageing and increasing multimorbidity of AS population, the need for a less invasive approach was clearly identified in the first European Heart Survey, where a significant number of patients, about 42%, were not referred or accepted for surgery^[2]. The introduction of percutaneous treatment for severe AS with transcatheter aortic valve implantation (TAVI) remains one of the latest and greatest achievements in interventional cardiology. Since Cribier *et al.*^[2] reported the first “proof-of-concept” case of TAVI in 2002, more than 200,000 patients have undergone this procedure in approximately 65 countries. Starting off as a new approach for high-risk patients, TAVI has nowadays proved to be the best strategy in frail patients and is becoming increasingly seen as a very interesting option for those with intermediate risk. Recently, increased operator experience and improved device systems have led to consider the extension of this therapeutic strategy also to low-risk patients. Thus, in this intriguing setting, this review summarizes the present and future indications of TAVI.

CURRENT GUIDELINES RECOMMENDATIONS

The current guidelines of the European Society of Cardiology (ESC)/European Association for Cardio-Thoracic Surgery (EACTS) on valvular heart diseases established that the choice for intervention, SAVR *vs.* TAVI, should be based on a careful evaluation of patients’ procedural risk and technical suitability, thus a precise assessment of risks/benefits balance of each modality^[3]. Importantly, local expertise and outcomes data for both surgical and percutaneous intervention must be carefully evaluated and the Heart Team has to play a fundamental role in the final therapeutic decision of AS patients. Thus, the selection of TAVI *vs.* SAVR should involve a multidisciplinary discussion between cardiologists, surgeons, imaging specialists, anesthesiologists, and other specialists if necessary^[3].

In view of this, according to ESC guidelines, TAVI is recommended in all patients with severe symptomatic AS and a predicted survival greater than one year who are not eligible for SAVR (Class IB)^[3]. Data supporting this indication has been presented in many European registries such as the CoreValve Extreme Risk Registry and from the randomized Placement of AoRTic TraNscathetER valves (PARTNER I B) study^[4-7]. However, in this setting, the definition of “inoperable patient” has been problematic because it comes from score systems usually used for surgical population (Society of Thoracic Surgeons - STS or EuroSCORE II), that are not able to capture all comorbidities that make a patient an inadequate candidate for conventional surgery. Over the last few years, it has become clear that other factors such as frailty and anatomical features (porcelain aorta, “hostile chest”, liver disease, substernal location of a mammary graft) needed to be considered. Thus, the central element to evaluate whether patients are at high risk for surgery remains clinical judgment: the ability to integrate a quantitative assessment based on the traditional surgical risk scores and other important clinical features observed in the “real world” AS population but not included within score systems.

The current ESC guidelines also recommend that TAVI should be considered an alternative to SAVR in severe AS patients who are at high risk for mortality and complications after conventional surgery, thus those with STS or EuroSCORE II $\geq 4\%$ or logistic EuroSCORE I $\geq 10\%$ ^[3]. Of note, TAVI should be favored in elderly patients eligible for transfemoral access as suggested by registries and two important randomized controlled trials comparing TAVI *vs.* SAVR: the PARTNER I A trial, using a balloon-expandable device, and the CoreValve High-Risk study with a self-expandable valve^[8,9]. Similar recommendations are reported by the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines for the management of patients with valvular heart disease^[10]. They recommend a global risk assessment resulting in a 4-group classification (low, intermediate, high and prohibitive risk) according to STS score value ($< 4\%$ in low risk, between 4% and 8% in intermediate risk, $> 8\%$ in high risk), presence or not of frailty, impairment in 1 or more major organ systems (no comorbidity in low risk, 1 organ system in intermediate risk, 2 in high

Table 1. Indications for aortic valve implantation according to the ESC/EACTS and ACC/AHA

ESC/EACTS Guidelines	ACC/AHA Guidelines
The choice for intervention must be based on careful individual evaluation of technical suitability and evaluating of risks and benefits of each modality. In addition, the operators' expertise and outcomes data for the given procedure must be taken into account (I C)	TAVR is recommended for symptomatic patients with severe AS and high risk for SAVR, depending on patient specific procedural risk, values and preferences (I A)
Aortic valve implantation is recommended in patients who are not suitable for SAVR as assessed by the Heart Team (I B)	TAVR is recommended for symptomatic patients with severe AS, extremely high risk for SAVR, and predicted post-procedure survival greater than 12 months (I A)
In patients who are at increased surgical risk (STS or EuroSCORE II $\geq 4\%$ or logistic EuroSCORE I $\geq 10\%$ or other risk factors not included in these scores (frailty, porcelain aorta, post-radiation chest), the choice between surgery or aortic valve implantation should be made by the Heart Team in consideration of individual patient features, with aortic valve implantation being encouraged in elderly patients suitable for transfemoral access (I B)	TAVR is a reasonable alternative to SAVR for symptomatic patients with severe AS and intermediate surgical risk, depending on patient-specific procedural risk, values and preferences (IIa B)
Aortic valve procedures should only be performed in centres with Cardiology Unit and Cardiovascular Surgery Unit on site and with structured collaboration between the two, including a Heart Team (heart valve centres) (IC)	For severely symptomatic patients with bioprosthetic stenosis or regurgitation at high of prohibitive risk for reoperation, and in whom improvement in hemodynamics is anticipated, valve in valve TAVR is feasible (IIa B)

ACC: American College of Cardiology; AHA: American Heart Association; AS: aortic stenosis; EACTS: European Association for Cardio-Thoracic Surgery; ESC: European Society of Cardiology; STS: Society of Thoracic Surgeons Risk Score; TAVR: transcatheter aortic valve replacement

risk) and procedural impediments (absent in low risk and minimal or possible in intermediate and high risk, respectively). The fourth group (prohibitive risk) doesn't consider STS risk score, but pre-operative risk of mortality and morbidity at 1 year $> 50\%$, ≥ 3 compromised major organ systems not to be improved postoperatively, severe frailty, or severe impediments linked to procedure^[10].

According to this classification, TAVI is recommended in patients with prohibitive surgical risk and is considered a reasonable alternative to conventional surgery in those with high risk. In Table 1, indications for TAVI according to the ESC/EACTS and ACC/AHA are reported.

HIGH-RISK PATIENTS

Untreated AS has been conventionally considered a terminal condition for patients refusing high-risk surgical valve replacement or those deemed not operable candidates by treating physicians^[11]. Surgical aortic valve replacement has demonstrated to improve symptoms and long-term prognosis; however, observational studies identified subgroups of patients, such as those elderly or with reduced ejection fraction, that are at increased surgical risk for procedural complications or death anyway^[12]. As regards these patients, a less invasive treatment would be a desirable alternative^[8] and, for this reason, over the last decade TAVI has been identified as the standard of care for high-surgical risk patients or for those considered inoperable by surgeons. TAVI has demonstrated the potential to decrease the morbidity associated with standard SAVR owing to the avoidance of a median sternotomy, cardiopulmonary bypass and cardioplegic arrest. Nevertheless, the selection process towards TAVI needs thoughtful consideration of risks and benefits of the procedure and a comparison of these factors with alternative therapies^[13]. The EACTS/ESC Guidelines recommend four main steps for patient selection before TAVI procedure: severity of valve stenosis and symptom confirmation, assessment of the technical feasibility, exclusion of contraindications, and accurate clinical examination for surgical risk assessment based on validated scores^[14].

Also given these guidelines, a EuroSCORE $> 20\%$ ^[15] or a STS $> 10\%$ ^[16] has been used to identify "high-risk" patients. It has also been recognized that factors such as frailty, associated with adverse outcomes and actu-

ally not incorporated within current models^[17], should be considered. Of note, frailty is related to different factors and several scores have been proposed to measure it. Therefore, Anand *et al.*^[18] indicate frailty evaluation like a marker of physiological reserve and concomitantly a prognostic index. An overall assessment by score systems also led to identification of patients in whom aortic valve replacement would be futile. Nishimura *et al.* recognized that valve interventions are likely to be futile in patients with a life expectancy less than 1 year or a likelihood of “survival with benefit” less than 25% at 2 years (improvement in quality of life or life expectancy or improvement in heart failure or angina class)^[19].

Several randomized trials reported similar outcome results between TAVI and SAVR in the high-risk population and the benefit of a percutaneous strategy in patients not suitable for surgery compared with optimal medical treatment. The PARTNER I was the first large randomized trial conducted using a balloon-expandable device (Edwards Sapien Valve) to test the effectiveness of TAVI and the publication of its 1-year outcomes has really redefined the conventional wisdom^[8,20]. Patients were divided into two groups: the first one with patients who were considered available for surgery although a high surgical risk (Cohort A, $n = 699$, STS risk score $> 10\%$ or a predicted risk of death by 30 days after surgery of 15% or higher derived from other comorbidities), and the second one (Cohort B, $n = 358$) with patients who have a real contraindication for surgery because of coexisting conditions that would be associated with a predicted probability of 50% or more of either death by 30 days after surgery or a serious irreversible condition^[21]. In the Cohort A, patients undergoing TAVI showed similar 1-year all-cause mortality rate compared with SAVR group (24.2% vs. 26.8%; $P = 0.001$ for non-inferiority)^[11]. Reported 5-year results were comparable as well, with a mortality rate observed in 67.8% of patients receiving TAVI vs. 62.4% of those treated with SAVR ($P = 0.76$)^[22]. The prevalence of stroke was 3.8% and 2.1%, respectively ($P = 0.2$), although for all neurologic events, the difference between TAVI and SAVR was significant ($P = 0.04$), including 4.6% for femoral artery access transcatheter replacement vs. 1.4% for open surgery ($P = 0.05$). In patients deemed not to be candidates for surgery (Cohort B), TAVI was superior to standard therapy with significantly reduced 1-year all-cause mortality (30.7% vs. 50.7%; $P = 0.001$)^[11]. Recently 5-year follow-up data for this cohort have been reported; principal findings were a mortality rate of 71.8% in the TAVI group compared with 93.6% of patients on medical therapy. Similarly, cardiovascular mortality (57.5% vs. 85.9%, HR 0.41, 95% CI: 0.31-0.55, $P < 0.0001$) and repeat hospitalizations (47.6% vs. 87.3%, $P < 0.0001$) were significantly lower in the TAVI arm at 5 years^[23]. The Medtronic CoreValve US Pivotal Trial confirmed these results; in this series, the self-expandable Medtronic CoreValve implantation was associated with a 40% reduction in the primary endpoint (combined of all-cause mortality and stroke) at 1-year follow-up in the “extreme risk” arm^[9]. Also, several registries reflecting real-life TAVI experience confirmed the effectiveness and safety of TAVI in elderly patients with AS, considered at high-risk and in whom the percutaneous approach appeared to be a reasonable and convincing option. The FRANCE 2 Registry enrolled all TAVI performed in 34 centers in France and Monaco, without selection bias^[5], including 3195 procedures; of those, 80.4% were percutaneous and 19.6% were surgical. The overall 30-day mortality was 9.7% (8.5% in the transfemoral group and 13.9% in the transapical group), a percentage closer to those previously reported by other registries^[24,25] but higher compared with randomized trials, probably related to high-risk profile of enrolled patients^[11]. On the other hand, the 1-year survival rate of 76% was comparable with randomized data. At the multivariate analysis, logistic EuroSCORE, NYHA functional class III or IV, the use of transapical approach and the presence of post-procedural periprosthetic regurgitation grade of 2 or more have been demonstrated independent predictors of mortality after 1-year from procedure.

Thus, according to these findings and guideline recommendations for the management of surgical high-risk patients, it is not surprising that this technique has found widespread use in this subset of patients.

INTERMEDIATE AND LOW RISK PATIENTS

Recently several studies have focused their attention on the large proportion of AS population defined at

intermediate-risk (STS risk score between 4% and 8%) and at low-risk (STS risk score < 4%)^[26,27]. Under current guidelines, the choice to perform TAVI for that group of patients should be allowed only after a specific cases selection by the Heart Team and, importantly, in centers with well-established procedural training. Many trials are ongoing to demonstrate real advantage of TAVI in low risk subset but others have already been published which show the true benefits for intermediate risk patients.

The PARTNER II was the first large randomized trial that evaluated the outcome of TAVI in intermediate surgical risk population^[28]. In this study Leon *et al.*^[28] randomized about 2000 patients with severe AS and STS score between 4% and 8% to TAVI [mean STS- Prediction of Mortality (PROM) 5.8%] or SAVR. At 24-month follow-up, the composite endpoint of all-cause death or disabling stroke was 19.3% in the TAVI group compared with 21.1% in those treated with SAVR ($P = 0.25$). Of note, a significant interaction between TAVI approach and mortality was observed, with transfemoral TAVI showing superiority over SAVR (HR 0.79; 95% CI: 0.62-1.00; $P = 0.05$). Larger aortic valve areas, lower rates of acute kidney injury, severe bleeding, and new-onset atrial fibrillation were also reported in TAVI group, whereas, SAVR caused fewer major vascular complications and less paravalvular aortic regurgitation^[28]. In the Surgical Replacement and Transcatheter Aortic Valve Implantation trial (SURTAVI), Reardon *et al.*^[29] confirmed the feasibility of TAVI in patients at intermediate risk (mean STS-PROM of 4.4%). They observed an incidence of the same end point chosen in the PARTNER II trial (overall death and stroke) of 12.6% in patients receiving TAVI and of 14% in those undergoing conventional surgery. A pre-specified analysis of the single components of the primary end point has shown a similar rate of all-cause death between the two treatment strategies (11.4% and 11.6%), whereas, at 24 months follow-up, a numerically lower rate of disabling stroke was found in the TAVI group compared with the surgical group, although the difference was not significant (4.5% *vs.* 2.6%)^[29]. Similar positive results have been demonstrated also in low-risk AS population. In the Nordic Aortic Valve Intervention Trial (NOTION), Thyregod *et al.*^[30] randomized 280 low-risk AS patients to TAVI or SAVR. The primary endpoint of death for any causes, myocardial infarction and stroke after 1 year was observed in 13.1% of the TAVI group and 16.3% of the SAVR group. The “intention to treat” analysis confirmed these results (11.3% *vs.* 15.7%, respectively in TAVI and SAVR groups). Moreover, the 5-year follow-up of the NOTION trial was recently reported at the latest ACC Congress^[31]. After five years, there were no differences in the incidence of the composite primary end point (39.2% of TAVI patients and 35.8% of SAVR patients). Looking at the endpoints individually, the rate of all-cause mortality was 27.7% for both SAVR and TAVI. In addition, stroke incidence was 10.5% in TAVI patients and 8.2% in those receiving SAVR, while 8.7% and 8.6% of patients had experienced a myocardial infarction in the TAVI and SAVR groups, respectively. These results were also observed for patients with STS score less than 4%^[31]. Similar evidence was reported by Wenaweser *et al.*^[32] comparing clinical outcomes of patients with intermediate/low-risk and high-risk patients undergoing TAVI. Patients with severe AS receiving TAVI showed a mortality rate of 3.9% and 2.4%, respectively for mid and low risk groups ($P < 0.001$); these data are extremely positive and promising, especially in consideration of the expected mortality estimates of 6.8% according to STS-PROM score. Finally, in the Italian registry Observational Study of Effectiveness of SAVR-TAVI Procedures for Severe Aortic Stenosis Treatment (OBSERVANT)^[33], enrolling patients surgical and TAVI treated, the 1-year mortality was not different in the two groups (13.6% *vs.* 13.8%; $P = 0.936$), even if in the first 90 days after aortic procedure, mortality was numerically greater in patients receiving SAVR compared with TAVI. Furthermore, in this last group, the rate of rehospitalization for cardiac symptoms and episodes of acute heart failure was lower (21.9% *vs.* 23.6% and 19.0% *vs.* 19.7%, respectively)^[33]. Several trials such as PARTNER 3 and NOTION 2 are ongoing and could probably confirm the effectiveness and safety of transcatheter aortic approach compared with surgery also in the low-risk subgroup.

However, although results of previously reported studies are very encouraging, nobody can demonstrate a statistically relevant result that favours choosing TAVI in every patient, irrespective of risk profile. Moreover,

major complications associated with TAVI should be considered such as the need for permanent pacemaker (PMK) (25.9% at 30 days in the SURTAVI trial and 18.5% at 1 year in the OBSERVANT trial) and the development of paravalvular leaks, with an incidence of about 1% according to the recent literature findings^[34]. Probably with increased operators' experience, longer learning curve and newer generation devices, these complications could be reduced. However, rehospitalization and mortality rates of patients undergoing PMK after TAVI were not higher compared with those who did not develop this complication. Moreover, only a small percentage of patients treated with TAVI has undergone a new procedure (valve-in-valve) or shifted to open surgery due to the development of large paravalvular leaks^[35]. On the other side, two important elements arise from all these studies in favor of TAVI: reduced hospitalization time (in days) and lower incidence of new onset atrial fibrillation (AF). Length to stay in hospital is significantly shorter for TAVI patients than SAVR: in particular, 4 vs. 10 days was reported by Garcia *et al.*^[36] and 8.9 vs. 12.9 days in the NOTION trial^[31]. Similar results were observed also in the OBSERVANT registry (8.8 vs. 12.6 days, $P < 0.001$)^[33]. In this century where spending review is a real concern, reduction of average length of stay in hospital is an important issue; obviously, no less important is an earlier patient's return to home with an overall positive advantage for public health (healthcare infection, lodging). On other hand, AF is the most frequent rhythm disease with several aspects that significantly deteriorate patient quality of life and long-term prognosis. Several studies showed that SAVR is burdened with a higher incidence of AF compared with TAVI; in the NOTION study, about 59.4% of patients developed AF after 1 year from surgery compared with only 21.2% of patients undergoing TAVI ($P < 0.001$)^[31]. Leon *et al.*^[28] confirmed these findings in the PARTNER 2 trial reporting an incidence of new onset AF of 11.3% and 27.3% in the TAVI and SAVR groups, respectively. In the SURTAVI trial, the incidence of AF was higher after SAVR (43% after 30 days) than in the TAVI group^[29].

Finally, another important issue that should be considered for TAVI as a routinely procedure especially in low-risk population is valvular degeneration. Actually, poor data are available to define the durability of prostheses in young patients with life expectancy > 20 years. Otherwise, there is not yet a clear definition of "prosthesis degeneration". One of those currently used, according to Valve Academic Research Consortium (VARC 2) definition, is based on specified echocardiographic criteria: mean aortic valve gradient ≥ 20 mmHg, EOA (effective orifice area) ≤ 0.9 - 1.1 cm², DVI (Doppler velocity Index) < 0.35 m/s, and/or moderate or severe prosthetic valve regurgitation^[34]. Thus, a specific trial is needed to evaluate long-term outcomes in a selected population with low-risk profile. Surely TAVI may be considered even as first option in a population with intermediate-risk and, where patient agrees, also in a low risk profile.

PATIENTS WITH BICUSPID AORTIC VALVE

Bicuspid aortic valve (BAV) is one of the most common congenital valve abnormalities, occurring in 0.7%-2% of the general population. BAV is related to a higher valve shear stress, favouring leaflet calcification and degeneration and AS and/or aortic regurgitation (AR) development^[37]. BAV has been considered for a long time as a relative contraindication to TAVI, first of all for the higher expected risk for relevant AR. Furthermore, the unfavorable anatomy of BAV may interfere with the appropriate positioning and expansion of the prosthetic valve, theoretically increasing the incidence of procedural complications as well as decreasing the efficacy and durability of the prosthetic valve^[8,20].

Different studies were conducted to evaluate the relative benefits of TAVI in patients with BAV. Bauer *et al.*^[38], within the German TAVI Registry, prospectively enrolled 1424 patients with severe AS undergoing TAVI from January 2009 to June 2010. They compared TAVI outcomes in patients with BAV ($n = 38$, 3%) and those with tricuspid aortic valve (TAV) (TAV; $n = 1357$, 97%). They observed that PMK implantation occurred more frequently in patients with TAV (17% vs. 35%, $P = 0.02$), whereas a greater rate of relevant AR was observed among patients with BAV after the transcatheter procedure (25% vs. 15%, $P = 0.05$). Of note, despite the higher risk for relevant AR among patients with BAV compared with those with TAV, 30-day and 1-year mortality rates were similar in both subsets of patients^[38]. A systematic review and meta-analysis conducted

by Phan *et al.*^[39] analyzed seven studies including a total of 2245 patients (149 with BAV and 2096 with TAV) undergoing TAVI. According to their results, no difference was observed in 30-day mortality between BAV and TAV groups. Moreover, no difference was found in post-TAVI mean peak gradients, grading of paravalvular leaks (moderate or severe) (25.7% vs. 19.9% respectively, $P = 0.29$), PMK implantations rate (18.5% vs. 27.9%, $P = 0.52$), life-threatening bleeding (8.2% vs. 13.9%, $P = 0.33$) and major bleeding (20% vs. 16.8%, $P = 0.88$). Furthermore, need for conventional open-heart surgery (1.9% for BAV group and 1.2% for TAV group, $P = 0.18$) and occurrence of vascular complications (8.6% and 10.1% respectively, $P = 0.32$) were also comparable between the two groups^[39]. Confirming these findings, Sannino *et al.*^[40] investigated the efficacy and safety of TAVI in BAV population. From January 2012 to February 2016, 823 consecutive patients with severe AS (735 with TAV and 77 with BAV) undergoing TAVI were retrospectively enrolled. Definition of a successful procedure was established assessing postprocedural valve function, thus measuring echocardiographic parameters such as mean gradient, peak velocity, effective orifice area and evaluating the presence of paravalvular leak at least of moderate degree. Safety was evaluated by 30-day and 1-year mortality for any causes, immediate postprocedural mortality and 30-day cardiovascular mortality, procedural success, pacemaker implantation, and development of procedural complications. No significant differences in in-hospital mortality (1.1% in BAV group vs. 0.8% in TAV group), 30-day cardiovascular mortality (3.4% vs. 2.3%), 30-day all cause mortality (3.4% vs. 3.1%) and 1-year all cause mortality (8.5% vs. 10.5%) were found between the two groups^[40].

In conclusion, according to the current evidence, bicuspid anatomy should not be excluded from TAVI: transcatheter valve replacement is deemed a safe and useful procedure both in inpatients with TAV and in those with BAV.

PATIENTS WITH PURE AORTIC REGURGITATION

Nowadays, TAVI procedure for native AR treatment has a marginal role, mainly as “off-label” application in very high-risk patients. TAVI for pure native AR has been shown to be more difficult and associated with lower procedural success, safety, and clinical efficacy rates^[41]. Possible explanations for this limited success are related to the concept that a certain amount of aortic annulus and/or valve calcification is necessary to anchor a balloon- or self-expandable transcatheter valve prosthesis into the annulus^[2]. However, limited data exist about safety and efficacy of TAVI in this specific subset of patients, mainly reported in several case reports and small clinical studies. Yoon *et al.*^[42] enrolled 331 patients with symptomatic, severe pure native AR undergoing TAVI across 40 participating centers between September 2007 and February 2017. The aim of this study was to compare the outcomes of TAVI with early- and new-generation prosthesis devices in symptomatic patients with pure AR. Compared to the early-generation devices, the new ones were associated with a significantly higher device success rate (81.1% vs. 61.3%, $P < 0.001$) due to lower rates of second valve implantation and post-procedural AR (more than moderate). The cumulative rates of all-cause and cardiovascular death at 1-year follow-up were similar and post-procedural AR was independently associated with worse outcomes^[42]. Similar findings were showed by Franzone *et al.*^[43] that confirmed TAVI as technically feasible in AR patients, with acceptable early morbidity and mortality rates^[43]. Overall, the rates of complications and of residual moderate or severe AR were low; the main complication was the need of a second valve implantation^[43]. Furthermore, in a large international registry, Sawaya *et al.*^[44] described their experience with TAVI when used to treat patients with severe AR, both those with native AR and those with failing bioprosthetic surgical heart valves ($n = 146$, 78 with native AR and 68 with failing surgical devices). Also in this study, TAVI for pure AR was associated with higher occurrence of device embolization/migration and significant paravalvular regurgitation especially with old-generation transcatheter heart valves. However, new-generation devices significantly improved procedural success and clinical efficacy compared to old ones, suggesting that also in patients with failing surgical prostheses, TAVI may represent a valuable therapeutic option (85% vs. 54% and 75% vs. 46%, respectively $P < 0.05$)^[44]. However, further studies and new

device technology are needed before considering TAVI as a reasonable treatment option for pure AR.

PATIENTS WITH DEGENERATIVE BIOPROSTHETIC SURGICAL VALVES: THE VALVE IN VALVE STRATEGY

As previously reported, TAVI is a useful procedure for the treatment of patients with native AS judged to have high/intermediate surgical risk^[20,45,46]. However, over the last years, another “off-label” use of TAVI is also emerging in practice: the so-called “valve in valve” (VIV)^[47,48]. Bioprostheses are known to have a limited durability, degenerating in a period of about 10-20 years^[49]. Although reoperation (“redo” surgery) is considered the gold standard treatment for this population, it represents a procedure associated with high risk of morbidity and mortality. Maganti *et al.*^[50] observed a poor 5-year survival ($67\% \pm 5\%$) in a cohort of patients aged 75 years or older who underwent redo valve surgery. According to these findings, transcatheter aortic VIV implantation has emerged as a viable and less-invasive technique to be used in this setting and to obviate the need for reoperation. Despite being a technique still in progress, performed especially in specialized centers, a worldwide register is now available (Global Valve-in-Valve Registry)^[51], aiming to evaluate the effectiveness and clinical results of this technique in a wide cohort of patients. Before the creation of this register, previous studies investigating the VIV technique included only a small number of cases and were therefore limited in providing conclusive results. Several technical aspects have to be considered in VIV-TAVI for a failing surgical bioprosthesis such as different radiopaque markers and different implantation techniques. Moreover, some complications, also potentially life threatening and dangerous, such as elevated postprocedural gradients and ostial coronary obstruction, have been reported anecdotally and sporadically, during this type of procedure^[52,53]. This registry started in December 2010, with a total of 38 participating centers (Europe, North America, Australia *etc.*). The register contains data and procedural results from the mentioned centres that have experience of TAVI with the use of both balloon and self-expandable devices. Preliminary results were reported including 202 patients with degenerated bioprosthetic valves (aged 77.7 ± 10.4 years; 52.5% men)^[51]. Bioprosthesis mode of failure was stenosis ($n = 85$; 42%), regurgitation ($n = 68$; 34%), or both ($n = 49$; 24%). Two devices have been implanted: Medtronic CoreValve® ($n = 124$) and Edwards SAPIEN® ($n = 78$). Procedural success was achieved in 93.1% of patients. Device malposition occurred in 15.3% of cases whereas ostial coronary obstruction was observed in 3.5% of patients. Post-procedural valve maximum and mean gradient were 28.4 ± 14.1 mmHg and 15.9 ± 8.6 mmHg respectively; about 95% of patients developed $\leq +1$ degree AR. All-cause 30-day mortality was 8.4%, whereas, 1-year follow-up showed a survival rate of 85.8%.

These preliminary data emerging from the Global Valve-in-Valve Registry allows us to consider the VIV strategy a possible alternative to conventional redo surgery in patients with degenerated bioprosthetic valves. Device malposition, ostial coronary obstruction, and post-procedural aortic stenosis remain important concerns against the routine application of this procedure. Thus, other studies and longer follow-up are needed to confirm its safety and efficacy in clinical practice.

CONCLUSION

TAVI may be considered as “first choice” and not as last chance in many patients with AS. TAVI has emerged as the standard of care in inoperable/high risk patients whereas several randomized trials have demonstrated similar results of TAVI compared with SAVR also in the intermediate/low risk subset. Moreover, the feasibility and safety of TAVI has been suggested also in patients with aortic bicuspid valve, pure AR and degenerative bioprosthetic surgical valves. Importantly, a careful risk assessment through surgical scores (STS, EuroSCORE *etc.*) is crucial, but other variables need to be considered such as frailty profile and specific anatomical elements (hostile chest, porcelain aorta). These factors are relevant for procedural success and long-term survival, but not included in the conventional scores possibly leading to underestimated risk classification. Surely improved procedural and technical experience, associated with an enhanced healthcare

organization, will cause a widespread extension of TAVI indication; on the other hand, prosthesis durability remains an important issue and a longer follow-up is needed to confirm the effectiveness of TAVI in different settings.

DECLARATIONS

Author's contributions

All authors provided substantial contributions to conception, design, drafting the article or revising it critically for important intellectual content.

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Ross J Jr, Braunwald E. Aortic stenosis. *Circulation* 1968;38:61-7.
2. Cribier A, Eltchaninoff H, Bash A, Borenstein N, Tron C, Bauer F, Derumeaux G, Anselme F, Laborde F, Leon MB. Percutaneous transcatheter implantation of an aortic valve prosthesis for calcific aortic stenosis: first human case description. *Circulation* 2002;106:3006-8.
3. Baumgartner H, Falk V, Bax JJ, De Bonis M, Hamm C, Holm PJ, Iung B, Lancellotti P, Lansac E, Rodriguez Muñoz D, Rosenhek R, Sjögren J, Tornos Mas P, Vahanian A, Walther T, Wendler O, Windecker S, Zamorano JL; ESC Scientific Document Group. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. *Eur Heart J* 2017;38:2739-91.
4. Duncan A, Ludman P, Banya W, Cunningham D, Marlee D, Davies S, Mullen M, Kovac J, Spyt T, Moat N. Long-term outcomes after transcatheter aortic valve replacement in high-risk patients with severe aortic stenosis: the U.K. Transcatheter Aortic Valve Implantation Registry. *JACC Cardiovasc Interv* 2015;8:645-53.
5. Gilard M, Eltchaninoff H, Iung B, Donzeau-Gouge P, Chevreul K, Fajadet J, Leprince P, Leguerrier A, Lievre M, Prat A, Teiger E, Lefevre T, Himbert D, Tchetché D, Carrié D, Albat B, Cribier A, Rioufol G, Sudre A, Blanchard D, Collet F, Dos Santos P, Meneveau N, Tirouvanziam A, Caussin C, Guyon P, Boschat J, Le Breton H, Collart F, Houel R, Delpine S, Souteyrand G, Favereau X, Ohlmann P, Doisy V, Grollier G, Gommeaux A, Claudel JP, Bourlon F, Bertrand B, Van Belle E, Laskar M; FRANCE 2 Investigators. Registry of transcatheter aortic-valve implantation in high-risk patients. *N Engl J Med* 2012;366:1705-15.
6. Walther T, Hamm CW, Schuler G, Berkowitsch A, Kötting J, Mangner N, Mudra H, Beckmann A, Cremer J, Welz A, Lange R, Kuck KH, Mohr FW, Möllmann H; GARY Executive Board. Perioperative results and complications in 15,964 transcatheter aortic valve replacements: prospective data from the GARY registry. *J Am Coll Cardiol* 2015;65:2173-80.
7. Popma JJ, Adams DH, Reardon MJ, Yakubov SJ, Kleiman NS, Heimansohn D, Hermiller J Jr, Hughes GC, Harrison JK, Coselli J, Diez J, Kafi A, Schreiber T, Gleason TG, Conte J, Buchbinder M, Deeb GM, Carabello B, Serruys PW, Chenoweth S, Oh JK; CoreValve United States Clinical Investigators. Transcatheter aortic valve replacement using a self-expanding bioprosthesis in patients with severe aortic stenosis at extreme risk for surgery. *J Am Coll Cardiol* 2014;63:1972-81.
8. Smith CR, Leon MB, Mack MJ, Miller DC, Moses JW, Svensson LG, Tuzcu EM, Webb JG, Fontana GP, Makkar RR, Williams M, Dewey T, Kapadia S, Babaliaros V, Thourani VH, Corso P, Pichard AD, Bavaria JE, Herrmann HC, Akin JJ, Anderson WN, Wang D, Pocock SJ; PARTNER Trial Investigators. Transcatheter versus surgical aortic-valve replacement in high-risk patients. *N Engl J Med*

- 2011;364:2187-98.
9. Adams DH, Popma JJ, Reardon MJ, Yakubov SJ, Coselli JS, Deeb GM, Gleason TG, Buchbinder M, Hermiller J Jr, Kleiman NS, Chetcuti S, Heiser J, Merhi W, Zorn G, Tadros P, Robinson N, Petrossian G, Hughes GC, Harrison JK, Conte J, Maini B, Mumtaz M, Chenoweth S, Oh JK; U.S. CoreValve Clinical Investigators. Transcatheter aortic-valve replacement with a self-expanding prosthesis. *N Engl J Med* 2014;370:1790-8.
10. Otto C, Kumbhani DJ, Alexander KP, Calhoon JH, Desai MY, Kaul S, Lee JC, Ruiz CE, Vassileva CM. 2017 ACC expert consensus decision pathway for transcatheter aortic valve replacement in the management of adults with aortic stenosis: a report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. *J Am Coll Cardiol* 2017;69:1313-46.
11. Kirtane AJ, Leon MB. The Placement of Aortic Transcatheter Valve (PARTNER) trial: clinical trials. *Circulation* 2012;125:3229-32.
12. Florath I, Albert A, Boening A, Ennker IC, Ennker J. Aortic valve replacement in octogenarians: identification of high-risk patients. *Eur J Cardiothorac Surg* 2010;37:1304-10.
13. Dewey TM, Brown D, Ryan WH, Herbert MA, Prince SL, Mack MJ. Reliability of risk algorithms in predicting early and late operative outcomes in high-risk patients undergoing aortic valve replacement. *J Thorac Cardiovasc Surg* 2008;135:180-7.
14. Vahanian A, Alfieri OR, Al-Attar N, Antunes MJ, Bax J, Cormier B, Cribier A, De Jaegere P, Fournial G, Kappetein AP, Kovac J, Ludgate S, Maisano F, Moat N, Mohr FW, Nataf P, Pierard L, Pomar JL, Schofer J, Tornos P, Tuzcu M, van Hout B, von Segesser LK, Walther T. Transcatheter valve implantation for patients with aortic stenosis: a position statement from the European Association of Cardio-Thoracic Surgery (EACTS) and the European Society of Cardiology (ESC), in collaboration with the European Association of Percutaneous Cardiovascular Interventions (EAPCI). *Eur J Cardiothorac Surg* 2008;34:1-8.
15. Roques F, Nashef SA, Michel P; EuroSCORE study group. Risk factors for early mortality after valve surgery in Europe in the 1990s: lessons from the EuroSCORE pilot program. *J Heart Valve Dis* 2001;10:572-7; discussion 577-8.
16. Ferguson TB Jr, Dziuban SW Jr, Edwards FH, Eiken MC, Shroyer AL, Pairorero PC, Anderson RP, Grover FL. The STS National Database: current changes and challenges for the new millennium. Committee to Establish a National Database in Cardiothoracic Surgery, The Society of Thoracic Surgeons. *Ann Thorac Surg* 2000;69:680-91.
17. Green P, Woglom AE, Genereux P, Daneault B, Paradis JM, Schnell S, Hawkey M, Maurer MS, Kirtane AJ, Kodali S, Moses JW, Leon MB, Smith CR, Williams M. The impact of frailty status on survival after transcatheter aortic valve replacement in older adults with severe aortic stenosis: a single-center experience. *JACC Cardiovasc Interv* 2012;5:974-81.
18. Anand A, Harley C, Visvanathan A, Shah ASV, Cowell J, MacLulich A, Shenkin S, Mills NL. The relationship between preoperative frailty and outcomes following transcatheter aortic valve implantation: a systematic review and meta-analysis. *Eur Heart J Qual Care Clin Outcomes* 2017;3:123-32.
19. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP 3rd, Guyton RA, O'Gara PT, Ruiz CE, Skubas NJ, Sorajja P, Sundt TM 3rd, Thomas JD; ACC/AHA Task Force Members. 2014 AHA/ACC Guideline for the management of patients with valvular heart disease: executive summary; a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014;129:e650.
20. Leon MB, Smith CR, Mack M, Miller DC, Moses JW, Svensson LG, Tuzcu EM, Webb JG, Fontana GP, Makkar RR, Brown DL, Block PC, Guyton RA, Pichard AD, Bavaria JE, Herrmann HC, Douglas PS, Petersen JL, Akin JJ, Anderson WN, Wang D, Pocock S; PARTNER Trial Investigators. Transcatheter aortic-valve implantation for aortic stenosis in patients who cannot undergo surgery. *N Engl J Med* 2010;363:1597-607.
21. Shroyer AL, Coombs LP, Peterson ED, Eiken MC, DeLong ER, Chen A, Ferguson TB Jr, Grover FL, Edwards FH; Society of Thoracic Surgeons. The Society of Thoracic Surgeons: 30-day operative mortality and morbidity risk models. *Ann Thorac Surg* 2003;75:1856-64.
22. Mack MJ, Leon MB, Smith CR, Miller DC, Moses JW, Tuzcu EM, Webb JG, Douglas PS, Anderson WN, Blackstone EH, Kodali SK, Makkar RR, Fontana GP, Kapadia S, Bavaria J, Hahn RT, Thourani VH, Babaliaros V, Pichard A, Herrmann HC, Brown DL, Williams M, Akin J, Davidson MJ, Svensson LG; PARTNER 1 trial investigators. 5-year outcomes of transcatheter aortic valve replacement or surgical aortic valve replacement for high surgical risk patients with aortic stenosis (PARTNER 1): a randomised controlled trial. *Lancet* 2015;385:2477-84.
23. Kapadia SR, Leon MB, Makkar RR, Tuzcu EM, Svensson LG, Kodali S, Webb JG, Mack MJ, Douglas PS, Thourani VH, Babaliaros VC, Herrmann HC, Szeto WY, Pichard AD, Williams MR, Fontana GP, Miller DC, Anderson WN, Akin JJ, Davidson MJ, Smith CR; PARTNER trial investigators. 5-year outcomes of transcatheter aortic valve replacement compared with standard treatment for patients with inoperable aortic stenosis (PARTNER 1): a randomised controlled trial. *Lancet* 2015;385:2485-91.
24. Thomas M, Schymik G, Walther T, Himbert D, Lefèvre T, Treede H, Eggebrecht H, Rubino P, Michev I, Lange R, Anderson WN, Wendler O. Thirty-day results of the SAPIEN aortic Bioprosthesis European Outcome (SOURCE) Registry: A European registry of transcatheter aortic valve implantation using the Edwards SAPIEN valve. *Circulation* 2010;122:62-9.
25. Moat NE, Ludman P, de Belder MA, Bridgewater B, Cunningham AD, Young CP, Thomas M, Kovac J, Spyt T, MacCarthy PA, Wendler O, Hildick-Smith D, Davies SW, Trivedi U, Blackman DJ, Levy RD, Brecker SJ, Baumbach A, Daniel T, Gray H, Mullen MJ. Long-term outcomes after transcatheter aortic valve implantation in high-risk patients with severe aortic stenosis: the U.K. aortic valve implantation (United Kingdom Transcatheter Aortic Valve Implantation) Registry. *J Am Coll Cardiol* 2011;58:2130-8.
26. Brown DL. Expanding indications for TAVR: The preferred procedure in intermediate-risk patients? *Cleve Clinic J Med* 2017;84:e10-4.
27. Stähli BE, Tasnady H, Lüscher TF, Gebhard C, Mikulicic F, Erhart L, Bühler I, Landmesser U, Altwegg L, Wischniewsky MB, Grünfelder J, Falk V, Corti R, Maier W. Early and late mortality in patients undergoing transcatheter aortic valve implantation: comparison of the Novel EuroSCORE II with Established Risk Scores. *Cardiology* 2013;126:15-23.
28. Leon MB, Smith CR, Mack MJ, Makkar RR, Svensson LG, Kodali SK, Thourani VH, Tuzcu EM, Craig Miller D, Herrmann HC,

- Doshi D, Cohen DJ, Pichard AD, Kapadia S, Dewey T, Babaliaros V, Szeto WY, Williams MR, Kereiakes D, Zajarias A, Greason KL, Whisenant BK, Hodson RW, Moses JW, Trento A, Brown DL, Fearon WF, Pibarot P, Hahn RT, Jaber WA, Anderson WN, Alu MC, Webb JG; PARTNER 2 Investigators. Transcatheter or surgical aortic-valve replacement in intermediate-risk patients. *N Engl J Med* 2016;374:1609-20.
29. Reardon MJ, Van Mieghem NM, Popma JJ, Kleiman NS, Søndergaard L, Mumtaz M, Adams DH, Deeb GM, Maini B, Gada H, Chetcuti S, Gleason T, Heiser J, Lange R, Merhi W, Oh JK, Olsen PS, Piazza N, Williams M, Windecker S, Yakubov SJ, Grube E, Makkar R, Lee JS, Conte J, Vang E, Nguyen H, Chang Y, Mugglin AS, Serruys PW, Kappetein AP; SURTAVI Investigators. Surgical or transcatheter aortic-valve replacement in intermediate-risk patients. *N Engl J Med* 2017;376:1321-31.
30. Thyregod HG, Steinbrüchel DA, Ihlemann N, Nissen H, Kjeldsen BJ, Petursson P, Chang Y, Franzen OW, Engstrøm T, Clemmensen P, Hansen PB, Andersen LW, Olsen PS, Søndergaard L. Transcatheter versus surgical aortic valve replacement in patients with severe aortic valve stenosis: 1-year results from the All-Comers NOTION Randomized Clinical Trial. *J Am Coll Cardiol* 2015;65:2184-94.
31. Thyregod HG. Five-year outcomes from the All-Comers Nordic Aortic Valve Intervention Randomized Clinical Trial in patients with severe aortic valve stenosis. *ACC Annual Congress*; 2018.
32. Wenaweser P, Stortecky S, Schwander S, Heg D, Huber C, Pilgrim T, Gloekler S, O'Sullivan CJ, Meier B, Juni P, Carrel T, Windecker S. Clinical outcomes of patients with estimated low or intermediate surgical risk undergoing transcatheter aortic valve implantation. *Eur Heart J* 2013;34:1894-905.
33. Tamburino C, Barbanti M, D'Errigo P, Ranucci M, Onorati P, Covelto RD, Santini F, Rosato S, Santoro G, Fusco D, Grossi C, Seccareccia F; OBSERVANT Research Group. 1-year outcomes after transfemoral transcatheter or surgical aortic valve replacement: results from the Italian OBSERVANT Study. *J Am Coll Cardiol* 2015;66:804-12.
34. Tarantini G, Nai Fovino L, Gers BJ. Transcatheter aortic valve implantation in lower-risk patients: what is the perspective? *Eur Heart J* 2018;39:1-12.
35. Thourani VH, Forcillo J, Szeto WY, Kodali SK, Blackstone EH, Lowry AM, Semple M, Rajeswaran J, Makkar RR, Williams MR, Bavaria JE, Herrmann HC, Maniar HS, Babaliaros VC, Smith CR, Trento A, Corso PJ, Pichard AD, Craig Miller D, Svensson LG, Kapadia S, Ailawadi G, Suri RM, Greason KL, Hahn RT, Jaber WA, Alu MC, Leon MB, Mack MJ; PARTNER Trial Investigators. Outcomes in 937 intermediate-risk patients undergoing surgical aortic valve replacement in PARTNER-2A. *Ann Thorac Surg* 2018;105:1322-9.
36. Garcia S, Kelly R, Mbai M, Gurevich S, Oestreich B, Yannopoulos D, Adabag S. Outcomes of intermediate-risk patients treated with transcatheter and surgical aortic valve replacement in the Veterans Affairs Healthcare System: a single center 20-year experience. *Catheter Cardiovasc Interv* 2018; doi: 10.1002/ccd.27478.
37. Tarantini G, Gasparetto V, Napodano M, Fraccaro C, Gerosa G, Isabella G. Valvular leak after transcatheter aortic valve implantation: a clinician update on epidemiology, pathophysiology and clinical implications. *Am J Cardiovasc Dis* 2011;1:312-20.
38. Bauer T, Linke A, Sievert H, Kahlert P, Hambrecht R, Nickenig G, Hauptmann KE, Sack S, Gerckens U, Schneider S, Zeymer U, Zahn R. Comparison of the effectiveness of transcatheter aortic valve implantation in patients with stenotic bicuspid versus tricuspid aortic valves (from the German TAVI Registry). *Am J Cardiol* 2014;113:518-21.
39. Phan K, Wong S, Phan S, Ha H, Qian P, Yan TD. Transcatheter aortic valve implantation (aortic valve implantation) in patients with bicuspid aortic valve stenosis--systematic review and meta-analysis. *Heart Lung Circ* 2015;24:649-59.
40. Sannino A, Cedars A, Stoler RC, Szerlip M, Mack MJ, Grayburn PA. Comparison of efficacy and safety of transcatheter aortic valve implantation in patients with bicuspid versus tricuspid aortic valves. *Am J Cardiol* 2017;120:1601-6.
41. Praz F, Windecker S, Huber C, Carrel T, Wenaweser P. Expanding indications of transcatheter heart valve interventions. *J Am Coll Cardiol Intv* 2015;8:1777-96.
42. Yoon SH, Schmidt T, Bleiziffer S, Schofer N, Fiorina C, Munoz-Garcia AJ, Yzeiraj E, Amat-Santos IJ, Tchetché D, Jung C, Fujita B, Mangieri A, Deutsch MA, Ubben T, Deuschl F, Kuwata S, De Biase C, Williams T, Dhoble A, Kim WK, Ferrari E, Barbanti M, Vollema EM, Miceli A, Giannini C, Attizzani GF, Kong WKF, Gutierrez-Ibanez E, Jimenez Diaz VA, Wijeysondera HC, Kaneko H, Chakravarty T, Makar M, Sievert H, Hengstenberg C, Prendergast BD, Vincent F, Abdel-Wahab M, Nombela-Franco L, Silaschi M, Tarantini G, Butter C, Ensminger SM, Hildick-Smith D, Petronio AS, Yin WH, De Marco F, Testa L, Van Mieghem NM, Whisenant BK, Kuck KH, Colombo A, Kar S, Moris C, Delgado V, Maisano F, Nietlispach F, Mack MJ, Schofer J, Schaefer U, Bax JJ, Frerker C, Latib A, Makkar RR. Transcatheter aortic valve replacement in pure native aortic valve regurgitation. *J Am Coll Cardiol* 2017;70:2752-63.
43. Franzone A, Piccolo R, Siontis GC, Lanz J, Stortecky S, Praz F, Roost E, Vollenbroich R, Windecker S, Pilgrim T. Transcatheter aortic valve replacement for the treatment of pure native aortic valve regurgitation: a systematic review. *JACC Cardiovasc Interv* 2016;9:2308-17.
44. Sawaya FJ, Deutsch MA, Seiffert M, Yoon SH, Codner P, Wickramarachchi U, Latib A, Petronio AS, Rodés-Cabau J, Taramasso M, Spaziano M, Bosmans J, Biasco L, Mylotte D, Savontaus M, Gheeraert P, Chan J, Jørgensen TH, Sievert H, Mocetti M, Lefèvre T, Maisano F, Mangieri A, Hildick-Smith D, Kornowski R, Makkar R, Bleiziffer S, Søndergaard L, De Backer O. Safety and efficacy of transcatheter aortic valve replacement in the treatment of pure aortic regurgitation in native valves and failing surgical bioprostheses: results from an International Registry Study. *JACC Cardiovasc Interv* 2017;10:1048-56.
45. Brown JM, O'Brien SM, Wu C, Sikora JA, Griffith BP, Gammie JS. Isolated aortic valve replacement in North America comprising 108,687 patients in 10 years: changes in risks, valve types, and outcomes in the Society of Thoracic Surgeons National Database. *J Thorac Cardiovasc Surg* 2009;137:82-90.
46. Ussia GP, Barbanti M, Petronio AS, Tarantini G, Ertori F, Colombo A, Violini R, Ramondo A, Santoro G, Klugmann S, Bedogni F, Maisano F, Marzocchi A, Poli A, De Carlo M, Napodano M, Fiorina C, De Marco F, Antonucci D, de Cillis E, Capodanno D, Tamburino C. Transcatheter aortic valve implantation: 3-year outcomes of selfexpanding CoreValve prosthesis. *Eur Heart J* 2012;33:969-76.

47. Rodé's-Cabau J, Dumont E, Doyle D, Lemieux J. Transcatheter valve-in-valve implantation for the treatment of stentless aortic valve dysfunction. *J Thorac Cardiovasc Surg* 2010;140:246-8.
48. Eggebrecht H, Schäfer U, Treede H, Boekstegers P, Babin-Ebell J, Ferrari M, Möllmann H, Baumgartner H, Carrel T, Kahlert P, Lange P, Walther T, Erbel R, Mehta RH, Thielmann M. Valve-in-valve transcatheter aortic valve implantation for degenerated bioprosthetic heart valves. *JACC Cardiovasc Interv* 2011;4:1218-27.
49. David TE, Ivanov J, Armstrong S, Feindel CM, Cohen G. Late results of heart valve replacement with the Hancock II bioprosthesis. *J Thorac Cardiovasc Surg* 2001;121:268-77.
50. Maganti M, Rao V, Armstrong S, Feindel CM, Scully HE, David TE. Redo valvular surgery in elderly patients. *Ann Thorac Surg* 2009;87:521-5.
51. Dvir D, Webb J, Brecker S, Bleiziffer S, Hildick-Smith D, Colombo A, Descoutures F, Hengstenberg C, Moat NE, Bekerredjian R, Napolitano M, Testa L, Lefevre T, Guetta V, Nissen H, Hernández JM, Roy D, Teles RC, Segev A, Dumonteil N, Fiorina C, Gotzmann M, Tchetché D, Abdel-Wahab M, De Marco F, Baumbach A, Laborde JC, Kornowski R. Transcatheter aortic valve replacement for degenerative bioprosthetic surgical valves: results from the global valve-in-valve registry. *Circulation* 2012;126:2335-44.
52. Seiffert M, Conradi L, Baldus S, Knap M, Schirmer J, Franzen O, Koschyk D, Meinertz T, Reichenspurner H, Treede H. Impact of patient-prosthesis mismatch after transcatheter aortic valve-in-valve implantation in degenerated bioprostheses. *J Thorac Cardiovasc Surg* 2011;143: 617-24.
53. Gurvitch R, Cheung A, Bedogni F, Webb JG. Coronary obstruction following transcatheter aortic valve-in-valve implantation for failed surgical bioprostheses. *Catheter Cardiovasc Interv* 2011;77:439-44.

Case Report

Open Access



Iatrogenic injury to axillary artery: rescued by endovascular repair

Chih-Chen Kao, Yao-Kuang Huang

Division of Thoracic and Cardiovascular Surgery, Chiayi Chang Gung Memorial Hospital, Chiayi 613, Taiwan.

Correspondence to: Dr. Yao-Kuang Huang, Division of Thoracic and Cardiovascular Surgery, Chiayi Chang Gung Memorial Hospital, Chiayi 613, Taiwan. E-mail: cckaomd@gmail.com

How to cite this article: Kao CC, Huang YK. Iatrogenic injury to axillary artery: rescued by endovascular repair. *Vessel Plus* 2018;2:18. <http://dx.doi.org/10.20517/2574-1209.2018.42>

Received: 4 Jun 2018 **First Decision:** 25 Jun 2018 **Revised:** 28 Jul 2018 **Accepted:** 30 Jul 2018 **Published:** 10 Aug 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

Most of axillo-subclavian artery injuries are due to violence. Iatrogenic injuries to such vessels are relatively rare. We hereby present the first report of pigtail catheter insertion for right upper chest wall hematoma drainage resulting in penetration of axillary artery and pseudoaneurysm formation. A 39-year-old male victim of motor vehicle accident developed right upper chest wall hematoma after initial conservative treatment. Subsequent admission was arranged and pigtail catheter drainage was performed under sonography guidance. The procedure caused penetrating injury to his right axillary artery with pseudoaneurysm formation. Endovascular repair and stent placement were performed. The patient was discharged within 2 weeks without significant sequelae. Non-catheterization procedure caused penetration of axillary artery was rarely seen in published reports. Our report described a case of axillary artery penetration resulted by pigtail catheter insertion which was never seen. We wish to emphasize on the jeopardy of non-vascular procedure on penetrating nearby vessels because of anatomical proximity.

Keywords: Trauma, vascular injury, endovascular surgery

INTRODUCTION

Thoracic cage and shoulder girdle provide a well protection of the proximate major vessels. Therefore, injuries to such vessels which are adjacent to thoracic inlet, including axillo-subclavian artery are fairly rare^[1]. On the other hand, the well protection of these vessels by local anatomy also posed a challenge for surgeons to approach them during open surgery. The rareness of such cases further increases the risk of open operation of axillo-subclavian vessel repair due to inexperience of even senior cardiovascular surgeons. Stab and gunshots are among the majority of mechanism causing injuries to axillo-subclavian artery^[2]. Iatrogenic injuries are relatively minor in proportion^[3]. Overall mortality of patients with axillo-subclavian artery injury



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



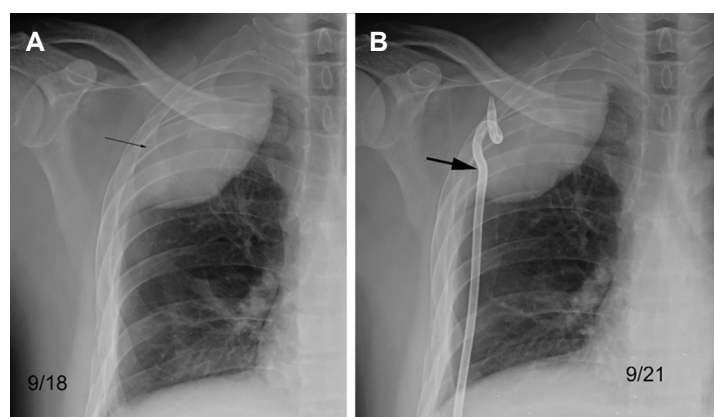


Figure 1. (A) Chest X-ray image reveals a huge hematoma or haemothorax shadow which crosses as many as 4 intercostal spaces. (B) The arrow points out the insertion site of the pigtail tube, which is very close to axillary artery

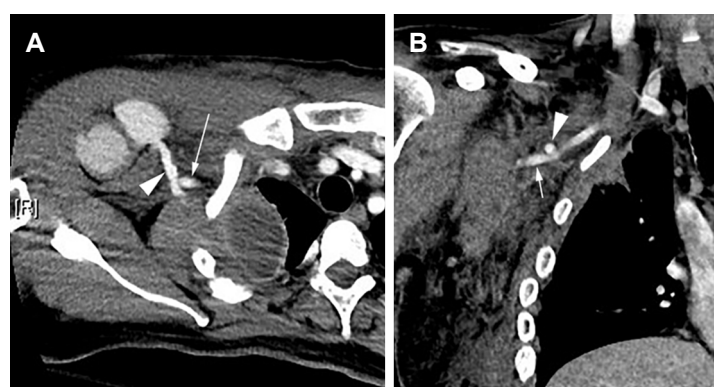


Figure 2. (A) Contrast-enhanced chest CT axial view reveals the penetrated axillary artery (arrow) and a track (arrowhead) tracing to the pseudoaneurysm. (B) Coronal view of the same lesion

who survived initial insult and reached the medical institutes receiving operation range from 5% to 30%^[4-6]. As a result, a growing trend of endovascular procedure for repairing injuries to axillo-subclavian artery appears in recent years^[3]. We herein present a case of penetrating injury to the right axillary artery resulted by pigtail catheter insertion. The patient was successfully treated with endovascular repair and discharged within 2 weeks without significant sequelae.

CASE REPORT

A 39-year-old male patient, victim of a motor vehicle accident, was admitted to our satellite hospital. Right 1st and 3rd -8th ribs fracture, minimal pneumothorax and right pleural effusion were diagnosed by chest computed tomograms (CT). Right upper chest wall hematoma was found in subsequent out-patient clinic follow up with CXR. He was then admitted, and thoracocentesis under sonography guidance was done. Grossly, the drainage fluid was bloody, which was suspected to be the result of musculoskeletal hemorrhage. Progressive chest pain with expansion of chest wall ecchymosis developed after drainage, and therefore a pigtail catheter with 12Fr. in diameter was inserted for drainage over right upper chest wall [Figure 1]. Nonetheless, the drainage amount was minimal and it was considered as a failure because the amount of pleural effusion remained unchanged on chest X-ray. The pigtail catheter was removed within 3 days of placement. However, upon removal, the doctors encountered major bleeding, up to 2000 cc. Emergent CT scan was done right after the incidence which illustrated contrast extravasation trace from right axillary artery leading to the tract of the previous inserted pigtail catheter, and the formation of a chest wall pseudoaneurysm [Figure 2]. General surgeons tried to approach the bleeder with open surgery but failed. The patient was then transferred to our hospital.

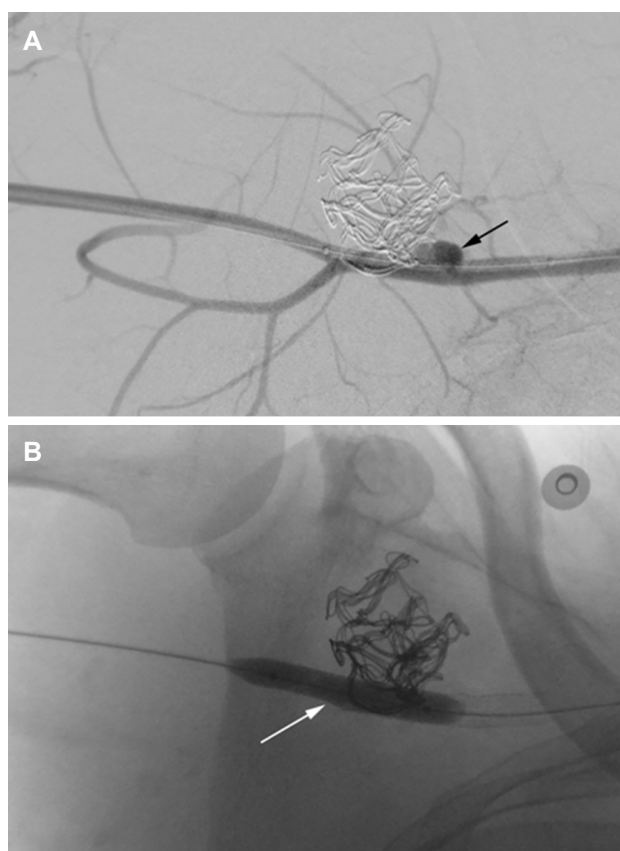


Figure 3. (A) Angiography shows the pseudoaneurysm formation. (B) The endoprosthesis (GORE® VIABAHN® Endoprosthesis, W.L. Gore and Associates, Inc. Flagstaff, AZ)

Right on transferal, emergent endovascular repair was scheduled, and an extravasation of contrast media was detected on right axillary artery intra-operatively. A cover stent (GORE® VIABAHN® Endoprosthesis, W.L. Gore and Associates, Inc. Flagstaff, AZ) with 8 mm in diameter and 5 cm in length was implanted through right brachial artery to facilitate arterial repair. Postdilatation was done with Rival 8 mm balloon. The pseudoaneurysm was debrided after the endovascular procedure [Figures 3 and 4]. The patient was discharged uneventfully within 2 weeks.

DISCUSSION

The complexity of anatomical structure of axillo-subclavian artery poses a potential risk for open surgical approach. Not to mention that injuries of axillo-subclavian artery are uncommon, and therefore even seasoned surgeons have limited experience on approaching the field under active bleeding condition^[4]. Furthermore, the patients who suffer from such injuries might be too critically ill and thus not suitable for highly invasive treatment^[7]. Under such circumstances, there is a growing trend of endovascular repair in axillo-subclavian artery injuries^[3,7-10]. Among such cases, iatrogenic-related injury accounts for 22.4%. Most of the iatrogenic-related penetrating injuries of axillo-subclavian arteries cases are caused by venous catheterization^[3]. Iatrogenic-related penetrating injuries to axillo-subclavian artery result in complications such as pseudoaneurysm, AV fistula and dissection^[3,11-13].

Our case was rare, and there was no similar report in previous publication. It was a case of penetrating injury to axillary artery by pigtail catheter insertion for chest wall hematoma. Pseudoaneurysm formation was revealed with subsequent CT and angiogram evaluation. For injuries to the axillo-subclavian artery, there were various kinds of endoprosthesis useful for repair. For the subgroup of penetrating injuries or iatrogenic



Figure 4. The wound of the patient. The white arrow points out the approach site of endovascular procedure which is the brachial artery near elbow. The black arrow is the insertion site of the pigtail tube while the white arrowhead points out the wound created by the surgeon at our satellite hospital in attempt to approach the bleeder

injuries to axillo-subclavian artery, there were very few cases repaired by endoprosthesis (GORE[®] VIA-BAHN[®] Endoprosthesis, W.L. Gore and Associates, Inc. Flagstaff, AZ) that we chose for our patient^[3,8,14,15].

Based on what was mentioned above, we wish to deliver a few messages. First of all, there is a potential risk of non-vascular procedure in causing axillary artery penetrating injury. In trauma such as our case, the site of hematoma was at upper chest which was very close to axillo-subclavian artery anatomically. When approaching the lesion with percutaneous procedures such as pigtail catheter drainage, penetrating injury could happen.

Secondly, we also demonstrated the safety and instantaneity of endovascular repair in case of penetrating axillo-subclavian artery with ruptured pseudoaneurysm. As mentioned above, the complexity of anatomical structure over axillo-subclavian artery makes it difficult to approach the vessels openly. While patients with such injuries might be too critically ill, open surgery might not be able to provide timely repair. In comparison, endovascular has the potential to provide a safer, quicker and more secure approach to repair of penetrating injuries to axillo-subclavian artery.

DECLARATIONS

Acknowledgments

Lu MS, Lin CC, Tseng YH helped to proofreading the draft.

Authors' contributions

Drafted the manuscript, and collected the proper images: Kao CC

Conceived of the study and helped to proofreading the draft: Huang YK

Read and approved the final manuscript: both authors

Availability of data and materials

The data in the study was collected from the medical record of the patient in Chiayi Chang Gung Memorial Hospital. The patient has agreed to provide his data for study.

Financial support and sponsorship

Huang YK has sources of funding including research number CMRPG380841, CMRPG6C0342, CMRP-G6B0503, NMRPG6D022, CMRPG6E0421.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

The patient has agreed to provide his data for study.

Consent for publication

Written informed consent was delivered to the patient and was signed by the patient himself. The patient was well explained and was aware of the publication of this case report and any accompanying images.

Copyright

© The Author(s) 2018.

REFERENCES

- Demetriades D, Chahwan S, Gomez H, Peng R, Velmahos G, Murray J, Asensio J, Bongard F. Penetrating injuries to the subclavian and axillary vessels. *J Am Coll Surg* 1999;188:290-5.
- Gill H, Jenkins W, Edu S, Bekker W, Nicol AJ, Navsaria PH. Civilian penetrating axillary artery injuries. *World J Surg* 2011;35:962-6.
- DuBose JJ, Rajani R, Gilani R, Arthurs ZA, Morrison JJ, Clouse WD, Rasmussen TE; Endovascular skills for trauma and resuscitative surgery working group. Endovascular management of axillo-subclavian arterial injury: a review of published experience. *Injury* 2012;43:1785-92.
- Demetriades D, Asensio JA. Subclavian and axillary vascular injuries. *The Surg Clin North Am* 2001;81:1357-73, xiii.
- Schaff HV, Brawley RK. Operative management of penetrating vascular injuries of the thoracic outlet. *Surgery* 1977;82:182-91.
- Degiannis E, Levy RD, Potokar T, Saadia R. Penetrating injuries of the axillary artery. *Aust N Z J Surg* 1995;65:327-30.
- Chemelli AP, Wiedermann F, Klocker J, Falkensammer J, Strasak A, Czermak BV, Waldenberger P, Chemelli-Steinguber IE. Endovascular management of inadvertent subclavian artery catheterization during subclavian vein cannulation. *J Vasc Interv Radiol* 2010;21:470-6.
- Pikwer A, Acosta S, Kolbel T, Malina M, Sonesson B, Akeson J. Management of inadvertent arterial catheterisation associated with central venous access procedures. *Eur J Vasc Endovasc Surg*. 2009;38:707-14.
- Kumar RM, Reddy SS, Sharma R, Mahajan R, Talwar KK. Endovascular repair of a traumatic axillary artery pseudoaneurysm. *Cardio-vasc Interv Radiol* 2009;32:598-600.
- Michaluk BT, Deutsch E, Moufid R, Panetta TF. Endovascular repair of an axillary artery pseudoaneurysm attributed to hyperextension injury. *Ann Vasc Surg* 2009;23:412 e415-9.
- Criado E, Marston WA, Ligush J, Mauro MA, Keagy BA. Endovascular repair of peripheral aneurysms, pseudoaneurysms, and arteriovenous fistulas. *Ann Vasc Surg* 1997;11:256-63.
- Kapadia S, Parakh R, Grover T, Agarwal S, Yadav A. Endovascular covered stent for management of arterial pseudoaneurysms after central venous access. *J Cardiothorac Vasc Anesth* 2007;21:99-102.
- Castelli P, Caronno R, Piffaretti G, Tozzi M, Lagana D, Carrafiello G, Cuffari S. Endovascular repair of traumatic injuries of the subclavian and axillary arteries. *Injury* 2005;36:778-82.
- Vinces FY, Sperling DC. Endovascular treatment of a combined pseudoaneurysm and arteriovenous fistula of the subclavian artery caused by a gunshot wound to the chest. *J Thorac Cardiovasc Surg* 2005;130:225-7.
- Chen AY, Laniado I Jr, Lin PH. Durability of the Viabahn stent graft after axillary artery pseudoaneurysm exclusion. *J Vasc Surg Cases Innov Tech* 2017;3:99-101.

Original Article

Open Access



Genetic variants of renin on the prevalence of diabetic nephropathy

Pulakes Purkait^{1,3,4}, Kalpataru Halder², Jammigumpula Masthanaiah Naidu³, Biswanath Sarkar⁴

¹Department of Anthropology, Panjab University, Chandigarh 160014, India.

²Department of Molecular Biology, Brahmananda Keshab Chandra College, BonHooghly, Kolkata 700108, India.

³Department of Anthropology, Andhra University, Visakhapatnam 530003, India.

⁴DNALaboratory, Anthropological Survey of India, Kolkata 700016, India.

Correspondence to: Dr. Pulakes Purkait, Department of Anthropology, Panjab University, Chandigarh 160014, India.
E-mail: pp.diabetes@gmail.com

How to cite this article: Purkait P, Halder K, Naidu JM, Sarkar B. Genetic variants of renin on the prevalence of diabetic nephropathy. *Vessel Plus* 2018;2:19. <http://dx.doi.org/10.20517/2574-1209.2018.16>

Received: 2 Mar 2018 **First Decision:** 29 Jun 2018 **Revised:** 19 Jul 2018 **Accepted:** 25 Jul 2018 **Published:** 14 Aug 2018

Science Editor: Alexander D. Verin **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Aim: Renin, a component of the Renin-Angiotensin-Aldosterone System (RAAS), is produced in the juxtaglomerular cells of the kidney. It is an important factor for the regulation of blood pressure and electrolyte balance and encoded by the REN gene. Recent studies suggest that the RAAS is a regulator of kidney functions. Individuals with REN variants have been associated with high blood pressure. We substantiated the hypothesis that genetic variants of REN gene have significant association with prevalence of nephropathy and in the development of nephropathy in type 2 diabetes mellitus (T2DM).

Methods: We enrolled to the study 718 consecutive subjects who were registered patients in two individual hospitals in Kolkata city, India. They consisted of 246 (34.26%) T2DM patients without nephropathy cases, 168 (23.40%) type 2 diabetes with nephropathy cases (T2DNH) and 304 (42.34%) healthy controls. Genotypes were assayed with genomic DNA for two known variants of the REN gene, i.e., rs16853055 and rs41317140 using sequencing methods.

Results: Association between the REN gene variants and prevalence of T2DM and T2DNH was tested. A significant association of T2DNH and variant rs41317140 was obtained and it was evident that the rs41317140 (C>T) shows a significant difference between T2DM and T2DNH ($\chi^2 = 4.92$; $P = 0.03$; OR = 0.6162; 95% CI: 0.4006-0.948). The results from the multiple model test that additive model predicted the association at genotype level and shows a significant difference between T2DM and T2DNH (OR = 0.6067; $P = 0.03$). There was no significant association between T2DNH or T2DM and variant rs16853055.



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Conclusion: Thus, it is concluded that a genetic variant of the REN gene should have a significant impact on the onset of type 2 diabetic nephropathy.

Keywords: Single nucleotide polymorphism, type 2 diabetes mellitus, nephropathy, Indian population

INTRODUCTION

Nephropathy is related to damage or disease of the kidney. Diabetic nephropathy is impairment to the kidney caused by hyperglycemia. In severe cases the kidney can fail. The kidneys filter waste from blood through its capillaries. Diabetes resulting in high blood sugar can destroy these tiny blood vessels (American Diabetes Association). Renal failure or kidney disease in diabetes is intervened by various biochemical pathways such as renin-angiotensin-aldosterone system (RAAS)^[1,2], aldose reductase-polyol^[3], di-acyl glycerol-protein kinase C^[4], advanced glycosylation-end products (AGE)^[5,6] and hexosamine pathway^[7]. The RAAS regulates blood pressure and water balance. Renin is secreted by the kidneys when blood pressure is low and it stimulates the production of angiotensin (Ang). Ang causes blood vessels to constrict, which results in high blood pressure. Experimental and clinical evidence recommend that the RAAS is a controller of kidney functions and is proposed to play an important role in the progression of nephropathy in type 2 diabetes mellitus^[8-10].

Renin is a component of the RAAS and it is a protein containing 406 amino acids together with a pre segment carrying 20-23 and a pro segment of 43-47 amino acids^[11-14]. In a number of steps, pro-renin is generated in the juxtaglomerular cells of the kidney by the elimination of 23 amino acids from C-terminus of prepro-renin, and is later converted into mature renin by removal of N-terminal fragment of pro-renin^[12,14]. Renin, an aspartyl protease, is encoded by the REN gene and is mapped to 1q25-q32 by in situ hybridization^[15]. It spans 12.5 kb in length of DNA and contains 8 introns^[16] and encodes 10 exons^[17]. It cleaves angiotensinogen and it converts it to Ang I after which, Ang I-converting enzyme (ACE) transforms it into Ang II, a potent vasoconstrictor. The concentrations of angiotensinogen circulating in the blood is abundant and perhaps more than 1000 times in excess of plasma Ang I and Ang II concentrations^[18]. Although with exception of some species, activity of renin thus is a key factor for the determination of the rate of Ang I formation in the plasma from enormous supplies of circulating angiotensinogen^[19,20]. Therefore, even small relative changes in the rates of Ang I formation perhaps lead to a large absolute difference in the circulating concentrations of Ang II. It is well known that renin is synthesized and stored in substantial quantities in the granules of juxtaglomerular cells and is released in response to various stimuli^[20,21]. Thus, large changes in plasma renin levels can rapidly changes the generation of Ang I. Therefore the polymorphism in the promoter region of REN gene may be of great significance in the changes Ang I. Ang I is easily converted to Ang II because the widespread presence of Ang converting enzyme on endothelial cells of many vascular beds including lung^[19,20,22]. The resultant increases in plasma Ang II exert powerful actions throughout the body through activation of AGTR1 receptors^[20,23]. In this context renin is also an important regulator of blood pressure and electrolyte balance. Individuals with REN polymorphisms have been associated with high blood pressure^[24,25], susceptibility to hypertension^[26-28] and end-stage renal disease^[29].

Keeping the existing body of knowledge in view, the aim of the present study was to investigate the distribution of genotype, allele frequency of REN gene polymorphism and its relationship with type 2 diabetic nephropathy patients in an Eastern Indian population.

METHODS

Subjects

Patients were recruited from registered patients list of two participating medical institutions of Kolkata, West Bengal. A standardized protocol was implemented to obtain data from each of the study participants.

Ethical committee clearance was obtained from the medical institutions prior to the recruitment of subjects in this study. An informed consent was obtained from all the participants prior to their recruitment for the study.

The study included 168 type 2 diabetic patients with nephropathy cases on hemodialysis (T2DNH), 246 type 2 diabetes patients without nephropathy cases (T2DM) and 304 controls (CON). The identification of type 2 diabetic and nephropathy patients was based on physician's recommendation or registered patient for dialysis. A detailed medical history of each patient was recorded. The unrelated controls were randomly selected and recruited from local community centers. Participants were born into Bengali families in Kolkata and the surrounding area.

Genotyping

Genomic DNA was prepared from fresh whole blood by using the conventional phenol-chloroform extraction method followed by ethanol precipitation^[30]. In this study, previously published primers 5'GCTGTCTTCTG GTGGTACTGCC3'(sense) and 5'TGCTGGCCATGAACTGGTTCTAGC3' (antisense) were used for the PCR based detections of single nucleotide polymorphisms (SNPs). PCR amplification was performed in a final volume of 10 μ L reaction mixture containing 50 ng of genomic DNA, 20 pmol of each primer, 10X Taq PCR buffer, 25 mM MgCl₂, 100 mM of each dNTPs and 0.5 U/ μ L of Red Taq polymerase. PCR amplification was performed in a DNA thermo cycler (Bio-Rad). PCR was carried out with an initial denaturing time at 95 °C for 5 min. Then the DNA was amplified for 35 cycles with denaturation at 94 °C for 1 min, annealing at 69 °C for 1:30 min and extension at 72 °C for 1:30 min and final extension 72 °C for 10 min. The PCR products were checked by 1% agarose gel electrophoresis with ethidium bromide staining and directly visualized in UV light. Only those PCR products that had a single amplification product with no evidence of non-specific amplification were used for DNA sequencing. The samples were analyzed on ABI 3730 genetic analyzer with a 48 capillary (Applied Biosystems, USA) to generate DNA sequences. Details described in our previous article^[31,32].

Statistical analysis

Allele frequencies were calculated for all the SNPs and were tested for Hardy-Weinberg equilibrium (HWE) and allelic association with the disease (Chi-Square test/Fisher exact test). Allelic and genotype association with the phenotypes was tested under different genetic models for both quantitative and qualitative traits by regression analysis and Fisher model test. Allele frequencies were calculated for the SNPs and tested for HWE and allelic association with disease (Fisher exact test, logistic regression and Fisher model tests) using PLINK software^[33]. For comparing the allelic distributions between study groups, the odds ratio (OR) with 95% confidence interval (CI) were also calculated. A level of $P < 0.05$ was assumed statistically significant.

Linkage disequilibrium (LD) between all the SNPs and also for associated SNP's was estimated using Haploview 4.2 software^[34]. The pair wise LD statistics D' and r^2 was calculated for all markers and also for associated SNP's. The Haploview 4.2 with default program or settings (Gabriel *et al.*^[35], 2002) was used to assess the linkage disequilibrium (D' and r^2) between each pair of SNPs.

RESULTS

The present study has focused on the 5' region with a special attention to rs41317140 (C to T) mutation. Through DNA sequencing seven SNPs were identified, out of which five new mutations were observed (data are not presented here) and two SNPs have been described previously as rs16853055 and rs41317140^[36-39]. The SNP rs16853055 is located at 3879 upstream from the start codon consisting of a C to A mutation. The rs41317140 (TaqI RFLP) is located at 4063 upstream from the start codon in 5' region with a substitution of C by T mutation. The sequence electropherogram of REN gene presented in [Figure 1](#) for the SNP rs16853055 indicating C \rightarrow A mutation in heterozygote CA condition and the SNP rs41317140 depicted in [Figure 2](#) is indicated with the red arrow, i.e., in heterozygote condition (CT).

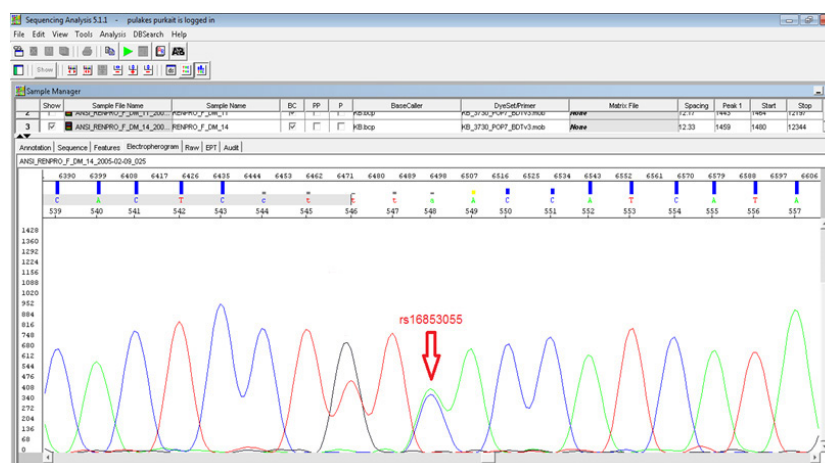


Figure 1. Sequence electropherogram of Renin gene for the SNP rs16853055

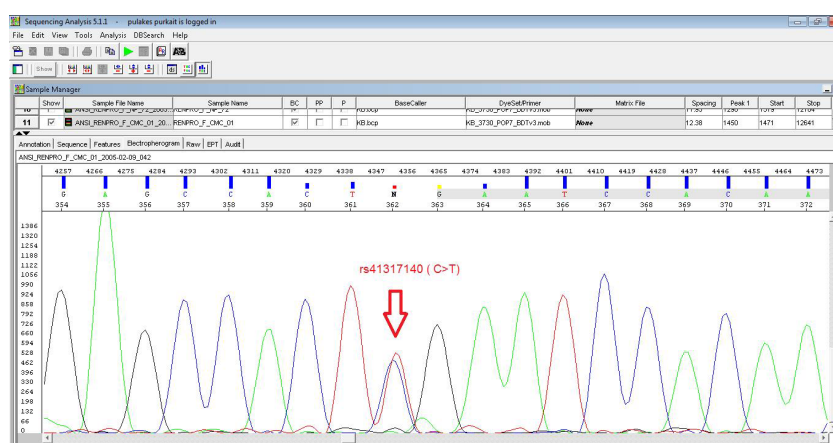


Figure 2. Sequence electropherogram of Renin gene for the SNP rs41317140

The genotype data of the Renin gene SNPs are presented in [Table 1](#). The SNP rs16853055 was found among 80 (26.32%) control, 66 (26.83%) T2DM and 34 (20.24%) T2DNH patients, whereas the SNP rs41317140 was observed among 74 (24.34%) control, 64 (26.6%) T2DM and 34 (20.24%) T2DNH patients while homozygote “TT” was found among 2.44% of T2DM patients and 0.66% control.

The results of HWE test are presented in [Table 2](#). From the HWE test it was found that the SNPs rs16853055 and rs41317140 were in HWE, indicating maintenance of allele frequency for control group of the study population. The Fisher exact test for allelic association of rs16853055 and rs41317140 of REN gene is presented in [Table 3](#). From the Fisher exact test, no significant differences were evident in the allele frequencies of the SNP rs16853055 between different combination of study groups that is case and control subjects. However, the rs41317140 (C>T) shows a significant difference between T2DM and T2DNH ($\chi^2 = 4.92$; $P = 0.03$; OR = 0.62; 95% CI: 0.4006-0.948) groups, indicating that a person with the SNP rs41317140 (C>T) will develop resistance for T2DM. Logistic regression analysis was performed to confirm the association at different genetic model and the results are presented in [Table 4](#) and exhibit that additive model predicted the association at genotype level and shows significant difference between T2DM and T2DNH groups (OR = 0.61; $P = 0.03$).

It is observed that all three models predicted the association but not to a significant extent for the SNP rs16853055. To the best of our knowledge still there is no literature with respect to any possible role of this polymorphic change with any health problem.

Table 1. Genotype distribution of RENIN gene variants among the study groups

SNP	Genotype	Control		T2DM		T2DNH	
		n = 304	%	n = 246	%	n = 168	%
rs16853055	A A	2	0.66	6	2.44	2	1.19
	C A	80	26.32	66	26.83	34	20.24
	C C	222	73.03	174	70.73	132	78.57
rs41317140	C C	228	75.00	176	71.54	134	79.76
	C T	74	24.34	64	26.02	34	20.24
	T T	2	0.66	6	2.44	0	0.00

Table 2. Hardy-Weinberg Equilibrium test for rs16853055 (C>A) and rs41317140 (C>T) of RENIN gene among the study groups

SNP	Alleles	Study groups	ObsHET	PredHET	HWpval	MAF
rs16853055	C:A	CON	0.263	0.238	0.0969	0.138
		T2D	0.242	0.241	1	0.14
		T2DM	0.268	0.267	1	0.159
		T2DNH	0.202	0.201	1	0.113
rs41317140	C:T	CON	0.243	0.224	0.1929	0.128
		T2D	0.237	0.23	0.7763	0.133
		T2DM	0.26	0.261	1	0.154
		T2DNH	0.202	0.182	0.3119	0.101

Alleles: major and minor alleles for this marker; CON: Control group; T2D: over all type 2 diabetes with and without nephropathy group; T2DM: type 2 Diabetes without nephropathy group; T2DNH: type 2 diabetes with nephropathy who are on hemodialysis group; ObsHET: marker's observed heterozygosity; PredHET: marker's predicted heterozygosity ($2 \times \text{MAF} \times (1 - \text{MAF})$); HWpval: Hardy-Weinberg equilibrium *P* value; MAF: minor allele frequency

Table 3. Fisher exact test for allelic association of SNPs rs16853055 (C>A) and rs41317140 (C>T) of RENIN gene among the study groups

SNP	A1	A2	Groups	F_A	F_U	CHISQ	P value	OR	L95	U95
rs16853055	A	C	CON vs. T2DM	0.1585	0.1382	0.899	0.34	1.175	0.8415	1.641
			CON vs. T2DNH	0.1131	0.1382	1.208	0.27	0.7955	0.5286	1.197
			T2DM vs. T2DNH	0.1131	0.1585	3.422	0.06	0.6768	0.4468	1.025
rs41317140	T	C	CON vs. T2DM	0.1545	0.1283	1.548	0.21	1.241	0.8827	1.746
			CON vs. T2DNH	0.1012	0.1283	1.52	0.21	0.765	0.4992	1.172
			T2DM vs. T2DNH	0.1012	0.1545	4.92	0.03*	0.6162	0.4006	0.948

SNP: single nucleotide polymorphism; A1: code for allele 1 (the more rare or "minor" allele based on the entire sample frequencies); A2: code for allele 2 (the more common or "major" allele); CON: Control group; T2D: over all type 2 diabetes with and without nephropathy group; T2DM: type 2 Diabetes without nephropathy group; T2DNH: type 2 diabetes with nephropathy who are on hemodialysis group; F_A: frequency of minor allele in affected individuals (case); F_U: frequency of minor allele in unaffected individuals (control); CHISQ: Chi-squared value for allelic association (with 1 df); *P*: the asymptotic *P*-value for chi-square test; OR: odds ratio; L95: lower bound of the 95% confidence; U95: upper bound of the 95% confidence; *Significant

DISCUSSION

The development of diabetic nephropathy is multifactorial^[40-43] and genetic predisposition has been anticipated to be an important factor in the development and progression of the disease. Apart from that, hypertension is presumed to be the single most important factor that accelerates the development of diabetic renal disease^[25,44,45].

Although the RAAS system has an important function in the controlling blood pressure, maintaining the stable equilibrium of Na⁺ ion and extracellular fluid volume^[46,47], more and more evidence also point out an influence towards the development of diabetic nephropathy. Particular RAAS gene polymorphisms were recognized as risk factors for type 2 diabetes mellitus complications, including hypertension^[31], coronary heart disease^[48], nephropathy^[48-50] and retinopathy^[51].

Many researchers have established the importance of tissue RAAS in the heart, vasculature, adrenal glands and brain as well as in the kidney^[52,53]. Though each organ system in the body has components of the RAAS,

Table 4. Logistic regression analysis of SNPs rs16853055 (C > A) and rs41317140 (C > T) of RENIN gene among the study groups

SNP	Study groups	Additive model				Dominant model				Recessive model			
		OR	L95	U95	P value	OR	L95	U95	P value	OR	L95	U95	P value
rs16853055	CON vs. T2DM	1.18	0.84	1.67	0.32	1.12	0.77	1.62	0.55	3.77	0.75	18.87	0.11
	CON vs. T2DNH	0.78	0.51	1.19	0.25	0.73	0.47	1.15	0.18	1.81	0.25	13.03	0.55
	T2DM vs. T2DNH	0.67	0.44	1.02	0.06	0.65	0.41	1.04	0.07	0.48	0.09	2.41	0.37
rs41317140	CON vs. T2DM	1.25	0.88	1.77	0.20	1.19	0.81	1.74	0.36	3.77	0.75	18.87	0.11
	CON vs. T2DNH	0.74	0.47	1.16	0.19	0.76	0.48	1.20	0.24	0.00	0.00	inf	0.99
	T2DM vs. T2DNH	0.61	0.39	0.94	0.03*	0.63	0.39	1.02	0.05	0.00	0.00	inf	0.99

SNP: single nucleotide polymorphism; A1: code for allele 1 (the more rare or "minor" allele based on the entire sample frequencies); A2: code for allele 2 (the more common or "major" allele); CON: control group; T2D: over all type 2 diabetes with and without nephropathy group; T2DM: type 2 Diabetes without nephropathy group; T2DNH: type 2 diabetes with nephropathy who are on hemodialysis group; F_A: frequency of minor allele in affected individuals (case); F_U: frequency of minor allele in unaffected individuals (control); CHISQ: Chi-squared value for allelic association (with 1 df); P: the asymptotic P-value for chi-square test; OR: odds ratio; L95: lower bound of the 95% confidence; U95: upper bound of the 95% confidence; *Significant

the kidney is unique, because it contains all the elements of the RAAS with compartmentalization in the interstitial networks and tubules as well as intracellular accumulation^[54]. In this regard, the adrenal glands along with the kidneys are distinctive because of their tissue concentrations of Ang II, which are much higher than can be explained by the concentrations transported by the arterial blood flow^[55]. There is considerable evidence that the major fraction of Ang II present in renal tissues is greater locally from Ang delivered to the kidney as well as from angiotensinogen locally produced by proximal tubule cells^[56,57]. Renin secreted by the juxtaglomerular apparatus cells and delivered to the renal interstitium and vascular compartment appear to be a most powerful controller for producing Ang I from Ang^[58,59]. To regulate the production of Ang II, renin is most important, because once Ang I is formed, conversion readily occurs as there are abundant amounts of Ang converting enzyme^[54] in circulation. In this regard the genetic variants of REN gene may have a vital role in the regulation of the gene expression as well as the regulation of Ang II production.

Previous findings show that renin gene polymorphism have been associated with diabetic nephropathy^[39,60-62], increased risk of vascular complications^[25,63], plasma renin activity (PRA)^[44], susceptibility to hypertension in a variety of ethnic groups^[24,25,27,64,65], T2DM^[66,67], and CRI^[68] although often with inconsistent results^[69,70]. A few studies have reported that the renin rs4131714 (-4063C/T) a promoter variant has no association with DN^[39,68,71], whereas the present study found a positive association between rs4131714 (-4063C/T) and T2DNH ($P = 0.02655$), which shows high linkage disequilibrium (LD) with rs16853055 ($D' = 0.936$; $LOD = 82.03$; $r^2 = 0.823$), [Figure 3]. However, the rs16853055 did not show any significant association with diabetes and diabetic nephropathy diseases. Deinum *et al.*^[39] also reported weak association of REN gene (Bgl I RFLP) polymorphism in the first intron with diabetic nephropathy along with increased level of plasma renin. The first intron is involved in the renin gene transcription regulation^[72] and therefore Bgl I RFLP may have some contribution in this regard. Likewise the renin rs4131714 (-4063C/T) promoter variant may also have some regulatory function in the expression of prorenin or renin which is subject to further study. Though we have not measured the level of plasma prorenin or renin which is beyond our scope, but one can illustrate that area. In any future study one can also investigate the precise role of rs16853055 (-3879C/A) polymorphism in connection with diabetic nephropathy, as it shows high linkage disequilibrium with rs4131714 (-4063C/T).

Genetic sketching of the functional genes helps to explore a particular disease as well as helps to recognize the tendency of that disease within a particular population that may eventually be of support to the doctors for recommending personalized medicine.

The present study has to be taken under consideration within its limitations; that it was limited to a specific ethnic group (Eastern Indian Bengali population). A larger study from different ethnic groups will be needed to confirm for any contribution of renin gene polymorphism to T2DM complications for development of

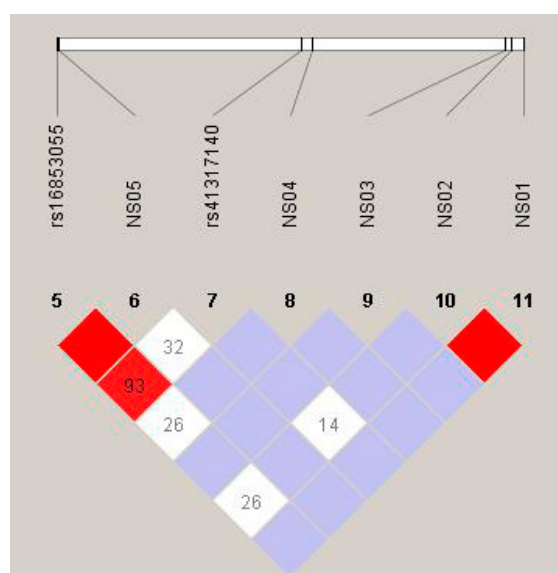


Figure 3. Linkage disequilibrium plot of Renin genes SNPs

renal problem or nephropathy. It is also worth mentioning here that this research work only deals with the association study (irrespective of gender) between diabetic nephropathy and the genetic variation of renin gene within its promoter sequence.

DECLARATIONS

Acknowledgments

We would like to thank the members of the study populations, patients and control participants for voluntarily taking part in this research work and donating their blood samples and cooperation during data collection. We would also like to thank to Dr. P Roychodhury, (Endocrinologist; Calcutta Medical college and Hospital), Dr. S. Bhattachariya, (Nephrologists; B. P. Poddar Hospital) for their cooperation during Patients selection, registration and medical data collection. We wish to express our deep gratitude to the Director, Anthropological Survey of India, for his kind permission to initiate the work and also for providing financial support.

Authors' contributions

Involved in the sequencing experiments, screening for gene mutations, performed the statistical analysis as well as participating in the write up of the manuscript: Purkait P

Contributed to preparation of the manuscript: Halder K

Supervised the project: Naidu JM

Supervised the project and compliance with Institutional ethical procedures: Sarkar B

Read and approved the final manuscript: all authors

Availability of data and materials

Reader can ask or mail to corresponding author for materials.

Financial support and sponsorship

This study was funded by the Anthropological Survey of India, Kolkata (fellowship to Dr. Pulakes Purkait as Junior Research Fellowship and Senior Research Fellowship).

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Ethical committee clearance was obtained from the respective medical institutions and Ethical committee of the Anthropological Survey of India, Government of India. Verbal and written well informed consent was obtained from all participants before they were eligible for recruitment into the study.

Consent for publication

Although the manuscript does not involve the use of live photographs of any of the participants, consent was obtained from them for the data to be published as at the time recruitment into the study.

Copyright

© The Author(s) 2018.

REFERENCES

1. Ruggenenti P, Craverdi P, Remuzzi G. The RAAS in the pathogenesis and treatment of diabetic nephropathy. *Nature Rev Nephrol* 2010; 6:319-30.
2. Chawla T, Sharma D, Sing A. Role of renin angiotensin system in diabetic nephropathy. *World J Diabetes* 2010;1:141-5.
3. Dunlop M. Aldose reductase and the role of polyol pathway in diabetic nephropathy. *Kidney Int Suppl* 2000;77:S3-12.
4. Noh H, King GL. The role of protein kinase C activation in diabetic nephropathy. *Kidney Int Suppl* 2007;(106):S49-53.
5. Forbes JM, Cooper ME, Oldfield MD, Thomas MC. Role of advanced glycation end products in diabetic nephropathy. *J Am Soc Nephrol* 2003;14:S254-8.
6. Buse MG. Hexosamines, insulin resistance and complications of diabetes: current status. *Am J Physiol Endocrinol Metab* 2006;290:E1-8.
7. Schleicher ED, Weigert C. Role of hexosamine biosynthetic pathway in diabetic nephropathy. *Kidney Int Suppl* 2000;77:S13-8.
8. Ruggenenti P, Bettinaglio P, Pinares F, Remuzzi G. Angiotensin converting enzyme insertion/deletion polymorphism and renoprotection in diabetic and nondiabetic nephropathies. *Clin J Am Soc Nephrol* 2008;3:1511-25.
9. Wang F, Fang Q, Yu N, Zhao D, Zhang Y, Wang J, Wang Q, Zhou X, Cao X, Fan X. Association between genetic polymorphism of the angiotensin converting enzyme and diabetic nephropathy: a meta-analysis comprising 26,580 subjects. *J Renin Angiotensin Aldosterone Syst* 2012;13:161-74.
10. Rahimi Z, Hasanvand A, Felehgari V. Interaction of MTHFR 1298C with ACE D allele augments the risk of diabetic nephropathy in Western Iran. *DNA Cell Biol* 31;553-9.
11. Imai T, Miyazaki H, Hirose S, Hori H, Hayashi T, Kageyama R, Ohkubo H, Nakanishi S, Murakami K. Cloning and sequence analysis of cDNA for human renin precursor. *Proc Natl Acad Sci U S A* 1983;80:7405-9.
12. Hsueh WA, Baxter JD. Human prorenin. *Hypertension* 1991;17:469-77.
13. Morales R, Wathier Y, Böcskei Z. Human prorenin structure sheds light on a novel mechanism of its autoinhibition and on its non-proteolytic activation by the pro(renin) receptor. *J Mol Biol* 2012;421:100-11.
14. Sparks MA, Crowley SD, Gurley SB, Mirososou M, Coffman TM. Classical renin-angiotensin system in kidney physiology. *Compr Physiol* 2014;4:1201-28.
15. McGill JR, Chirgwin JM, Moore CM, McCombs JL. Chromosome localization of the human renin gene (REN) by in situ hybridization. *Cytogenet Cell Genet* 1987;45:55-7.
16. Hobart PM, Fogliano M, O'Connor BA, Schaefer IM, Chirgwin JM. Human renin gene: structure and sequence analysis. *Proc Natl Acad Sci U S A* 1984;81:5026-30.
17. Cohen-Hagueneauer O, Soubrier F, Van Cong N, Serero S, Turleau C, Jegou C, Gross MS, Corvol P, Frézal J. Regional mapping of the human renin gene to 1q32 by in situ hybridization. *Ann Genet* 1989;32:16-20.
18. Navar LG, Nishiyama A. Intrarenal formation of angiotensin II. *Contrib Nephrol* 2001;(135):1-15.
19. Ichihara A, Kobori H, Nishiyama A, Navar LG. Renal renin-angiotensin system. *Contrib Nephrol* 2004;143:117-30.
20. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev* 2006;86:747-803.
21. Schweda F, Kurtz A. Cellular mechanism of renin release. *Acta Physiol Scand* 2004;181:383-90.
22. Navar LG. The kidney is blood pressure regulation and development of hypertension. *Med Clin North Am* 1997;81:1165-98.
23. Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JA, Smith RD. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 1993;45:205-51.
24. Moore N, Dicker P, O'Brien JK, Stojanovic M, Conroy RM, Treumann A, O'Brien ET, Fitzgerald D, Shields D, Stanton AV. Renin gene polymorphisms and haplotypes, blood pressure, and responses to renin-angiotensin system inhibition. *Hypertension* 2007;50:340-7.
25. Mansego ML, Redon J, Marin R, González-Albert V, Martín-Escudero JC, Fabia MJ, Martínez F, Chaves FJ. Renin polymorphisms and haplotypes are associated with blood pressure levels and hypertension risk in postmenopausal women. *J Hypertens* 2008;26:230-7.
26. Ahmad U, Saleheen D, Bokhari A, Frossard PM. Strong association of a renin intronic dimorphism with essential hypertension. *Hypertens Res* 2005;28:339-44.
27. Frossard PM1, Malloy MJ, Lestrangant GG, Kane JP. Haplotypes of the human renin gene associated with essential hypertension and stroke. *J Hum Hypertens* 2001;15:49-55.

28. Chiang FT, Hsu KL, Tseng CD, Lo HM, Chern TH, Tseng YZ. Association of the renin gene polymorphism with essential hypertension in a Chinese population. *Clin Genet* 1997;51:370-4.
29. Sarkar S, Gupta V, Kumar A, Chaudhary M, Diyundi S, Sehajpal PK, Thangaraj K, Rajender S. M235T polymorphism in the AGT gene and A/G18-83 substitution in the REN gene correlate with end-stage renal disease. *Nephron* 2015;129:104-8.
30. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
31. Purkait P, Halder K, Thakur S, Ghosh Roy A, Raychaudhuri P, Bhattacharya S, Sarkar BN, Naidu JM. Association of angiotensinogen gene SNPs and haplotypes with risk of hypertension in eastern Indian population. *Clin Hypertens* 2017;23:12.
32. Purkait P, Roy AG, Halder K, Suthar P, Raychaudhuri P, Bhattacharya S, Sarkar BN, Naidu JM. Sex-based association of CYP11B2 (-344 C/T) polymorphism in Indian type 2 diabetic patients. *Int J Diabetol Vasc Dis Res* 2015;3:89-93.
33. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a toolset for whole-genome association and population-based linkage analysis. *Am J Hum Genet* 2007;81:559-75.
34. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263-5.
35. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. *Science* 2002;296:2225-9.
36. Frossard PM, Gonzalez PA, Fritz LC, Ponte PA, Fiddes JC, Atlas SA. Two RFLPs at the human renin (ren) gene locus. *Nucleic Acids Res* 1986;14:4380.
37. Frossard PM, Lestringant GG, Malloy MJ, Kane JP. Human renin gene BglII dimorphism associated with hypertension in two independent populations. *Clin Genet* 1999;56:428-33.
38. Naftilan AJ, Williams R, Burt D, Paul M, Pratt RE, Hobart P, Chirgwin J, Dzau VJ. A lack of genetic linkage of renin gene restriction fragment length polymorphisms with human hypertension. *Hypertension* 1989;14:614-8.
39. Deinum J, Tarnow L, van Gool JM, de Bruin RA, Derkx FH, Schalekamp MA, Parving HH. Plasma renin and prorenin and renin gene variation in patients with insulin-dependent diabetes mellitus and nephropathy. *Nephrol Dial Transplant* 1999;14:1904-11.
40. Hasegawa G, Nakano K, Sawada M, Uno K, Shibayama Y, Ienaga K, Kondo M. Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. *Kidney Int* 1991;40:1007-12.
41. Heesom AE, Hibberd ML, Millward A, Demaine AG. Polymorphism in the 5'-end of the aldose reductase gene is strongly associated with the development of diabetic nephropathy in type1 diabetes. *Diabetes* 1997;46:287-91.
42. Parving HH, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P; Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group. The effect of irbesartan on the development of diabetic nephropathy in patient with type 2 diabetes. *N Engl J Med* 2001;345:870-8.
43. Purkait P, Halder K, Roy AG, Sarkar BN, Naidu JM. GSTM1 null genotype associated with type 2 diabetic nephropathy patients among Indian population. *World J Pharm Res* 2014;3:4452-63.
44. Hasimu B, Nakayama T, Mizutani Y, Izumi Y, Asai S, Soma M, Kokubun S, Ozawa Y. Haplotype analysis of the human renin gene and essential hypertension. *Hypertension* 2003;41:308-12.
45. Viswanathan V. Prevention of diabetic nephropathy: a diabetologist's perspective. *Indian J Nephrol* 2004;14:157-62.
46. O'Donnell CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, Myers RH, Levy D. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. *Circulation* 1998;97:1766-72.
47. Manrique C, Lastra G, Gardner M, Sowers JR. The renin angiotensin aldosterone system in hypertension: roles of insulin resistance and oxidative stress. *Med Clin North Am* 2009;93:569-82.
48. Lin J, Hu FB, Qi L and Curhan GC. Genetic polymorphisms of angiotensin-2 type 1 receptor and angiotensinogen and risk of renal dysfunction and coronary heart disease in type 2 diabetes mellitus. *BMC Nephrol* 2009;10:9.
49. Chang HR, Cheng CH, Shu KH, Chen CH, Lian JD, Wu MY. Study of the polymorphism of angiotensinogen, angiotensin-converting enzyme and angiotensin receptor in type II diabetes with end-stage renal disease in Taiwan. *J Chin Med Assoc* 2003;66:51-6.
50. Ezzidi I, Mtiraoui N, Kacem M, Chaieb M, Mahjoub T, Almawi WY. Identification of specific angiotensin-converting enzyme variants and haplotypes that confer risk and protection against type 2 diabetic nephropathy. *Diabetes Metab Res Rev* 2009;25:717-24.
51. van Ittersum FJ, de Man AM, Thijssen S, de Knijff P, Slagboom E, Smulders Y, Tarnow L, Donker AJ, Bilo HJ, Stehouwer CD. Genetic polymorphisms of the renin-angiotensin system and complications of insulin-dependent diabetes mellitus. *Nephrol Dial Transplant* 2000;15:1000-7.
52. Mitchell KD, Navar LG. Intrarenal actions of angiotensin II in the pathogenesis of experimental hypertension, in hypertension: pathophysiology, diagnosis and management. In: Laragh JH, Brenner BM, editors. New York: Raven Press; 1995. p. 1437-50.
53. Navar LG, Prieto-Carrasquero MC, Kobori H. Renal renin-angiotensin system. In: Kastin AJ, editor. Handbook of Biologically Active Peptides. Academic Press; 2006. p. 1235-42.
54. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev* 2007;59:251-87.
55. Ingert C, Grima M, Coquard C, Barthelmebs M, Imbs JL. Contribution of angiotensin II internalization to intrarenal angiotensin II levels in rats. *Am J Physiol Renal Physiol* 2002;283:F1003-10.
56. Rosivall L, Navar LG. Effects on renal hemodynamics of intra-arterial infusions of angiotensins I and II. *Am J Physiol* 1983;245:F181-7.
57. Komlosi P, Fuson AL, Fintha A, Peti-Peterdi J, Rosivall L, Warnock DG, Bell PD. Angiotensin I conversion to angiotensin II stimulates

- cortical collecting duct sodium transport. *Hypertension* 2003;42:195-9.
58. Hackenthal E, Paul M, Ganten D, Taugner R. Morphology, physiology, and molecular biology of renin secretion. *Physiol Rev* 1990;70:1067-116.
 59. Schnermann JB, Traynor T, Yang T, Huang YG, Oliverio MI, Coffman T, Briggs JP. Absence of tubuloglomerular feedback responses in AT1A receptor deficient mice. *Am J Physiol* 1997;273:F315-20.
 60. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Lecerf L, Poirier O, Danilov S, Parving HH. Lack of relationship between an insertion/deletion polymorphism in the angiotensin I converting enzyme gene and diabetic nephropathy and proliferative retinopathy in IDDM patients. *Diabetes* 1995;44:489-94.
 61. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Ricard S, Poirier O, Parving HH. Angiotensinogen gene polymorphisms in IDDM patients with diabetic nephropathy. *Diabetes* 1996;45:367-9.
 62. Takahashi K, Yamamoto H, Hirose T, Hiraishi K, Shoji I, Shibasaki A, Kato I, Kaneko K, Sasano H, Satoh F, Totsune K. Expression of (pro)renin receptor in human kidneys with end-stage kidney disease due to diabetic nephropathy. *Peptides* 2010;31:1405-8.
 63. Kostulas K, Brophy VH, Moraitis K, Manolescu A, Kostulas V, Gretarsdottir S, Cheng S, Hillert J. Genetic profile of ischemic cerebrovascular disease and carotid stenosis. *Acta Neurol Scand* 2008;118:146-52.
 64. Chiang FT, Hsu KL, Tseng CD, Lo HM, Chern TH, Tseng YZ. Association of the renin gene polymorphism with essential hypertension in a Chinese population. *Clin Genet* 1997;51:370-4.
 65. Sun B, Williams JS, Pojoga L, Chamathi B, Lasky-Su J, Raby BA, Hopkins PN, Jeunemaitre X, Brown NJ, Ferri C, Williams GH. Renin gene polymorphism: its relationship to hypertension, renin levels and vascular responses. *J Renin Angiotensin Aldosterone Syst* 2011;12:564-71.
 66. Purkait P, Suthar PC, Purohit VK, Naidu JM, Sarkar BN. Renin- Angiotensin- Aldosterone System gene polymorphisms in Type 2 Diabetic patients among the Mewari population of Rajasthan. *Int J Biol Med Res* 2013;4:3128-34.
 67. Pulakes Purkait, Raychodhury P, Bandhyopadhy S, Naidu JM, Sarkar BN. Analysis of Aldosterone Synthase Gene Promoter (-344 C>T) Polymorphism in Indian Diabetic Nephropathy Patients. *J Diabetes Metab* 2013;4:271.
 68. Prasad P, Tiwari AK, Kumar KM, Ammini AC, Gupta A, Gupta R, Sharma AK, Rao AR, Nagendra R, Chandra TS, Tiwari SC, Rastogi P, Gupta BL, Thelma BK. Chronic renal insufficiency among Asian Indians with type 2 diabetes: I. Role of RAAS gene polymorphisms. *BMC Med Genet* 2006;7:42.
 69. Berge KE, Berg K. No effect of a BglII polymorphism at the renin (REN) locus on blood pressure level or variability. *Clin Genet* 1994;46:436-8.
 70. Chiang FT, Hsu KL, Tseng CD, Lo HM, Chern TH, Tseng YZ. Association of the renin gene polymorphism with essential hypertension in a Chinese population. *Clin Genet* 1997;51:370-4.
 71. Mtiraoui N, Ezzidi I, Turki A, Chaieb M, Mahjoub T, Almawi WY. Renin-angiotensin-aldosterone system genotypes and haplotypes affect the susceptibility to nephropathy in type 2 diabetes patients. *J Renin Angiotensin Aldosterone Syst* 2011;12:572-80.
 72. Germain S, Philippe J, Fuchs S, Lengronne A, Corvol P, Pinet F. Regulation of human renin secretion and gene transcription in Calu-6 cells. *FEBS Letters* 1997;407:177-183.

Review

Open Access



Advantages and disadvantages of total arterial coronary artery bypass graft as compared to venous coronary artery bypass graft

Dickson Dewantoro¹, Antonio Nenna², Umberto Satriano², Massimo Chello², Cristiano Spadaccio¹

¹Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK.

²Department of Cardiovascular Surgery, Università Campus Bio-Medico di Roma, Rome 00128, Italy.

Correspondence to: Dr. Cristiano Spadaccio, Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Agamemnon St, Glasgow, Clydebank G81 4DY, UK. E-mail: cristianospadaccio@gmail.com

How to cite this article: Dewantoro D, Nenna A, Satriano U, Chello M, Spadaccio C. Advantages and disadvantages of total arterial coronary artery bypass graft as compared to venous coronary artery bypass graft. *Vessel Plus* 2018;2:20. <http://dx.doi.org/10.20517/2574-1209.2018.50>

Received: 26 Jun 2018 **First Decision:** 17 Jul 2018 **Revised:** 23 Jul 2018 **Accepted:** 26 Jul 2018 **Published:** 16 Aug 2018

Science Editor: Mario F. L. Gaudino, Cristiano Spadaccio **Copy Editor:** Jun-Yao Li **Production Editor:** Huan-Liang Wu

Abstract

Considering the plethora of literature about surgical revascularization, this review aims to discuss the most recent studies about the effects of total arterial coronary artery bypass graft (TACABG) compared with CABG that involves venous graft (VCABG) in patients with multivessel coronary artery disease. Patients were sampled from published papers that studied various aspects involving TACABG or VCABG. Resulting samples were used to compare the complexity and 5 years' outcomes of TACABG to VCABG in the revascularization of coronary arteries. TACABG provides a better prognosis with average all-cause mortality within 5 years of 5.35% as compared to VCABG with average of all-cause mortality within 5 years of 9.1%. Furthermore, assumption of deep sternal wound infection from TACABG, especially when bilateral internal thoracic arteries were used, is very technique-dependent, as reports have been showing that the rate of such infection to occur is less than 1%. TACABG was concluded to wield a better prognosis within both short- and long- terms, although more research need to be done to prove its use in left main disease.

Keywords: Total arterial, internal thoracic artery, coronary artery disease, myocardial revascularization, coronary artery bypass graft

INTRODUCTION

Coronary heart disease, or coronary artery disease (CAD), commonly caused by atherosclerosis, as mentioned by World Health Organization, has markedly lower prevalence within populations with lower life expectancy. However, the presence of risk factors contributes greatly towards the prevalence of CAD, and 3.8



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



million men and 3.4 million women died of CAD each year^[1]. Therefore, there is urgent need for prevention, early recognition, and proper management of CAD in order to reduce mortality while also improving quality of life.

Percutaneous coronary intervention (PCI) provides a way for emergency intervention of CAD in the setting of acute coronary syndromes and provides a less invasive intervention of CAD in stable patients. According to an international, open-label, multicenter randomized trial that compared everolimus-eluting stents with coronary artery bypass graft (CABG) for the management of patients with left main disease, the 3 years' rate of death from any cause, stroke, or myocardial infarction of PCI was higher than the CABG group at 15.4% and 14.7% respectively. However, these differences did not seem to be very significant^[2].

CABG is part of a routinely done revascularization intervention to manage CAD by using grafted vessels to revascularize vessels distal from the blockage. Various grafts can be obtained from different sources, which include veins (especially saphenous vein) and arteries (such as radial artery and internal mammary arteries). This article is written to discuss the advantages and disadvantages of using each type of grafts based on results provided by existing studies. This article is mainly aimed at comparing total arterial coronary artery bypass graft (TACABG) with CABG that involves venous graft (VCABG) in term of benefits for patients who suffered from CAD from the surgeon's perspective. In common clinical practice, left anterior descending is grafted with an arterial conduit, generally the left internal thoracic artery; other grafts could be performed using arterial or venous conduits, with single grafts, sequential grafts or composite grafts. In TACABG, all grafts are arterial and therefore no veins are used for revascularization, while in VCABG at least one graft derives from a vein. In brief, differences between arterial and venous grafts will be discussed, in terms of harvesting and complications, and then the results of the most significant clinical trials will be summarized.

DIFFERENCES IN HARVESTING BETWEEN VENOUS AND ARTERIAL GRAFTS

Grafts: complexity pre-operatively and peri-operatively

Complexity of each graft can be assessed by comparing the requirements for preparations of procedures, time taken to do the surgery, and skills required to perform the surgery. When surgeons decided to choose radial artery as a conduit, they need to make sure that the compensating ulnar artery is working properly, thus, there is a need to do a modified Allen's test. This is not the case for saphenous venous graft, which also has the advantage of being longer and easier to handle^[3].

During the operation, the standard procedure, according to a retrospective multicenter study, is for all patients to undergo median sternotomy for the open-heart surgery. Firstly, when internal thoracic arteries (ITAs) (also known as internal mammary arteries, IMAs) are required, they are obtained in a skeletonized or semi-skeletonized manner. Secondly, when radial arteries (RAs) are required, they are supposed to be done through sharp dissection to provide open atraumatic entry and the arterial extraction would then be supplemented by the use of low-power cautery or harmonic scalpel. Lastly, when saphenous vein grafts (SVGs) are required, open entry technique in the lower leg is done while avoiding the thigh vein^[4,5].

While trying to find out the average time taken to do each types of CABG, there was no studies that specifically show the total time taken for each procedure. However, operative time can be interpreted as the sum of perfusion time (Cardio-pulmonary Bypass time) and cross clamp time^[6]. This information is available from a retrospective study about the effectiveness of total arterial revascularization. In the study, the mean cross-clamp time for total arterial revascularization and non- total arterial revascularization are 60.6 and 63.8 min respectively; and the perfusion times for each groups are 80.2 and 90.7 respectively^[4]. Even though patients are given prophylaxis antibiotics, it is logical that increased open surgery time is equal to increased risk of infection.

Radial artery: between intrinsic limitations and clinical effectiveness

A few papers have summarized the important limitations of radial artery that need to be taken into consid-

eration while being chosen as a conduit^[7,8]. As discussed above, there is a requirement for the adequacy of ulnar flow in order to act as a collateral blood supply. This can be assessed by modified Allen's test and complemented by pulse oximetry and echo-Doppler. Also, calcified radial arteries or those with diameter of less than 2 mm are generally excluded from harvesting. There might be sensory abnormalities and motor weakness in the forearm after removal of radial arteries, and there is a requirement for the use of vasodilators as radial arteries are infamous for their striking spastic reactions to vasoconstrictors and hypothermia. While skeletonization is described to provide a longer graft with larger diameters, less spasms, and better patency frequencies, it increases the harvesting time and the risk of severe graft injury^[8].

However, a recent metanalysis evaluated 534 patients with radial-artery grafts and 502 patients with saphenous-vein grafts, concluding that as compared with the use of saphenous-vein grafts, the use of radial-artery grafts for CABG resulted in a lower rate of adverse cardiac events and a higher rate of patency at 5 years of follow-up. At follow-up angiography, the use of radial-artery grafts was also associated with a significantly lower risk of occlusion (hazard ratio: 0.44); lower incidence of myocardial infarction (hazard ratio: 0.72) and a half incidence of repeat revascularization^[9].

POST-OPERATIVE OUTCOMES

The post-operative outcome of a procedure is crucial in deciding on whether such procedure is worth doing, especially in term of benefits and harm for the patients. Several studies have compared the outcome of coronary artery revascularization that will be compared in this article (considering only CABG) and comparing the data based on whether CABG done in the study is TACABG or VCABG.

Patency

In general, when comparing ITA and SV when they were acting as conduits, several studies such as a follow-up Cooperative Studies Trial done by Goldman *et al.*^[10], showed that ITA had better patency as shown by the 10-year angiogram of the study mentioned. The 10-year patency was 61% for SVG and 85% for ITA. However, the number of patients has been declining during the 10 years' period, so that at 1 week the study cohort consisted of 1025 patients but at 10 years follow up the study cohort declined to just 85 patients. However, from the study's graft, it is shown that the percentage of patent grafts has always been higher in ITA as compared to SVG^[10]. Another study done in order to determine the post-CABG prognostic factors for atherosclerosis progression that further supports the superiority of arterial grafts as the saphenous vein conduit's patency, due to it being prone to develop atherosclerosis, may act as a limiting factor for better prognosis of revascularization^[11].

This is confirmed by a prospective study aiming to find out the patency of right ITA (RITA) as compared to other conduit vessels. The study showed that, at 10 years, the patency of RITA is at least 90%; RA is 70%; and SVG is 50%^[12]. Thus, confirming that arterial conduits are more patent than saphenous venous conduits.

Long-term clinical outcomes

Mortality and serious adverse events are the key points when comparing TACABG with VCABG. However, no direct comparison could be made using current clinical data due to the lack of tailored studies, but TACABG and VCABG could be indirectly evaluated with the results of some trials.

According to a non-blinded prospective, randomized, open-label, non-inferiority trial published in 2016, out of 592 patients with left main disease, with mean age of 66.2 years, the 5-year Kaplan-Meier outcome estimated for all-cause mortality is 32 patients (9%); major adverse Cardiac and Cerebrovascular Events (MACCE) occurred in 80 patients (18%); total revascularization rate is 10%, and stroke incidence was 2%^[13]. During this study, there seem to be no propensity score analysis done but the 5-year Kaplan-Meier estimates were stratified into groups based on SYNTAX score in order to reduce propensity bias.

Another randomized trial that aims at comparing single and bilateral ITA conduits has been used to provide data for the outcome of VCABG. The data taken from this trial will be the outcome for the single ITA conduit to reduce the bias provided by using both ITA in term of sternal healing and long-term patency of graft. In this trial, there were 1554 patients with average age of 63.5 years that underwent single ITA graft plus supplementary venous or arterial conduits. The 5-year outcome found from patients follow up in term of MACCE is 198 (12.7%), all-cause mortality was 8.4%, total revascularization rate was 6.6% and stroke incidence was 3.2%. As mentioned in the report, even though this trial involved statistical corrections and propensity matching, there is still chance of bias in terms of patient and operator selection. In this trial, it was mentioned that a post-hoc analysis of the SYNTAX trial compared 5-year outcomes in 456 patients who received a second arterial conduit with those in 963 patients who underwent single ITA grafting with additional vein grafts, in which propensity score adjustment was done, showing that MACCE were 23.3% in arterial group and 21.4% in venous group ($P = 0.04$). However, the all-cause mortality was 9.1% in the arterial group and 9.5% in the venous group ($P = 0.19$)^[11,14]. From this analysis, single ITA with supplementary vein graft has better MACCE outcome as compared to total arterial revascularization. However, the starting number of arterial group is half of that venous group, showing a tighter population choice which lead to selection bias, even though propensity score has been done. On the other hand, the venous group done worse in term of all-cause mortality, although no differences were found with regards to cardiovascular mortality.

Special population: patients with left main disease

Left main CAD is the highest-risk lesion subset of ischemic heart disease and has traditionally been an indication for coronary artery bypass grafting (CABG). Significant (defined as a greater than 50% angiographic narrowing) left main disease is found in 4 to 6% of all patients who undergo coronary arteriography, and it is associated with multivessel CAD about 70% of the time. While trying to find outcome analysis available to show the result of total arterial CABG in left main disease, it has shown to be a challenge as there are not much of such data available. The closest data is that from RAPCO study^[15]. In this study, radial artery was compared to either right ITA or saphenous vein and patency was then compared. The group of patients in which the result is being used here, is that of 140 patients with an average age of 60.1 years old with total arterial revascularization of their cardiac arteries. The only available results are all cause of mortality (2%) and revascularization with PCI (2%). RAPCO study is a prospective, randomized, single-center trial^[15]. Even though the study was able to provide criteria in order to reduce selection bias due to its prospective nature, but by being a single-center trial, it may not necessarily be representative of the population in general [Table 1].

A retrospective multicenter analysis comparing TACABG to VCABG, has shown that TACABG is associated with higher peri-operative as well as long-term survival^[4]. In the study, the Kaplan-Meier survival within 5 years for TACABG group is 91.3% as compared to 90.1% in the VCABG patients ($P < 0.01$). Although it may seem that the survival rates are not significantly different, when compared to those of the 10 years, there seems to be a widening gap between the two groups, in which the survival for TACABG is 85.4% while VCABG is 81.2% ($P < 0.01$)^[4]. This study should not show a significant selection bias due to propensity score matching. Although, its nature of being a retrospective analysis may lead to some unmodifiable selection bias.

DISCUSSION

Evidence from the literature show that arterial conduits have better prognosis as compared to venous conduits when used in the revascularization of coronary arteries. However, there are certain aspects of venous conduits that are non-inferior than arterial ones.

Firstly, in term of preparation, there is a requirement to check that any of the vessels being used are intact and of satisfactory quality to provide the best outcome from undergoing CABG. While it is required to check for the patency of ulnar artery when radial artery is being used, there seem to be no requirement to

Table 1. 5-year outcome of patients in TACABG and VCABG in patients with left main disease

	NOBLE*	VCABG	TAR Non-TAR group	TACABG	
		ART Single-graft group		RAPCO**	TAR TAR group
Average age (years)	66.2	63.5	64.7	60.1	64.4
Number of patients	592	1554	6232	140	6232
MACCE	80 (18%)	198 (12.7%)*	N/A	N/A	N/A
All-cause mortality	32 (9%)	130 (8.4%)	9.9%***	3 (2%)	8.7%***
Cardiac death	15 (3%)	N/A	N/A	N/A	N/A
Vascular death	1 (< 1%)	N/A	N/A	N/A	N/A
Non-procedural myocardial infarction	10 (2%)	N/A	N/A	N/A	N/A
Revascularisation (total)	47 (10%)	103 (6.6%)	N/A	N/A	N/A
Revascularisation with PCI	45 (10%)	N/A	N/A	3 (2%)	N/A
Revascularisation with CABG	2 (< 1%)	N/A	N/A	N/A	N/A
Target lesion revascularisation	36 (8%)	N/A	N/A	N/A	N/A
Target LMCA revascularisation	33 (9%)	N/A	N/A	N/A	N/A
De novo lesion revascularisation (new lesion in non-grafted segment)	11 (3%)	N/A	N/A	N/A	N/A
Symptomatic graft occlusion or definite stent thrombosis	15 (4%)	N/A	N/A	N/A	N/A
Stroke	7 (2%)	49 (3.2%)	N/A	N/A	N/A

*Composite death, myocardial infarction, and stroke; **RAPCO only take RA conduit; ***Derived from Kaplan-Meier survival. CABG: coronary artery bypass graft; TACABG: total arterial CABG; VCABG: CABG that involves venous graft; PCI: percutaneous coronary intervention; LMCA: left main coronary artery; MACCE: major adverse cardiac and cerebrovascular events; TAR: total-arterial revascularization; NOBLE/ART/RAPCO refer to names of clinical trials

check for saphenous vein collateral circulation. However, it has been shown that checking for saphenous vein through the use of Doppler ultrasound improves the prognosis of SVG^[16].

Secondly, in term of operation time, the total of cross-clamp time and perfusion time is shorter in the TACABG than in VCABG (as has been discussed above), a retrospective study has shown that the operative time taken for total arterial revascularization was 30 minutes longer^[17]. This is true especially when bilateral ITA or RA was used. The paper further added that the additional time taken was due to the extra conduit harvest and not to actual grafting procedure^[17].

Also, BIMA could be performed using two different configurations, in situ versus Y-graft. A recent study evaluated whether graft configuration might affect long-term outcomes in 2150 patients using a propensity-score approach^[18]. Late mortality and incidence of MACCES were similar between groups, and therefore the clinical outcome of BIMA grafting is independent of surgical configuration. However, Y-grafting increases the flexibility of BIMA grafting and should be taken into account when a surgical strategy for myocardial revascularization needs to be planned^[18].

Thirdly, it is important to consider the short term post-operative outcome of a surgical procedure. An example of this is the healing of any surgical wounds inflicted during CABG procedure, especially in high risk patients (such as those with diabetes mellitus)^[19]. One of the main topic of interest is the healing outcome of the sternum and chest wall after the collections of ITA, especially if bilateral ITAs were harvested. However, through careful harvesting of such grafts while preserving pleural cavities' integrity, it reduces the post-operative morbidity as well as lowering hospital cost^[19,20]. One of the fear of TACABG is deep sternal wound infection (DSWI), especially if bilateral ITA was used. However, reports from various studies have shown that there were low rates of DSWI, that is lower than 1%, in TACABG^[4,21-24]. The incidence of DSWI may be significantly higher after the harvest of both internal thoracic arteries in the elderly, with an odds risk of 1.86 ($P < 0.01$)^[25]. However, the risk of deep sternal wound infection can be minimized in diabetic patients undergoing CABG by performing ITA harvested in a skeletonized manner with meticulous attention to preserving sternal blood flow. Pedicled harvest is to be discouraged when utilizing both ITA owing to a significant

increase in the risk of postoperative DSWI^[26,27]. In fact, a recent meta-analysis showed that skeletonized ITA appears to reduce the incidence of postoperative SWI in comparison with pedicled ITA after CABG, with this effect being modulated by the presence of diabetes^[28]. In the sensitivity analysis, the difference in favour of skeletonized ITA was also observed in subgroups such as diabetic, bilateral ITA and diabetic with bilateral ITA; also, there was a difference in the type of study, since non-randomized studies together demonstrated the benefit of skeletonized ITA in comparison with pedicled ITA, but the randomized studies together did not show this difference^[28]. To summarize, strategies that reduce DSWI target the modifiable risk factors that include microbiological factors, appropriate antibiotic prophylaxis, tight glycemic control, while surgical strategies reduce DSWI following BIMA harvest include techniques of IMA harvesting with lesser devascularization of sternum using skeletonized, semiskeletonized and modified pedicle harvest are associated with greater preservation of sternal blood supply and sternal closure and stability techniques^[29]. Antibiotic prophylaxis given to patients pre- and post-operatively helps further reduce the chance of wounds infection, improving the surgical prognosis. Diabetes acts as a risk factors for the development of atherosclerosis as it accelerates the formation of atheroma^[30]. Its presence in patients who were undergoing revascularization for atherosclerosis also increased the risk of post-operative complications. A retrospective study with propensity score matching compared total arterial revascularizations to procedures that involved venous grafts in the revascularization of atherosclerosis in diabetic patients^[17]. While the rate of perioperative mortality (within 30-days post-operatively) was similar at 1.2% in total arterial CABG group as compared to 1.4% in the non-total arterial CABG group, nonetheless the rate of late mortality (mean of 4.9 years) was less among the total arterial CABG groups at 10.2% as compared to the non-total arterial CABG group at 12.2%^[17]. Thus, adding a point towards the advantage of undergoing total arterial CABG.

Fourthly, the long-term outcome (that is mainly patient's survival rate) is also an important factor to consider before choosing a procedure. From previous discussion, it has been shown that TACABG provides a better prognosis than VCABG. However, the long-term outcome of TACABG only showed the survival rate and did not elaborate on the MACCE that would be more relevant to the finding. Then, there was also no mentioning of revascularization in this group. However, based on literature and what was known about the patency of SVG as compared to arterial grafts, it could be concluded that total arterial grafting would yield a better outcome whenever it is possible to be done. A study confirmed that by using only SVG as compared to the use of ITA, there was 1.61 times greater risk of death throughout 10 years post-operation^[31], thus further favoring arterial conduits^[32,33].

A window for development into the surgical skills that may be beneficial to patients that are undergoing CABG is by doing such procedures through off-pump method. A paper querying about The Society of Thoracic Surgeons National Cardiac Database showed that off-pump coronary artery bypass was associated with a significant reduction in risk of death, stroke, acute renal failure, mortality or morbidity, and hospital stay as compared to on-pump coronary artery bypass^[34]. This does not only benefit patients (which would be one of the main points in considering on a procedure), but also help cut cost for the healthcare system (which makes the other critical point that need to be balanced together with patient's benefit and long term outcome). However, such procedures require experience as surgeons will be required to perform the surgery while the heart is still beating and thus avoiding the use of cardiopulmonary bypass pump. This would set an example in which a procedure that would be beneficial for both the patients and healthcare system requires significant investment, that is the amount of training need to be done by surgeons.

The challenge of increasing TACABG procedure lies on assuring surgeons that this procedure provides a greater benefit for patients and the healthcare system. Although there would be some exceptions for the procedure, in which VCABG can be used as an alternative when possible. In term of left main disease, there need to be more studies and results published to show the outcome (both long- and short-term) of using TACABG in the revascularization of left main disease.

CONCLUSION

TACABG takes a longer preparation as compared to non-total arterial CABG. However, whenever it is possible to perform TACABG, the short- and long-term survival were better as compared to VCABG. In addition, the patency of arterial conduits has been shown to be longer lasting and less prone to damage as compared to venous conduits. While there was a similar 30-day outcome in both TACABG and VCABG groups, the long-term mortality rate was higher in the VCABG group as compared to TACABG. Among the arteries available to be conduits, internal thoracic arteries by far provides the best outcome, even for those with higher risk of complications.

DECLARATIONS

Authors' contributions

Manuscript conception: Dewantoro D, Spadaccio C

Literature search, writing the draft: Dewantoro D, Satriano U

Critical revision of the manuscript, writing the revised version: Nenna A, Spadaccio C

Critical revision of the manuscript: Chello M

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Mackay J, Mensah GA, Greenlund K. The atlas of heart disease and stroke. World Health Organization, 2004.
2. Stone GW, Sabik JF, Serruys PW, Simonton CA, Généreux P, Puskas J, Kandzari DE, Morice MC, Lembo N, Brown WM 3rd, Taggart DP, Banning A, Merkely B, Horkay F, Boonstra PW, van Boven AJ, Ungi I, Bogáts G, Mansour S, Noiseux N, Sabaté M, Pomar J, Hickey M, Gershlick A, Buszman P, Bochenek A, Schampaert E, Pagé P, Dressler O, Kosmidou I, Mehran R, Pocock SJ, Kappetein AP; EXCEL Trial Investigators. Everolimus-eluting stents or bypass surgery for left main coronary artery disease. *N Engl J Med* 2016;375:2223-35.
3. Al-Sabti HA, Al Kindi A, Al-Rasadi K, Banerjee Y, Al-Hashmi K, Al-Hinai A. Saphenous vein graft vs. radial artery graft searching for the best second coronary artery bypass graft. *J Saudi Heart Assoc* 2013;25:247-54.
4. Tatoulis J, Wynne R, Skillington PD, Buxton BF. Total arterial revascularization: achievable and prognostically effective-a multi-center analysis. *Ann Thorac Surg* 2015;100:1268-75; discussion 1275.
5. Anyanwu AC, Saeed I, Bustami M, Ilsley C, Yacoub MH, Amrani M. Does routine use of the radial artery increase complexity or morbidity of coronary bypass surgery? *Ann Thorac Surg* 2001;71:555-9; discussion 559-60.
6. Maruthappu M, Duclos A, Lipsitz SR, Orgill D, Carty MJ. Surgical learning curves and operative efficiency: a cross-specialty observational study. *BMJ Open* 2015;5:e006679.
7. Gaudino M, Crea F, Cammertoni F, Mazza A, Toesca A, Massetti M. Technical issues in the use of the radial artery as a coronary artery

- bypass conduit. *Ann Thorac Surg* 2014;98:2247-54.
8. Samak M, Fatullayev J, Sabashnikov A, Zeriuoh M, Schmack B, Ruhparwar A, Karck M, Popov AF, Dohmen PM, Weymann A. Total arterial revascularization: bypassing antiquated notions to better alternatives for coronary artery disease. *Med Sci Monit Basic Res* 2016; 22:107-14.
 9. Gaudino M, Benedetto U, Fremes S, Biondi-Zoccai G, Sedrakyan A, Puskas JD, Angelini GD, Buxton B, Frati G, Hare DL, Hayward P, Nasso G, Moat N, Peric M, Yoo KJ, Speziale G, Girardi LN, Taggart DP; RADIAL Investigators. Radial-Artery or Saphenous-Vein Grafts in Coronary-Artery Bypass Surgery. *N Engl J Med* 2018;378:2069-77.
 10. Goldman S, Zadina K, Moritz T, Ovitt T, Sethi G, Copeland JG, Thottapurathu L, Krasnicka B, Ellis N, Anderson RJ, Henderson W; VA Cooperative Study Group #207/297/364. Long-term patency of saphenous vein and left internal mammary artery grafts after coronary artery bypass surgery: results from a Department of Veterans Affairs Cooperative Study. *J Am Coll Cardiol* 2004;44:2149-56.
 11. Domanski MJ, Borkowf CB, Campeau L, Knatterud GL, White C, Hoogwerf B, Rosenberg Y, Geller NL. Prognostic factors for atherosclerosis progression in saphenous vein grafts: the postcoronary artery bypass graft (Post-CABG) trial. *Post-CABG Trial Investigators. J Am Coll Cardiol* 2000;36:1877-83.
 12. Tatoulis J, Buxton BF, Fuller JA. The right internal thoracic artery: the forgotten conduit--5,766 patients and 991 angiograms. *Ann Thorac Surg* 2011;92:9-15; discussion 15-7.
 13. Mäkilä T, Holm NR, Lindsay M, Spence MS, Erglis A, Menown IB, Trovik T, Eskola M, Romppanen H, Kellerth T, Ravkilde J, Jensen LO, Kalinauskas G, Linder RB, Pentikainen M, Hervold A, Banning A, Zaman A, Cotton J, Eriksen E, Margus S, Sørensen HT, Nielsen PH, Niemelä M, Kervinen K, Lassen JF, Maeng M, Oldroyd K, Berg G, Walsh SJ, Hanratty CG, Kumsars I, Stradins P, Steigen TK, Fröbert O, Graham AN, Endresen PC, Corbascio M, Kajander O, Trivedi U, Hartikainen J, Anttila V, Hildick-Smith D, Thuesen L, Christiansen EH; NOBLE study investigators. Percutaneous coronary angioplasty versus coronary artery bypass grafting in treatment of unprotected left main stenosis (NOBLE): a prospective, randomised, open-label, non-inferiority trial. *Lancet* 2016;388:2743-52.
 14. Parasca CA, Head SJ, Mohr FW, Mack MJ, Morice MC, Holmes DR Jr, Feldman TE, Colombo A, Dawkins KD, Serruys PW, Kapteina AP; SYNTAX Investigators. The impact of a second arterial graft on 5-year outcomes after coronary artery bypass grafting in the synergy between percutaneous coronary intervention with TAXUS and cardiac surgery trial and registry. *J Thorac Cardiovasc Surg* 2015; 150:597-606.e592.
 15. Buxton BF, Raman JS, Ruengsakulrach P, Gordon I, Rosalion A, Bellomo R, Horrigan M, Hare DL. Radial artery patency and clinical outcomes: five-year interim results of a randomized trial. *J Thorac Cardiovasc Surg* 2003;125:1363-71.
 16. Luckraz H, Lowe J, Pugh N, Azzu AA. Pre-operative long saphenous vein mapping predicts vein anatomy and quality leading to improved post-operative leg morbidity. *Interact Cardiovasc Thorac Surg* 2008;7:188-91; discussion 191.
 17. Tatoulis J, Wynne R, Skillington PD, Buxton BF. Total arterial revascularization: a superior strategy for diabetic patients who require coronary surgery. *Ann Thorac Surg* 2016;102:1948-55.
 18. Di Mauro M, Iacò AL, Allam A, Awadi MO, Osman AA, Clemente D, Calafiore AM. Bilateral internal mammary artery grafting: in situ versus Y-graft. Similar 20-year outcome. *Eur J Cardiothorac Surg* 2016;50:729-34.
 19. Buxton BF, Hayward PA. The art of arterial revascularization-total arterial revascularization in patients with triple vessel coronary artery disease. *Ann Cardiothorac Surg* 2013;2:543-51.
 20. Bonacchi M, Prifti E, Giunti G, Salica A, Frati G, Sani G. Respiratory dysfunction after coronary artery bypass grafting employing bilateral internal mammary arteries: the influence of intact pleura. *Eur J Cardiothorac Surg* 2001;19:827-33.
 21. Tranbaugh RF, Dimitrova KR, Lucido DJ, Hoffman DM, Dincheva GR, Geller CM, Balaram SK, Ko W, Swistel DG. The second best arterial graft: a propensity analysis of the radial artery versus the free right internal thoracic artery to bypass the circumflex coronary artery. *J Thorac Cardiovasc Surg* 2014;147:133-40.
 22. Parsa CJ, Shaw LK, Rankin JS, Daneshmand MA, Gaca JG, Milano CA, Glower DD, Smith PK. Twenty-five-year outcomes after multiple internal thoracic artery bypass. *J Thorac Cardiovasc Surg* 2013;145:970-5.
 23. Puskas JD, Sadiq A, Vassiliades TA, Kilgo PD, Lattouf OM. Bilateral internal thoracic artery grafting is associated with significantly improved long-term survival, even among diabetic patients. *Ann Thorac Surg* 2012;94:710-5; discussion 715-6.
 24. Raza S, Sabik JF, 3rd, Masabni K, Ainkaran P, Lytle BW, Blackstone EH. Surgical revascularization techniques that minimize surgical risk and maximize late survival after coronary artery bypass grafting in patients with diabetes mellitus. *J Thorac Cardiovasc Surg* 2014;148:1257-64; discussion 1264-56.
 25. Deo SV, Altarabsheh SE, Shah IK, Cho YH, McGraw M, Sarayyepoglu B, Medalion B, Markowitz AH, Park SJ. Are two really always better than one? Results, concerns and controversies in the use of bilateral internal thoracic arteries for coronary artery bypass grafting in the elderly: a systematic review and meta-analysis. *Int J Surg* 2015;16:163-70.
 26. Deo SV, Shah IK, Dunlay SM, Erwin PJ, Locker C, Altarabsheh SE, Boilson BA, Park SJ, Joyce LD. Bilateral internal thoracic artery harvest and deep sternal wound infection in diabetic patients. *Ann Thorac Surg* 2013;95:862-9.
 27. Glineur D, Kuschner CE, Grau JB. Bilateral internal thoracic artery graft configuration and coronary artery bypass grafting conduits. *Curr Opin Cardiol* 2016;31:625-34.
 28. Sá MP, Ferraz PE, Escobar RR, Vasconcelos FP, Ferraz AA, Braile DM, Lima RC. Skeletonized versus pedicled internal thoracic artery and risk of sternal wound infection after coronary bypass surgery: meta-analysis and meta-regression of 4817 patients. *Interact Cardiovasc Thorac Surg* 2013;16:849-57.
 29. Sajja LR. Strategies to reduce deep sternal wound infection after bilateral internal mammary artery grafting. *Int J Surg* 2015;16:171-8.
 30. Chait A, Bornfeldt KE. Diabetes and atherosclerosis: is there a role for hyperglycemia? *J Lipid Res* 2009;50 Suppl:S335-9.
 31. Loop FD, Lytle BW, Cosgrove DM, Stewart RW, Goormastic M, Williams GW, Golding LA, Gill CC, Taylor PC, Sheldon WC. Influen-

- ence of the internal-mammary-artery graft on 10-year survival and other cardiac events. *N Engl J Med* 1986;314:1-6.
32. Gaudino M, Puskas JD, Di Franco A, Ohmes LB, Iannaccone M, Barbero U, Glineur D, Grau JB, Benedetto U, D'Ascenzo F, Gaita F, Girardi LN, Taggart DP. Three arterial grafts improve late survival: a meta-analysis of propensity-matched studies. *Circulation* 2017; 135:1036-44.
 33. Glineur D, D'hoore W, Price J, Dorméus S, de Kerchove L, Dion R, Noirhomme P, El Khoury G. Survival benefit of multiple arterial grafting in a 25-year single-institutional experience: the importance of the third arterial graft. *Eur J Cardiothorac Surg* 2012;42:284-90; discussion 290-1.
 34. Polomsky M, He X, O'Brien SM, Puskas JD. Outcomes of off-pump versus on-pump coronary artery bypass grafting: Impact of preoperative risk. *J Thorac Cardiovasc Surg* 2013;145:1193-8.

Review

Open Access



Recent advances in cerebral cavernous malformation research

Akhil Padarti, Jun Zhang

Department of Biomedical Sciences, Texas Tech University Health Science Center El Paso, El Paso, TX 79905, USA.

Correspondence to: Dr. Jun Zhang, Department of Biomedical Sciences, Texas Tech University Health Science Center, 5001 El Paso Drive, El Paso, El Paso, TX 79905, USA. E-mail: jun.zhang2000@gmail.com

How to cite this article: Padarti A, Zhang J. Recent advances in cerebral cavernous malformation research. *Vessel Plus* 2018;2:21. <http://dx.doi.org/10.20517/2574-1209.2018.34>

Received: 15 May 2018 **First Decision:** 23 Jul 2018 **Revised:** 1 Aug 2018 **Accepted:** 15 Aug 2018 **Published:** 28 Aug 2018

Science Editor: Aaron S. Dumont **Copy Editor:** Huan-Liang Wu **Production Editor:** Cai-Hong Wang

Abstract

Cerebral cavernous malformations (CCM) are manifested by microvascular lesions characterized by leaky endothelial cells with minimal intervening parenchyma predominantly in the central nervous system predisposed to hemorrhagic stroke, resulting in focal neurological defects. Till date, three proteins are implicated in this condition: CCM1 (KRIT1), CCM2 (MGC4607), and CCM3 (PDCD10). These multi-domain proteins form a protein complex via CCM2 that function as a docking site for the CCM signaling complex, which modulates many signaling pathways. Defects in the formation of this signaling complex have been shown to affect a wide range of cellular processes including cell-cell contact stability, vascular angiogenesis, oxidative damage protection and multiple biogenic events. In this review we provide an update on recent advances in structure and function of these CCM proteins, especially focusing on the signaling cascades involved in CCM pathogenesis and the resultant CCM cellular phenotypes in the past decade.

Keywords: Cerebral cavernous malformation, cerebral cavernous malformation signaling complex, angiogenesis, endothelial cells, cellular function, microvessel lesions, protein structure, function domain, motif

INTRODUCTION

Cerebral cavernous malformations (CCMs) are vessel dilatations within microvascular beds in the brain that are predisposed to hemorrhagic stroke. These microvascular malformations are present in 0.5% in the general population^[1]. These lesions are characterized by densely packed tortuous microvessels outlined with deficient interstitial brain parenchyma^[2,3], increasing the propensity of these vascular lesions for leakage^[4]. These microvascular lesions are predominantly found in the central nervous system (CNS) but are also known to affect skin and liver. Although it is highly prevalent^[1], a vast majority (approximately 70%) are asymptomatic^[5].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Even in patients affected by disease within the same family, there is a wide range of clinical presentations which are primarily determined by the number and size of lesions. This suggests that additional genetic modifiers or non-genetic factors may exist in the pathogenesis of the disease^[6]. As of date, *CCM1* (*KRIT1*) on chromosome 7q, *CCM2* (*MGC4607/OSM/Malcavernin*) on chromosome 7p, and *CCM3* (*PDCD10/TFAR15*) on chromosome 3q are genes that are known to cause the familial form of CCM. It is estimated that 1 in 200 people harbor a potential mutation in one of three *CCM* genes and the frequency increases to 1 in 70 in the Hispanic population^[7]. An epidemiological study showed that 94% of all familial forms and 57% of all sporadic forms of CCM result from mutations in one of these three genes^[8], raising a possibility that mutations in other genetic loci might exist^[9,10]. Recently, many polymorphisms were found in patients with the sporadic form of the disease, further strengthening the argument that the presence of other genetic risk factors that can contribute to the sporadic cases of CCM is possible^[11]. However, endeavors to identify novel *CCM* loci have so far failed. It was proposed that the lesions formed in the familial form through a “second hit” mutation while sporadic vascular lesions occurred due to somatic mutations in both alleles caused by mutagens, radiation, or other factors. However, the familial form has an increased propensity for lesions since only one functional allele is present. Therefore, the familial form characteristically develops neurological manifestations earlier with multiple brain lesions, while the sporadic form develops late neurological manifestation with solitary brain lesions^[12,13].

As mentioned earlier, at least one of these three genes (*CCM1*, 2, 3) is disrupted in most CCM cases in humans^[14]. The three *CCM* proteins interact to form a protein complex^[15-19] which further interacts with other proteins^[19-22]. This *CCM* protein complex, referred to as the *CCM* signaling complex (CSC)^[19], has affinity toward a wide range of ligands and such interactions are involved in cell adhesion, migration, and apoptosis^[15,16,19,23-25]. Current evidence suggests that as core CSC proteins, *CCM* proteins act as scaffolds for many signal molecules and spatiotemporally regulate localization and activity of these proteins, with none possessing innate catalytic activity^[26]. Homozygous mutations in any of the three *CCM* proteins are nonviable, indicating their essential role in biogenesis as phenotype suppressors^[11-13]. We will mainly focus on these three core CSC proteins in this review.

CLINICAL PRESENTATION

All forms of human *CCM* mutations induce lesions in the CNS. These lesions are used to follow the course of disease (clinically symptomatic or not) both in the laboratory and in the clinical setting through MRI. A *CCM* variant observed in the skin tissue is known as hyperkeratotic cutaneous capillary venous malformation (HCCVM)^[27]. Other cutaneous manifestations include café-au-lait spots, cutaneous venous malformation, and cavernous hemangiomas^[28,29]. Genetic analysis showed that HCCVMs were only found in patients with a frameshift mutation resulting in a premature stop codon in exon 1 of *CCM1*. *CCM1* mutations have also been associated with hepatic angiomas^[30]. As for clinical manifestation, *CCM1* and *CCM2* familial forms are similar while *CCM3* familial form has several unique characteristics. *CCM1* and *CCM2* mutations result in spinal cavernous angiomas^[28]. Unlike *CCM1* and *CCM2* mutations which manifest later, *CCM3* mutations manifest as early as age 20^[31]. *CCM3* cases have the heaviest disease burden characterized by numerous CNS lesions, with an increased risk of bleeding^[32,33]. In addition to cutaneous manifestations with premature termination codon mutation in exon 1 of *CCM1*^[34], *CCM3* mutation cases also present scoliosis^[33], mental retardation^[35], and meningiomas^[31]. No other genetic locus for familial forms of CCM has been identified yet^[36]. However, there are still patients suffering from CCM with normal *CCM1*, *CCM2*, and *CCM3* genetic screen results. Even utilizing next-generation sequencing to screen the whole genome of CCM patients with known and unknown mutations, no causative mutation among all three *CCM* loci was detected for these CCM patients^[37], further suggesting the existence of a potential new *CCM* locus.

PROTEIN STRUCTURE

CCM1 is the largest of the three *CCM* proteins (736 amino acids) and the most common *CCM* gene mutated^[7,38]. 50% of all familial forms of CCM are due to mutations of *CCM1*^[36,39]. The penetrance of *CCM1* mutation

is around 88%^[40]. A recurrent *CCM1* missense mutation (Q455X) is prevalent in the Hispanic population, hence it is named common Hispanic mutation^[7]. It is present in high frequency in southwestern US^[41]. From N-terminal to C-terminal, *CCM1* protein contains a NUDIX domain, three NPXY/F motifs, Ankyrin repeat domain, a FERM domain, nuclear export signal (residues 551-562)^[18,25,42] and nuclear localization signals (residues 46-51, 569-572)^[23,42-44].

CCM1 C-terminal FERM domain

Located at the C-terminus (residues 420-736), the FERM domain contains three subdomains (F1-F3). The F1 subdomain of the FERM domain folds into an ubiquitin-like fold, while the F2 subdomain folds into an acyl-CoA binding fold. Several proteins are known binding partners to this region of *CCM1*^[45]. The F3 subdomain of FERM domain, a bona fide PH domain, binds to NPXY motifs. There have been several reports on this type of interaction in *CCM1*^[19,46-48]. Some reports indicate that the C-terminal FERM domain binds to its first NPXY motif intramolecularly, thereby allowing the *CCM1* protein to adopt a closed conformation. They further suggested that in the open conformation, the *CCM1* binds to ICAP1 α and localizes it to the cytoplasm and to the nucleus. In the closed conformation, the protein's binding site is inaccessible to ICAP1 α and results in increased *CCM1* binding to microtubules through the NUDIX domain, retaining ICAP1 α in the cell membrane. In support of this theory, it was further suggested that cellular binding partners of *CCM1* to the F1/F2 subdomain of the FERM domain or NUDIX domain may drive this conformational change in *CCM1*^[47,48]. However, new evidence showed that unlike most N-terminal FERM domain-containing proteins, *CCM1* (containing a C-terminal FERM domain) does not seem to follow the auto-inhibition mechanism to undergo an intramolecular folding. In fact, both dynamic light scattering (DLS) and native protein gel electrophoresis showed an increased tendency of *CCM1* to undergo CCM3 - like oligomerization through intermolecular binding between F3 subdomain of FERM domain and 3 NPXY motifs among partnering *CCM1* proteins over intramolecular binding of F3 subdomain of FERM domain - NPXY motifs within *CCM1*^[19].

CCM1 ankyrin repeat domain

The ankyrin repeat domain (ARD) domain (residues 288 and 419) in *CCM1* is composed of 4 ankyrin repeats. This ARD packs onto the N-terminal side of the F1 subdomain of the FERM domain. Each repeat is identical and composed of two α -helices joined by a β -hairpin. The binding site is between the α -helices. These four Ankyrin repeats stack vertically into an "L"-shaped fold. The fourth repeat contains a tripeptide insert (G401, N402, and N403) in the binding site stabilized by a conserved W404. This tryptophan residue sits in a highly conserved hydrophobic pocket. X-ray crystallography showed that the ARD domain is bound tightly to the FERM domain. This highly conserved interaction is particularly mediated by the convex surfaces of Ankyrin repeats 2 and 3 in ARD domain and the β 2 strand and α 2 helix in F1 subdomain in the FERM domain. This interaction is stabilized by 10-12 hydrogen bonds over 993 Å². The ARD domain was termed as the F0 subdomain in the FERM domain due to its proximity to the FERM domain. The presence of another domain in such as position has been found in other FERM domain - containing proteins, i.e., Talin and Kindlin^[49,50]. Many proteins such as RAP1 and HEG1 are able to bind to the FERM domain with little to no structural change of the ARD domain required. The ARD in *CCM1* is different from other ARD in proteins such as DARPin due to its inability to bind to β -tubulin. *CCM1* was suggested to be a tubulin binding protein, however both structural studies and binding assays show that the ARD domain is not utilized for this interaction^[51,52]. Till now, there are no known binding partners to the ARD domain in *CCM1*.

Multiple NPXY motifs in CCM1 protein

CCM1 also contains 3 NPXY motifs (residues 192-195, NPAY; 231-234, NPLF; 250-253, NPYF) in the central portion of the protein which provide important interactions with phosphotyrosine binding (PTB), PH, FERM domains, etc. The first motif, which is the only one that can be phosphorylated, has a remarkably strong binding affinity to ICAP1 α ^[20,25]. NPXY motifs 2 and 3 can only bind to DAB-like PTB domains, including *CCM2*^[18,19].

CCM1 NUDIX domain

The NUDIX domain is found in the N-terminus (residues 1-170) of CCM1 and it contains a stretch of basic residues that potentially interacts with microtubules. NUDIX domains are usually found in hydrolases that bind to a variety of substrates. The NUDIX fold in CCM1 is a centrally positioned β -sheet with flanking α -helices. Traditionally, the NUDIX domain contains a NUDIX box which contains the G_xE_xREU_xEE_xGU motif^[53]. However, CCM1 doesn't contain a traditional NUDIX motif, yet the tertiary structure of N-terminus of CCM1 still adopts a NUDIX fold. The catalytic residues found in other NUDIX domains are missing in the CCM1 NUDIX domain^[54]. Therefore, the function of the NUDIX domain in CCM1 may be different from other NUDIX domain-containing proteins. Despite the similarities, superimposition of the X-ray crystal crystallography of the NUDIX domain with known substrates (81 in total) revealed no potential binding partners. Therefore, the function of this domain is still uncertain^[54]. However, sequence analysis has elucidated the presence of several known sequences such as potential tubulin binding sequence^[48] and a nuclear localization sequence (NLS)^[42] within the NUDIX domain.

CCM2 is the second largest of the CCM proteins, 444 amino acids in length and contains a PTB domain at the N-terminus and a harmonin homology (HH) domain at the C-terminus^[3]. 20% of all familial forms of CCM are due to mutations of CCM2^[36,39], however, the penetrance of CCM2 mutation was reported to be 100%^[40]. CCM2 is found ubiquitously expressed in the endothelial cells (EC) from various organs^[55,56]. Despite the lack of a recognizable NLS and NES, CCM2 is found in both the nucleus and cytoplasm due to its interaction with CCM1^[18,23,57]. In the absence of functional CCM1, CCM2 is not localized to the cell junction, however, this function is recovered with the addition of wild-type CCM1, implying that binding to CCM1 is essential for localization of CCM2 to the cell junction^[58].

CCM2 HH domain

CCM2 functions as the scaffold in the CSC with binding sites for both CCM1 and CCM3^[16]. The HH domain at the C-terminus (residues 283-375) is comprised of 6 packed α -helices termed H1*, H1, H2, H3, H4, and H5 in that order from N-terminus to C-terminus. The H1* is a short α -helix with 3 amino acid residues and H4 is a 3_{10} helix that contains 13 residues. This domain is stabilized by several intramolecular interactions (i.e., R346 to E314, R354 to E366, and P355 to F356). This C-terminal domain bears structural similarity to harmonin protein and therefore termed HH domain. Although there is structural similarity, CCM2 HH domain is able to bind to neither Cadherin 23 (a validated binding partner of Harmonin) nor to CCM3. This HH domain exists in two conformations, monomeric and dimeric. The dimeric form has an increased affinity for dimerization; however, there are no sufficient data to affirm the occurrence of dimeric CCM2 *in vivo* yet^[45].

CCM2 PTB domain

The N-terminus of the CCM2 contains a DAB-like PTB domain^[59]. This domain contains 2 β -sheets composed of 7 β -strands, with α -helices capped at both ends. It was shown that this PTB domain binds to the NPXY motifs present in CCM1. Yeast two-hybrid assays showed that CCM2 PTB domain was able to bind to a CCM1 construct that contained the second and third NPXY motifs but not the first motif^[17,18].

CCM3 is the smallest of the 3 CCM proteins with 212 amino acids. CCM3 mutations tend to result in the most aggressive form of the disease^[8]. 10% of all familial forms of CCM are due to mutations in CCM3 gene^[36,39]. The penetrance of CCM3 mutation is approximately over 60%^[40]. In one study that sought for promoter variants for the CCM genes, two protective single nucleotide polymorphisms were identified in the promoter region of CCM3 (rs9853967 and rs11714980) to be associated with CCMs, while no causative variants were identified in the promoter regions of CCM1 or CCM2, among the selected CCM patient cohort. These variants could partially explain the range of disease burden seen in CCM^[60]. CCM3 is localized to the *cis*-face of the Golgi body^[61]. Its interactions with phospholipids, PtdIns^[3-5], were believed to facilitate the translocation of CCM3 to the plasma membrane^[62]. CCM3 is a 2-domain-containing protein where both domains are conjoined by a flexible hinge region^[63]. The tertiary structure of CCM3 is a V-shaped structure.

After some invertebrate analogs of CCM3 were identified and studied, it was found that CCM3 is the most evolutionarily conserved of the three CCM proteins^[64,65]. However, CCM1 and CCM2 are predominantly found in the vertebrates^[63,66].

CCM3 N-terminal dimerization domain

CCM3 exists as a homodimer in the cell due to presence of the dimerization domain. It is made up of four α -helices: α_1 , α_2 , α_3 , and small α_4 . These four α -helices interlock with another set of four α -helices from a partner CCM3 dimerization domain. This dimerization domain is also used to bind to GCKIII kinases forming a heterodimer. The interactions between GCKIII and flexible hinge regions of CCM3 control the equilibrium between CCM3 homodimer and CCM3-GCKIII heterodimer^[45].

CCM3 C-terminal focal adhesion targeting - homology domain

The C-terminus of the CCM3 protein contains a focal adhesion targeting-homology domain (FAT-H) domain. This domain is commonly found in some tyrosine kinases such as Pyk2 and FAK. It is also composed of four α -helices: α_5 , α_6 , α_7 , and α_8 . This domain is utilized to bind to large variety proteins including CCM2 and phosphatidylinositides^[62].

NEWLY IDENTIFIED CELLULAR COMPONENTS OF CSC COMPLEX

CCM1 is highly expressed in EC cells during embryogenesis^[67]. It is localized ubiquitously throughout the cell including the nucleus^[17,20,38,42,68].

CCM1 C-terminal FERM domain

CCM1 was shown to bind to a small GTPase Rap1 utilizing its FERM domain through X-ray crystallography^[43,44] and yeast two-hybrid^[69]. This interaction localizes CCM1 to the periphery of the cell to facilitate signaling at the cell-cell junctions^[48]. In contrary, the release of CCM1 from the plasma membrane is due to its interaction with ICAP1 α . This complex localizes to the nucleus^[17]. Rap1 is a GTPase that has many cellular functions such as maintaining cell-cell contacts and integrin-mediated cell adhesion^[70]. Rap1 is activated by GTPase-activating proteins and CCM1 has a large affinity towards this activated form^[71]. Disruption of RAP1 and CCM1 interaction results in the absence of CCM1 localization to the cell membrane particularly the adherens junction^[71]. Superimposition of Rap1 and CCM1 showed that no conformational change was required for bindings to occur^[52]. The FERM domain contains 3 subdomains F1, F2, and F3. RAP1 binding site in the FERM domain overlaps F1 and F2 subdomains. HRas is another GTPase that binds to the same binding pocket as RAP1. However, in competition assays, RAP1 binds more strongly to CCM1 over HRas due to specific residues in the F2 subdomain of CCM1. This stronger binding affinity to RAP1 is dependent on the interaction of K570 on CCM1 with E45 on RAP1, with respective mutations significantly decreasing binding. Further analysis of the X-ray crystallography of CCM1 and RAP1 showed that the switch II region in RAP1 doesn't contribute to any binding affinity. This is a result of the interaction between Y419 on CCM1 and F64 on RAP1 which destabilizes the switch II region^[43]. Rap1 acts as an inhibitor of CCM1 interaction with microtubules^[48]. The inhibition mechanism is the binding of Rap1, releasing CCM1 from the cell membrane and spatially blocking the interaction between CCM1 and microtubules leading to an overall increase in stability of the cell junction^[72]. Rap1 is found in mice in two forms: Rap1a and Rap1b. Individual deletions of either protein have minimal effect on embryogenesis. However, a combined deletion is embryo lethal. It results in the perineural vessel dilation and hemorrhage, demonstrating the importance of Rap1 in EC cells maturation and angiogenesis. *Rap1* deletion was shown to reduce VEGFR2 signaling^[73]. Unlike *CCM1* deletions which result in malformed branchial arches, the formation of patent branchial arches can still be seen in *Rap1* deletion, suggesting that CCM1 acts along alternate pathways for vessel formation^[74]. CCM1 FERM domain also binds to HEG1 protein, which further enables CCM1 localization at the cell junction. HEG1 is a transmembrane protein that contains an NPxY motif on its cytoplasmic tail. The binding pocket of HEG1 in CCM1 does not overlap with RAP1. The binding strength of HEG1 to

CCM1 is independent of binding to RAP1. HEG1 interacts with the hydrophobic pocket between F1 and F3 subdomain. The interaction between HEG1 and CCM1 is trifold: F1 subdomain through polar interactions, F3 subdomain through hydrophobic interaction, and α 2A helix between F1 and F3 subdomain. Analysis of electron density shows that the C-terminal dipeptide on HEG1 of tyrosine-phenylalanine is critical for their binding. This binding is not in the category of the classical PH (F3 subdomain) - NPxY motif interaction. In fact, the binding pocket is similar to the inositol phosphate binding site in the PH domain^[75]. Similar to the binding of RAP1, the binding of HEG1 doesn't illicit any conformational change in the FERM domain^[43]. The binding of CCM1 with HEG1 and RAP1 is essential for appropriate cardiovascular development, and disruption of these interactions was shown to cause the phenotype of CCM^[71]. *Heg1*-null zebrafishes manifest with the same cardiovascular phenotype as *Ccm1* null mutants^[76]. Infusion of CCM1 mutants lacking the binding ability to RAP1 and HEG1 into *Ccm1* null zebrafish could not reverse the cardiovascular phenotype^[71,76], indicating the essential role of the interaction.

Multiple NPXY motifs in CCM1 protein

CCM1 binds to ICAP1 α and acts a competitive inhibitor of ICAP1 α and β 1-integrin interactions^[20,68]. ICAP1 α contains a PTB domain that binds to β 1-integrin and modulate β 1-integrin mediated cellular function^[15,23-25,54]. Mutagenesis of the T778 and V790 or N792 and Y795 of the cytoplasmic tails of β 1-integrin prevents binding to ICAP1 α . However, the exact consequence of this interaction is not fully agreed upon. The overwhelming theory is that CCM1 acts to sequester ICAP1 α resulting in increased levels of β 1-integrin activation because ICAP1 α is a potent repressor of β 1-integrins^[15,23-25,54]. However, one study suggests that CCM1 interaction with ICAP1 α stabilizes ICAP1 α , therefore increasing β 1-integrin activation. This effect is profound when low levels of CCM1 results in paradoxically increased β 1-integrin activation. However, appropriate amount of β 1-integrin is necessary for development of vascular sinusoids^[77], cell cycle^[78,79], and bone development^[80,81]. ICAP1 α PTB domain is a DAB-like PTB domain where the interaction between ICAP1 α and CCM1 is independent of the phosphorylation status of the NPXY motif. Binding of CCM1 or β 1-integrin to ICAP1 α doesn't result in any structural changes^[82]. CCM1 binding to ICAP1 α through a bidentate interaction with the ICAP1 α PTB domain. This interaction is identical to ICAP1 α and β 1-integrin interaction thereby resulting in competitive inhibition^[15,23-25]. CCM1 utilizes the first NPXY motif and a RR region to bind to ICAP1 α PTB domain^[83]. The RR region is a novel site that binds to the N-terminus of NPXY motif. This N- terminal region adopts an α -helix confirmation that binds to the loop between β 1 and β 2 and well as β 5 and β 6 and α 1 helix of the PTB domain^[82]. The interaction between the PTB domain and the RR site is mediated by the conserved arginine residues on the CCM1 (R179 and R185), binding to polar side chains such as the carbonyl groups of D146, D93, and Q96 on ICAP1 α . The interaction of the PTB domain and the NPxY motif is mediated by the interaction of N192 and Y195 on CCM1 to the ICAP1 α binding site of PTB domain, L135, I138, I139, and C184. Mutation in the NPXY binding sites on ICAP1 α inhibits binding to cytoplasmic tail of β 1 integrin. However, mutation in the RR binding sites (D146, D93, and Q96) did not affect binding with cytoplasmic tail of β 1 integrin. Therefore, unlike CCM1-ICAP1 α interaction, the binding between ICAP1 α and β 1-integrin is not a bidentate interaction^[54].

CCM1 binds to CCM2 and SNX17 through the utilization of the second and third NPXY motifs^[18,19,59,84]. Biochemical studies demonstrate that CCM2 utilizes its PTB domain to bind to the third NPXY motif of CCM1. Several leucine residues in CCM2 (L113, L115, L155, L198, and L213) are paramount for adequate binding to CCM1. Several residues downstream to the NPXY motif such as V244 and V248 are also important. Even a conservative mutation like V244L was found to significantly decrease binding. An X-ray crystallography of CCM2 with the third NPxY motif of CCM1 was determined. However, Co-IP shows that both the second and third NPxY motifs are required to bind to CCM1^[18]. Additionally, biochemical studies do not show an increased affinity with a construct containing both the second and third NPxY motifs over just the third NPxY motif^[59].

CCM2 PTB domain

Some PTB domains can bind to phospholipids and it was expected that such an affinity in CCM2 PTB domain will transport CCM2 complex to the plasma membrane, however no consensus has been reached in that regard. One study stated that CCM2 PTB domain is able to bind to phospholipids^[85], while other studies suggested the opposite^[3,19]. CCM2 PTB domain has been shown to interact with two proteins, TrkA and Smurf1. TrkA is present in neurons. The PTB domain of CCM2 binds to the cytoplasmic tail of TrkA^[86]. This complex binds with CCM3 and STK25 to form a large complex. This may happen through a direct mechanism where CCM2 brings CCM3 or an indirect mechanism. However, the formation of this complex leads to apoptosis. This pathway is found to be especially important in the field of prognostic and therapeutic aspects of neuroblastoma and medulloblastoma^[87]. CCM2 PTB domain was also reported to bind to Smurf1 HECT domain. This interaction brings Smurf1 to the plasma membrane^[88]. This interaction has been related to RhoA degradation. The lack of RhoA degradation results in uncurbed activation of Rho-associated coiled coil-forming kinase (ROCK) which leads to stress fiber formation^[89]. One report stated that CCM2 protein in macrophages is found adjacent to newly synthesized actin polymers. This localization of CCM2 is compatible with the predicted function of regulating RhoA because RhoA is active in actively synthesizing actin polymers^[22].

CCM2 C-terminal HH domain

A four nucleotide duplication of the last exon of CCM2 was introduced to create a mutation disrupting the structure of the HH domain at the C-terminus. This mutated form of CCM2 protein was able to bind to CCM1 and CCM3, however unable to bind to MEKK3, a Map3 kinase, suggesting that the HH domain mediates the interaction between CCM2 and MEKK3^[90].

CCM2 KARET domain and LD motif

Some reports showed that CCM2 interacted with CCM3 through a KARET domain at the C-terminus of CCM2 (residues 228-444), which overlaps with HH domain^[16,17,63,86,87]. However, it was recently shown through pull-down assays that the binding pocket was located in the middle of CCM2 (residues 223-238), between the N-terminal PTB domain and C-terminal HH domain. This region was determined to contain a LD motif, which binds to FAT-H domain on CCM3. The FAT-H domain of CCM3 contains a hydrophobic pocket termed HP1 which is the binding site for CCM2. The LD motif adopts a 3.5 turn α -helix parallel to α 7-helix in CCM3. The interaction is largely made up of hydrophobic residues from CCM2 (T225, I226, F228, L229, A232, I233, G236, and A237) binding to hydrophobic residues from CCM3 (I131, I134, A135, I138, L142, V168, F174, L178, S171, K132, and K139). These hydrophobic residues are also extremely conserved through evolution, indicating their significance^[63]. CCM2-CCM3 complex formation reciprocally protects the proteins from degradation. Either CCM2 or CCM3 depletions result in concurrent decreases in the reciprocal protein. Furthermore, CCM2 and CCM3 mutated cells grew slower than wild-type ones. This mutant phenotype can be rescued with the addition of CCM2 or CCM3. Overexpression of CCM2 in CCM3-depleted cells did not restore cellular proliferation, however overexpression of CCM3 in CCM2-depleted cells did, indicating that the CCM3 may have a greater contribution to cell proliferation^[91].

CCM2-Like protein

Recently CCM2-Like (Ccm2l) protein was identified in zebrafish. This protein bears large sequence similarity to CCM2. It contains a PH domain as opposed to a PTB domain, found in CCM2. Our lab showed that PTB domains and PH domains can have overlapping abilities^[19]. Despite the similarities, the function of Ccm2l and CCM2 are not identical. The N-terminus of CCM1, which contains the NPxY motifs, can bind to Ccm2l, potentially through the PH domain. Although, it is still unknown which of the three NPxY motifs are used by Ccm2l for binding. Ccm2l, which is selectively expressed in EC^[92], sequesters CCM1 by acting as a competitive inhibitor, however, it is unable to bind to CCM3. The ability to bind to CCM1 results in the inhibition of CCM2 mediated junctional stability. During embryogenesis, Ccm1 is expressed in the notochord while Ccm2 is expressed heavily in tissue anterior to the notochord. Interestingly, Ccm2l is found in both regions. Despite these differences, Ccm2l knockout zebrafish exhibits cardiac phenotype, i.e.,

cardiomegaly. This phenotype is seen to a milder extent in *Ccm1*, *Ccm2*, and *Heg1* null mutants. The effect of *Ccm2l* is more pronounced in the heart than large vessels. In fact, there is partial phenotypic rescue with *Ccm2* over-induction in *Ccm2l*-null zebrafish. Therefore, it is concluded that *Ccm2l* acts in the *Heg*-*Ccm* pathway^[93]. It is still unclear whether CCM2L is involved in the pathogenesis of CCM^[26].

CCM3 dimerization

The N-terminus of one CCM3 can bind to another CCM3 in the native state, driven by identical hydrophobic residues, L44, A47, I66, and L67^[94]. An important set of binding partners of CCM3 are GCKIII kinases such as Mst4 and Stk25. These kinases contain an N-terminal catalytic domain and C-terminal regulatory domain. The C-terminal regulatory domain of GCKIII can bind to dimerization domain in CCM3. The tertiary structure of the C-terminus of GCKIII is similar to the N-terminus of CCM3 and can compete with and replace a CCM3 in the CCM3 homodimer. These kinases adopt an independent V-shaped domain as well. Each side of the V in the GCKIII protein is made up of several α -helices. Mutations in the hydrophobic residues that removed CCM3 dimerization effectively also inhibit GCKIII and CCM3 binding^[95]. However, CCM3 has higher binding affinity to GCKIII kinases over CCM3 homodimer. There exists a long linker peptide in CCM3 between $\alpha 3$ and $\alpha 4$ helix along the CCM3 protein. In the homodimer state, this region folds into α -helix and merges with $\alpha 4$ forming an extended helix. This extended helix is stabilized by hydrophobic residues found in the antiparallel $\alpha 1$ helix in the partner CCM3 within the homodimer. Hydrophobic residues (V72, F76, L80, M83) in the extended $\alpha 4$ helix interact with hydrophobic residues (V25, A24, P21, M20, V18, and M17) in $\alpha 1$ helix of partner CCM3. This stabilizing interaction doesn't occur in the GCKIII-CCM3 complex, because the corresponding $\alpha 1$ helix in GCKIII doesn't contain any hydrophobic residues. In the interaction between CCM3-GCKIII, the linker region between $\alpha 3$ and $\alpha 4$ helices in CCM3 is less structured and able to adopt a flexible conformation. This allows hydrophobic residues (F76, L80, and M83) in $\alpha 4$ helix in CCM3 to fall into a hydrophobic pocket formed by $\alpha 1$ and $\alpha 3$ helix in CCM3 through intra-molecular binding. This twisting of the CCM3 causes in the full N-terminal face of CCM3 to interact with the full C-terminal face of GCKIII, which doesn't occur in the homodimer of CCM3 due to the inherent bend in the CCM3 tertiary structure^[63,96]. Therefore, there is a larger binding area between CCM3 and GCKIII. This larger-interface interaction present results in a much higher binding affinity^[95].

Knockout models of Stk24 and Stk25 caused cardiovascular disease similar to that observed in CCM3 knockout models^[97]. STK25, a serine/threonine kinase, controls RhoA activation, which is a GTPase. Loss of RhoA results in stress fiber formation^[98]. It has been well documented that CCM phenotype causes increased stress fiber formation; however several reports contradicted this finding^[99,100]. Increase RhoA activation leads to increased ROCK. ROCK is another serine/threonine kinase that phosphorylates several proteins: myosin light chain, MLC phosphatase, and LIM kinase. MLC phosphatase decreases the cross linking of myosin and actin, the source of fiber contractility. Phosphorylation by ROCK inhibits MLC phosphatase. In contrast, ROCK dependent phosphorylation of LIM kinase results in activation. Active LIM kinase catalyzes phosphorylation of cofilin, which inhibits cofilin activity that regulates actin depolymerization. Both pathways, LIM kinase and MLC phosphatase, lead to stress fiber formation. ROCK inhibition results in regression of stress fiber^[26]. Increases in ROCK activity have been recorded in CCM lesions. Interestingly, increases in ROCK activity have been seen in histologically normal blood vessels in CCM1 deficient mice, suggesting possible involvement of ROCK signaling in the pathogenesis of CCM lesions^[101].

CCM3 C-terminal FAT-H domain

CCM3 contains a FAT-H domain at the C-terminus that is used to bind to CCM2. The surface of the domain contains a hydrophobic patch termed hydrophobic patch 1 (HP1), which is found between $\alpha 7$ and $\alpha 8$ helix. This is the site for binding various proteins such as CCM2^[63], striatins^[61], and paxillin^[102]. For interaction of both striatins and paxillin, CCM3 recognizes LD motifs that adopt helical structures. Compared to CCM2-CCM3 complex, CCM3-paxillin complex has a smaller surface area, the LD motif is smaller (~ 2 turns), and the LD motif helix is less parallel to $\alpha 7$ helix in CCM3^[91]. Paxillin is known to bind to FAT-H domains

in FAK and Pyk2^[63]. Through fluorescence images, it was determined that paxillin and CCM3 were co-localized to the plasma membrane at the leading edge^[102]. The functionality of this binding is still unknown, one hypothesis is that the formation of some CCM3-GCKIII complexes is under the control of paxillin phosphorylation. Therefore, paxillin may be sequestering CCM3 from activity^[103].

CELLULAR SIGNAL TRANSDUCTION

CCM1 plays a role in Notch signaling

Cells with increased CCM1 activity show overexpression of HEY1 and DLL4, two major players in notch signaling. Notch signaling increases PI3K/AKT signal pathway and activated AKT leads to suppression of ERK1/2 by dephosphorylation. CCM1 deficient cells and CCM lesions show increased phosphorylation of ERK1/2^[104]. AKT phosphorylation is also important in regulating the expression of SOD2, which is an important free radical scavenger in the cell. SOD2 is upregulated with increases in reactive oxygen species through AKT phosphorylation. In the absence of CCM1, AKT phosphorylation is decreased^[72,105], leading to decreased expression of SOD2, and therefore increasing oxidative damage in the cell^[106]. CCM1 is also an inducer of SOD2 through interaction of ND1. ND1 is an important actin stabilization protein that binds to CCM1. This interaction increases the expression of SOD2. Therefore, CCM1 can prevent oxidative damage and cell death through complex induction of SOD2^[107].

CCM1 involved in KLF4/KLF2 signaling pathways

Both *in vivo* studies with *Ccm1* knockout mice and *in vitro* studies with CCM1 silencing in human brain EC (hCMEC) showed elevation of KLF4 nuclear signal. KLF4 has been reported to play an important role in EC in biogenesis of veins and angiogenesis in general. Combined silencing of both KLF4 and CCM1 significantly decreases the disease mortality (75% reduction of mouse mortality) and modest improvement of vascular lesions (reduced vascular density in retina). The prototypical lesions in CCM lack mesenchymal intervening tissue, which is due to increased proliferation and dysfunctional migration, both of which are mediated with KLF4 inhibition. In this signaling pathway, CCM1 binds and sequesters MEKK3, which in turn activates MEK5, which subsequently activates ERK5^[108,109]. ERK5 is a known inducer of KLF4 in EC cells^[110-112]. KLF4 is a transcription factor which activates BMP6 and decreases SMAD1 phosphorylation. It has been shown that SMAD is activated in CCM deficient condition and leads to active BMP6 and TGF- β . These two downstream proteins mitigate the histological manifestation of CCM, i.e., lack of intervening parenchyma^[113]. KLF2 is another transcription factor that is induced by a similar signaling cascade^[114]. KLF2 is responsible for the cardiac manifestations and increased angiogenesis seen in CCM^[115,116]. KLF4/KLF2 are transcription factors that suppress expression of thrombospondin1 (TSP1) which functions as an angiogenic inhibitor^[117]. Loss of TSP1 was found to exacerbate CCM phenotype in *Ccm1* deficient mice. Abnormalities of cell-cell junction are found to be the initial manifestation of CCM1. It was found that decreases in Claudin-5 and ZO-1 levels were the first to be observed before changes in VE-cadherin levels. The perturbed expression of these cell junction proteins can be rescued in *Ccm1* knockout mice with exogenous TSP1 (3TSR). 3TSR was found to decrease both VEGR2 phosphorylation leading to decreased angiogenesis and increased TGF- β activation. Furthermore, this treatment decreases the lesion burden in mice. Therefore, there is a possibility to utilize KLF4 inhibitors, ERK5 inhibitors, or exogenous TSP1 for potential therapeutic applications in CCM in the near future^[118].

CCM1 is a key player in integrin signaling

β 1-integrin signaling is an important regulator in many cellular functions such as cellular migration and adhesion^[15,24,25,57]. These functions are especially important for EC cells. ICAP1 α is a repressor of β 1-integrin signaling^[46]. Our previous results indicate that CCM1 binds to ICAP1 α and modulates ICAP1 α and β 1-integrin interaction. Depletion of CCM1 and ICAP1 α synergistically inhibits extracellular signal-regulated kinase/mitogen-activated protein (ERK/MAP) kinase pathway activation on cell survival^[15,23-25,57,72]. We hypothesized that CCM1 regulated recruitment of ICAP1 α to the cell membrane in proximity to focal adhesions, which

may be critical for the maintenance of cellular architecture as well as regulation of β 1-integrin-mediated signaling^[25]. The follow-up studies found that ICAP1 α deficiencies result in many osteoblastic defects which are the direct results of β 1-integrin activation. CCM1 acts as a competitive inhibitor for the interaction of ICAP1 α and β 1 integrin. The lack of inhibition of ICAP1 α leads to excessive inhibition of β 1-integrin which is thought to cause the leaky vasculature found in CCM^[54]. CCM1 has been reported to be responsible for localization of ICAP1 α to the nucleus, through the use of the N-terminus NLS^[42]. It was shown that only in the presence of intact CCM1- ICAP1 α interaction and functional NLS in CCM1 does ICAP1 α localize to the nucleus^[47]. However, ICAP1 α also contains a NLS and drives CCM1 localization to the nucleus. In the absence of ICAP1 α , CCM1 is evenly spread throughout the cell, but in the presence of ICAP1 α , the CCM1 localizes to the nucleus. Alanine walking in NLS1, NLS2, and NES in CCM1 showed that only NLS1 affected CCM1 localization. NLS2 and NES mutation showed identical CCM1 localization to wild-type CCM1. However, functional ICAP1 α is able to translocate NLS1 mutated CCM1 into the nucleus. However, the N-terminus of CCM1 is unable to translocate ICAP1 α with a deficient NLS. The addition of CCM1 to ICAP1 α -silenced cells results in CCM1 accumulation only in the cytoplasm, suggesting that ICAP1 α drives localization of CCM1, not the other way around^[119].

CCM proteins modulate VEGF signaling

Mutations in CCM1 and CCM3 were found to increase translocation of β -catenin from the cytosol to the nucleus. This leads to increased expression of various proteins such as VEGF-A, which can be further reversed by the addition of a β catenin transcription inhibitor. The increased level of VEGF-A activates VEGFR2, which is shown by increased VEGFR2 phosphorylation. This increases the endothelial cell (EC) permeability, leading to vascular leakage. This phenotype can be rescued with the addition of VEGFR2 inhibitors in both *in vitro* and *in vivo* conditions. Interestingly, VEGF inhibition blocked the formation of stress fiber formation. Therefore, the stress fiber formation is caused to some extent by VEGF signaling. Furthermore, enhanced VEGF signaling results in increased cellular migration. A wound-healing assay showed that CCM1 deficient cells had a 25% increase in migration compared to the cultured cells treated with VEGF. However, this phenotype can be reversed in the CCM1-null cells with the treatment of VEGF inhibitors. VEGFR2 phosphorylation results in downstream phosphorylation of β -catenin and VE-cadherin, which results in disruption of interaction with α -catenin^[120] and p120 catenin^[121] respectively. This results in translocation of β -catenin into the nucleus for further downstream effects. However, VEGF inhibitors were not sufficient to inhibit the β -catenin and VE-cadherin disassociation seen in CCM1 deficiency, suggesting that other mechanisms are involved for the disassociation^[122].

CCM2 modulates MAPK signaling

CCM2 leads to downstream activation of p38 MAPK, which is upregulated in osmotic shock. It is still unclear for the role of the CCM2 in the p38 MAPK activation pathway. One report stated that CCM2 localizes to the cell membrane where it facilitates binding MEKK3 and RAC1 leading to activation of MAPK^[17,22]. Another report showed that CCM2 is able to bind to F-actin, suggesting that CCM2 forms a complex that links RAC1-dependent actin reorganization to p38 MAPK signal pathway^[123]. Another report showed that the signaling pathway is through phospholipase C (PLC). The complex of CCM2-RAC1 causes a change in PLC cascade, leading to MAPK activation^[124]. While another report showed that CCM2 affects the JNK and MKK signaling leading to an alternative pathway to promote MAPK activation^[89].

CCM3 plays a role in Notch signaling

In recent years, several mechanisms for the pathogenesis of CCMs have been proposed such as decreased Notch signaling^[125], increased VEGF signaling^[126], or increased ERK activity in the deficiency of CCM3^[127]. It has been recently reported that CCM3 affects EC function by regulation of DLL4^[128]. Down-regulation of CCM3 resulted in decreased expression of DLL4 and Notch4, but no change was observed in Notch1. This was shown in both cell lines and brain tissue in CCM patients. In fact, the vascular phenotype found in CCM3 mutants can be replicated through mutations in DLL4. Aberrant DLL4/notch signaling results in

overexpression of vasodilators during angiogenesis. The phenotype of hyperangiogenesis in CCM3 deficient condition can be rescued by overexpression of DLL4. Abnormal Notch signaling leads to many downstream effects. Notch signaling is also reported to be involved in the regulation of expression of VEGF receptors, to modulate vascular bed architecture^[129] and angiogenesis^[39,130]. In fact, CCM3 deficient cells increase the expression levels of VEGF which affects cell survival through ERK1/2 activity^[131]. ERK1/2 kinase was reported to be upregulated in CCM3 deficient lesions, which can be reversed when DLL4 function is rescued through induction of recombinant DLL4. Therefore, CCM3-mediated notch signaling also affects ERK1/2 and VEGF functions leading to abnormalities in EC cells. The finding that CCM3 deficient phenotype can be rescued through DLL4 overexpression creates a promising venue for future pharmacotherapy^[126].

TLR4 signaling in CCM pathogenesis

It was found that induction of gram negative bacterial abscesses in Ccm1 and Ccm2 deficient mice significantly increased the phenotypic severity of CCM lesions. The effect was increased in mice with hematogenous infections of gram-negative bacteria. In fact, exposure to just lipopolysaccharide (LPS) was sufficient for significant lesion formation in the mice. It is well known that LPS response is mediated by TLR4 pathway^[132] and that MEKK3 deficiency terminates the signal^[133]. MEKK3-KLF2/4 pathway has already been implicated in CCM lesion formation^[90], which is further validated by decreased expression of KLF2/KLF4 with an LPS injection, suggesting the existence of TLR4-MEKK3-KLF2/4 pathway. Furthermore, heterozygous TLR4 mutants had a significantly decreased CCM lesion formation and homozygous mutants had a complete resolution of CCM lesions. This suggests that TLR4 signaling drives CCM lesions development. In fact, genetic polymorphisms in TLR4 (rs10759930) and CD14 (rs778587) (a TLR4 co-receptor) that result in increased expression of respective proteins do result in increased CCM lesions. These findings were further supported by lack of CCM lesion formation in mice that were surgically removed as fetuses and grown in sterile conditions. Therefore, exogenous stimulation of TLR4 may be involved in CCM lesion formation. Two additional experiments were also performed to prove post-natal suppression of lesion can be achieved: (1) TLR4 antagonists can decrease lesion severity in mice through decreased TLR4 signaling; (2) the course of antibiotics also decreases lesion severity. These antibiotics altered the nature of the microbiome in the mice for decreased TLR4 stimulation. This report provides a new avenue for potential CCM pharmacotherapy^[134].

CELLULAR FUNCTIONS AND BIOGENESIS

Angiogenesis

CCM lesions are hallmarked by abnormally increased EC proliferation. There are many proposed mechanisms for angiogenesis implicated in CCM. ICAP1 α is involved in NOTCH signaling. Therefore, loss of CCM1 or ICAP1 α results in increased angiogenesis^[104]. CCM3 is also reported to be involved in NOTCH signaling resulting in increased angiogenesis^[126]. CCM proteins also modulate MAP kinase activity which in turn modulates angiogenesis^[89]. It is also reported that CCM3 increases VEGF receptor concentrations, thereby resulting in increased EC proliferation^[135]. However, there is still controversy of the mechanistic pathways for control of angiogenesis by the CCM proteins^[23,136].

Microvascular integrity

CCM lesions are not only hallmarked by the presence of abnormal EC proliferation and migration but also increase the leakage predisposing the lesions to hemorrhage. There are several explanations for this phenomenon. ANKS1B is a novel PTB domain containing protein that binds to the third NPXY motif in CCM1. No change of EC cell proliferation, migration or sprouting was observed in ANKS1B-deficient EC, suggesting that ANKS1B-deficiency did not affect the CCM1 activity or Notch signaling. However, these EC cells had decreased transendothelial resistance (TER), which cannot be rescued by ROCK inhibitors. This observation indicates that ANKS1B regulated EC cell adhesion without involving RhoA signaling. Further, increased TER was achieved by overexpression of ANKS1B in CCM1 depletion, indicating that CCM1 is not involved in ANKS1B signaling either^[137]. Another explanation is that the increased vascular permeability

is due to stress fiber formation in CCM lesions. CCM proteins are responsible for inhibiting Rho kinase. However, unopposed activation of Rho kinase results in increased phosphorylation of myosin light chain, which was also seen in CCM lesions^[58]. Another theory is the dysfunction of RAP1 and CCM1. CCM1 acts as a scaffold for RAP1 and is important for cell junction protein β -catenin and VE-Cadherin, thereby stabilizing the cell membrane. VE-Cadherin is a part of the adherens junction^[138]. Therefore, dysfunction of VE-Cadherin affects cell contacts. β -catenin is a nuclear factor. Dysfunction of CCM proteins results in increased translocation of β -catenin in the nucleus that lead to EC proliferation^[139].

The vascular permeability seen in *Ccm1* knockout mice can be rescued by SOD2 and catalase infusions with antibody-mediated targeting to the endothelium. TNF- α was able to induce vascular permeability in the arterioles in these rescued mice. However, TNF- α is unable to induce vascular permeability in *Ccm1* null mice. It was also shown that in the absence of CCM1, TNF- α was unable to generate ROS. This suggests that TNF- α function involves CCM1. Yet, CCM lesions have shown to have elevated ROS. This is a result of increased activity of NADPH oxidases (NOX). Both *in vitro* and *in vivo* CCM lesions showed significant up-regulation of NOX4. NOX4 is not only a source of ROS, but also an enhancer for downstream activation of NF- κ B. This can be rescued by treatment of NOX4 inhibitors. Furthermore, broad spectrum NF- κ B inhibitor (i.e., N-(E)-p-coumaroyl-3-hydroxyanthranilic acid, YAv1) inhibits NF- κ B activation due to NOX4 or TNF- α , which can rescue the defective endothelial barrier seen in CCM^[140]. These inhibitors could potentially be utilized for CCM pharmacotherapy in the future.

CCM1 protects cells from oxidative damage

All three CCM proteins follow a knudsonian pattern of inheritance. Therefore, a second mutation is necessary for development of lesions and reactive oxidative species are a source of DNA mutations. Initially, *Ccm* mouse models were made with *Ccm1* and *Ccm2* heterozygous mutant mice. However, these mice never showed any CCM lesion phenotype, so another mutation, *Msh2*, was developed into these mice. *Msh2* is a DNA damage-repair protein that decreases DNA mutations. Only mice with heterozygous *Ccm1* and homozygous *Msh2* deletion showed considerable vascular CCM lesions^[101]. *Ccm1* is a regulator of FoxO1, through an unknown mechanism. This transcription factor induces the transcription of *Sod2* and *Sirt1*, two important anti-oxidants in the cell^[106]. It was also shown that CCM1 can regulate Rho GTPase, by interacting with ND1-L in the presence of oxidative stress^[107]. Another potential pathway modulated by CCM1 to limit oxidative stress is JNK/c-Jun signaling. In *CCM1*-null cells, there is an overexpression of C-Jun which can be reversed by reintroduction of CCM1. Therefore, CCM1 protects the cells from downstream oxidative stress of C-Jun redox pathways^[141]. The cells lacking CCM1 also have increased activity of COX-2, a mediator of inflammatory pathways in the cells^[142]. This is consistent with the *in vivo* data that *Ccm1* knockout mice had hyper-exaggerated response to inflammatory agents^[143]. Therefore, it can be concluded that inflammation and oxidative stress are involved in CCM lesion formation^[144]. CCM1 provides protection against oxidative stress in the cell by utilizing anti-inflammatory and anti-oxidant pathways^[106].

CCM is associated with increased ROS that mediate cellular damage. The cell usually responds through up-regulation of anti-oxidant enzymes. *CCM1* depletion induced lesions show increased levels of Nrf2 transcription factor and GLO1 enzyme, both having important anti-oxidant functions in the cell. This results in a paradoxical increase in cell death due to protective mechanisms. However, chronically activated anti-oxidant mechanisms result in impairment of regular redox reactions in the cell. Many other vascular diseases have overactivation of Nrf2^[145,146]. It was shown that chronic Nrf2 activation as seen in CCM is paradoxically associated with increased ROS production. This, in accordance with other studies, shows that the anti-oxidative effects of Nrf2 are only seen at certain concentration levels^[147]. Loss of function (LOF) of CCM1 results in increased JNK signaling, which has been shown to lead to increased Nrf2 activity^[148,149]. JNK inhibitors resulted in decreased Nrf2 activation and restored ability of autophagy. Impaired autophagy is seen in CCM lesions and rescue of this phenotype suggest that it is mediated by JNK signal pathway. Similar

to Nrf2, supraphysiological concentrations of Glo1 result in increased sensitivity to oxidative stressors^[150,151]. These cells also have decreased levels of heat shock protein (HSP), Hsp70 and Hsp27, which have a protective role in the cell by increasing cellular capacity to handle stressors. The sequential effects by Glo1 compound to increase sensitivity to stressors and decrease tolerance, result in greater propensity towards intrinsic cell death in CCM1 deficient condition^[152].

Appropriate valvulogenesis is predicated on CCM1-CCM2 complex

The fluid stress on the EC cells is paramount for appropriate differentiation of the heart. It is shown that abnormalities in blood flow through alteration of KLF2a/b activity affect *heg1*. *Heg1* is localized to areas of myocardium with increase fluid forces in zebrafish^[153]. It was found the increased *Heg1* expression stabilized *Ccm1* in these cells. Therefore, overexpression of *Heg1* resulted in increased *Ccm1* leading to decreased *Klf2a* activity. This decreased *Klf2a* activity desensitizes the cells to the fluid forces resulting in appropriate valvulogenesis. In the absence of *Ccm1*, the endocardial cushions do not develop into functional valves. Induced expression of *Ccm1* to endocardial cushions in *Ccm1* deficient zebrafish resulted in development of valves. These cells were shown to increase Notch signaling and decrease *Klf2a* activity with the targeted expression of *Ccm1*. Similar valvular defects were also seen with the inhibition of Notch activity^[154]. Therefore, CCM proteins are involved in appropriate valve development^[155].

CCM2 and CCM3 function coordinately in gonadogenesis

Ccm2 and *Ccm3* gene expressions are upregulated in adult mouse testis and ovaries, correlating with CCM2 and CCM3 protein expression, suggesting the involvement of *Ccm2* and *Ccm3* in the regulation of gonadogenesis. The expression pattern of CCM2 changes through embryogenesis. In the prenatal testis stage, CCM2 is mainly expressed in the interstitial cells of Leydig with little expression in gonocytes. Throughout gonad maturation, CCM2 begins to be expressed in spermatocytes, followed by the expression of CCM3. In ovaries, CCM2 is found in the oocyte nucleus at birth. Overtime this expression is decreased while the expression of CCM2 is increased in adult granulosa cells. The CCM2 in granulosa cells is expressed solely in the cytoplasm based on the spatiotemporally differential expression patterns of CCM2 and CCM3 in the testis and ovaries; it is plausible that CCM2 and CCM3 proteins may have different physiological roles during testicular and ovarian development^[156]. Homozygous *Ccm3* mutants in a *C. elegans* model rendered them sterile. These *Ccm3* mutant worms had multinucleated germ cells that showed hypoproliferation, which may be caused by altered expression of Rab-11. *Ccm3* promotes endocytic recycling by interacting with Rab-11. Defective endocytic recycling could result in decreased expression of Glp-1, a mediator of Notch signaling, and Rme-2, a mediator of protein endocytosis. *Ccm3* deficient germ cells have defective late-stages of cytokinesis leading to multinucleate giant cells. Polarity of *C. elegans* is modulated by non-muscle myosin^[157], while non-muscle myosin distribution is regulated by *Ccm3*. *Ccm3*-null embryos have aberrant expression of Par-6 and Par-2, both of which are polarity proteins. Therefore, it can be concluded that embryonic polarity is mediated by *Ccm3*^[158].

CCM2 plays a role in the cardiac phenotype seen in CCM

As we described before, CCM2 binds to MEKK3 through the HH domain, leading to increased expression in KLF2, KLF4, ADAMTS4, and ADAMTS5^[115]. These expression changes can be detected in the earliest stage of CCM lesions. The increased KLF4 and KLF2 were not only found in CCM lesions but also blood vessels in the cerebellum in *Ccm1* knockout mice. These increased expressions were also reported in both sporadic and familial forms of human CCMs. Also, their early presence suggests that KLF4/KLF2 may be involved in the formation of lesions. MEKK3 is a MAP3 kinase that controls KLF2/KLF4 activity which is especially critical in cardiogenesis. *Ccm1* null mutant mouse model is embryonic lethal, but *Map3k3* haploinsufficiency is able to rescue this lethality. To determine the temporality of the Rho activation vs. MEKK3 activation, CCM1 depleted EC cells treated with hydroxyfasudil (a Rho inhibitor) was unable to normalize the levels of KLF4/KLF2 while normalized KLF4/KLF2 levels in CCM1 null cells resulted in normal level of Rho activation,

suggesting that the KLF4/KLF2 signaling is upstream of the Rho activation in CCM lesions^[90]. Increased KLF4/KLF2 expression can lead to increased ADAMTS that functions to cleave proteoglycan matrix such as versican. Therefore, the up-regulation of ADAMTS can lead to disruption of intervening parenchyma around the blood vessel resulting in the formation of a cavernoma^[12,13].

CCM3 is a regulator of cell apoptosis

CCM3 has been linked to both apoptosis and cell survival pathways. Initially, CCM3 was discovered as a protein for granulocyte apoptosis. One proposed mechanism is that CCM3 binds with VEGFR2 resulting in increased stabilization of the receptor. Decreased CCM3 results in increased degradation of VEGFR2 leading to decreased VEGF stimulation^[135]. Furthermore, CCM3 is implicated in translocation of MST4 to the periphery of the cell where it activates ERM proteins. These ERM proteins are anti-oxidative and thereby prolong cell survival^[159].

CCM3 plays a role in exocytosis

CCM3 is known to interact with STK24 and UNC13D, a known vesicle fusion regulator, in neutrophils. STK24 is an inhibitor of neutrophil vesicle exocytosis. STK24 deficient neutrophils release larger amounts of enzymes through exocytosis. STK24 localizes to neutrophil granules. There are two pools of granules in neutrophils: readily available and reserved. STK24 is associated with increased release of the reserved pool. UNC13D is a protein that binds to vesicles to promote their exocytosis. STK24 inhibits UNC13D^[160,161]. CCM3 has a dual effect on neutrophil exocytosis. CCM3 binds to STK24 and stabilizes it to increase neutrophil exocytosis. However, CCM3 also increases the binding of UNC13D to liposomes through a calcium mediated mechanism, which is only seen in high intracellular concentrations. This inactivates excess UNC13D, resulting in decreased exocytosis. Simply stated, CCM3 is important for maintaining equilibrium of neutrophil exocytosis. Loss of CCM3 increases exocytosis of granules in neutrophils. This has been shown in renal ischemia-reperfusion injury model where reperfusion resulted in increased damage, suggesting that CCM3 deficiency results in an increased oxidative damage due to neutrophil exocytosis^[162].

CCM3 controls EC proliferation. Weibel-Palade bodies are granules in EC cells that contain angiopoietin-2 (ANGPT2)^[163,164]. ANGPT2 binds to a tyrosine kinase receptor, TIE-2, and regulates the formation of EC cell-cell junction in angiogenesis. *Ccm3* knockout mice were shown to have increased Angpt2 expression. Furthermore, TIE-2 showed more phosphorylation in areas such as cerebellum and retina, areas classically known to form CCM lesions. As explained previously, CCM3 is a mediator of exocytosis in neutrophils through UNC13B. It was seen to mediate exocytosis in EC as well. EC cells were shown to have increased exocytosis of granules which can be rescued by the suppression of UNK13B. ANGPT2 transcription or translation was not affected by CCM3 silencing, suggesting that this is not regulated at the transcriptional level. This is consistent with the theory that CCM3 blocks exocytosis of ANGPT2. A decreased CCM lesion burden was observed in *Ccm3* knockout mice with the introduction of ANGPT2 antibodies, reaffirming the involvement of TIE2 signaling in the CCM lesion formation. This finding provides another venue for potential pharmacotherapy^[90].

CCM3 might have multiple cellular functions through its partners

CCM3 interacts with STK25 or MST4 to form the STRIPAK complex^[165] which localizes to the cis-face of the Golgi complex^[61]. At this location, it plays a role in appropriate positioning of the Golgi. GCKIII kinases are activated by homodimerization and resulting in its autophosphorylation, but activation is tightly regulated by CCM3^[95]. Dysfunction of CCM3 results in the malposition of Golgi complex and centrosome^[166]. Migration is essential for proper placement of EC cells during angiogenesis. Increased expression of CCM3 causes over migration of EC cell^[162]. Therefore, dysfunction of this process could be involved in the formation of CCM lesions.

CCM3 stabilizes intracellular bridges

Certain cells such as germ cells have cytoplasmic connections that regulate cell-cell communication and coordination. Anillin proteins such as ANI-1 and ANI-2 regulate the length of these projections. ANI-1 is known to decrease bridge length, while its antagonist, ANI-2, increases bridge length^[167]. It was found that GCK-1 that is regulated by CCM3 binds to ANI-1. Therefore, deficiencies in CCM3 and GCK-1 result in a decrease in intracellular bridge size. This results in multiple histological defects in the gonads such as reduced distal arm length, rachis diameter, and brood size. Fluorescence imaging studies showed that CCM3 localizes to the bridges. Co-deletions of CCM3/GCK-1 and ANI-1 resulted in increased bridge number, suggesting a similar pathway between GCK-1/CCM3 and ANI-1. Non-muscle myosin II (NMMII) is responsible for constriction of bridges. However, unopposed activation of NMMII causes hyperconstriction and results in destabilization of bridges^[168]. CCM3/GCK1 deletion resulted in increased localization of NMMII to the intracellular bridges and ANI-1 binds to NMMII. Therefore, it was postulated that intracellular bridges is regulated by CCM3-GCKI-ANI-1- NMMII signaling cascade. Yet, co-deletion of these genes did not affect bridge size. Therefore, it is likely that GCK1/CCM3 affects intracellular bridges through other signaling pathways.

CCM lesions have defective autophagy

CCM3, along with CCM1 and CCM2, are involved in many signaling pathways that result in increased production of ROS: Sirt1/FoxO1, JNK/c-JUN, β -catenin, and TGF- β pathways^[146,169-171]. This oxidative stress will damage organelles in the cell. However, inadequate autophagy mechanisms hinder cell recovery ability leading to progression of disease. CCM lesions show defect in autophagy through increased activity of mTOR. Inhibitors of mTOR were shown to reverse the defect in autophagy suggesting that mTOR is involved in the process, which provides another set of pharmacotherapeutic agents in CCM.

CCM lesions have differentially expressed miRNA

The composition of micro RNAs (miRNAs) in CCM lesions was analyzed through an mRNA expression screen. These results were supported by RT-qPCR. Compared to normal controls, it was found that 10 miRNAs were upregulated and 42 miRNAs was downregulated in CCM lesions. A more stringent analysis showed 5 miRNAs that were very significantly downregulated. Using bioinformatics, potential binding mRNAs to these 5 miRNAs were identified. One of the miRNAs had a potential 981 binding partners. Several proteins already implicated in CCM lesions were found to be targets of these miRNAs including MLLT4, VEGFA, MAPK1, RAC1, RHOA, FOXO1, ENG, SMURF1, and HEYL^[17,83,99,106,126,139]. It was concluded that three miRNAs (let-7b-5p, miR-361-5p, and miR-370-3p) can potentially be involved in the pathogenesis of CCM^[172].

CCM3 was frequently implicated in tumorigenesis

CCM3 was initially identified as a tumor-associated apoptotic protein^[21]. Several cases of meningiomas have been reported in patients with dysfunctional CCM3, suggesting that CCM3 could potentially act as a tumor suppressor^[31,173,174]. One report stated that CCM3 deficient EC cells can continuously proliferate in cell cultures. In fibroblasts, CCM3 deficient cells can grow several more generations before entering senescence, comparing to wild-type cells. This suggests that the depletion of CCM3 delays cell senescence. Gene enrichment analysis showed a decreased production of cytokines in CCM3 deficient EC cells. Cytokine production was not inducible with TNF- α in these cells. It was found that these cells have a defect in C/EBP β activity. C/EBP β expression was upregulated in CCM3 deficient cells, which delays the progression of cells into senescence. Therefore, the lack of C/EBP β activity is the likely driven factor in delaying the cells into senescence. Gene enrichment analysis showed decreased expression level of lysosome gene set. Senescent cells have increased autophagy for unutilized organelles and CCM3 deficient cells do not show increased activity of autophagy. Growth of CCM3 depleted cells in minimum nutrient media showed impairment of autophagy. Therefore, CCM3 deficiency increases C/EBP β activity that, in turn, impairs cell senescence, resulting in declined cellular autophagy^[175]. More research is needed to elucidate the underlined relationship between CCM3-mediated senescence and meningiomas.

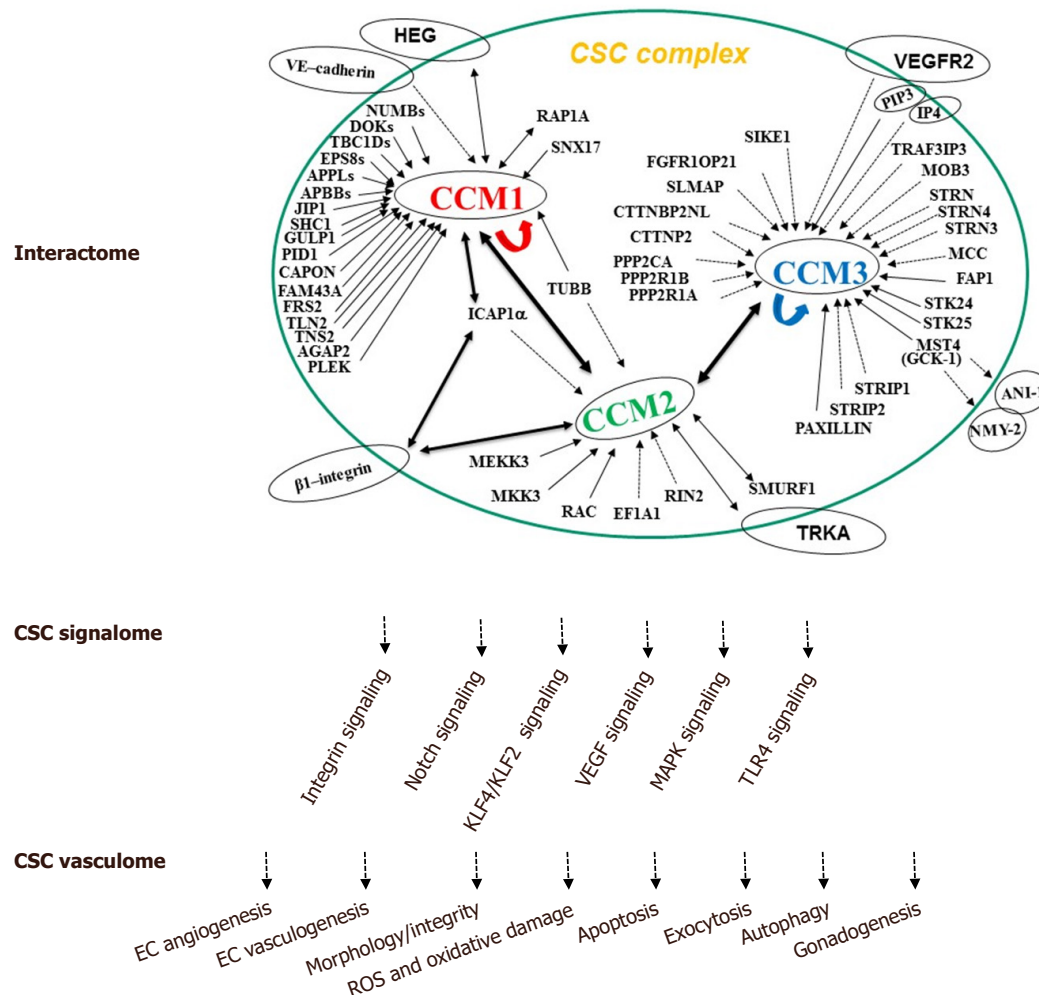


Figure 1. The cellular roles of the CSC complex. The schematic diagram summarizes the known CSC interaction protein partners (interactome), defined CSC-modulated signaling pathways (signalome), and distinct molecular and cellular functions of CSC complex (vasculome) with our current understanding of CSC cellular functions. CCM: cerebral cavernous malformations; CSC: CCM signaling complex; EC: endothelial cells

CCM3 mRNA 3'UTR was found to be able to bind to Mir-103, a microRNA that was found to be associated with prostate cancer. This microRNA was found to be down-regulated in prostate cancer. Furthermore, *in vivo* studies showed that up-regulation of Mir-103 restored cells to senescence. The *in vitro* studies of cell culture showed that Mir-103 plays an important role for G1/S cellular checkpoint. When Mir-103 binds to CCM3 mRNA, it targets the CCM3 transcript for degradation. The decreased expression of CCM3 in the cell leads to increased apoptosis. Further, overexpression Mir-103 in normal cells resulted in increased apoptosis. Likewise, down-regulation of CCM3 observed in a prostate cancer cell line resulted in increased apoptosis^[176], suggesting that CCM3 may play a role as oncogene or tumor suppressor (depending on cell signaling), in the tumorigenesis.

In summary, CCM proteins form a signaling complex (CSC) that have been demonstrated to play major roles in the regulation of multiple cell structures and signaling mechanisms involved in fundamental physiological and biogenic functions, as well as in cell responses to various environmental stressors. We have detailed these recent findings in the review, with a diagrammatic summary of major functions of CSC in three categories: interactome, signalome, and vasculome [Figure 1].

DECLARATIONS

Author's contributions

Akhil Padarti drafted the manuscript under the supervision of Jun Zhang.

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

- Otten P, Pizzolato GP, Rilliet B, Berney J. 131 cases of cavernous angioma (cavernomas) of the CNS, discovered by retrospective analysis of 24,535 autopsies. *Neurochirurgie* 1989;35:82-3, 128-31.
- Cavalcanti DD, Kalani MY, Martirosyan NL, Eales J, Spetzler RF, Preul MC. Cerebral cavernous malformations: from genes to proteins to disease. *J Neurosurg* 2012;116:122-32.
- Fisher OS, Zhang R, Li X, Murphy JW, Demeler B, Boggon TJ. Structural studies of cerebral cavernous malformations 2 (CCM2) reveal a folded helical domain at its C-terminus. *FEBS Lett* 2013;587:272-7.
- Tanriover G, Sozen B, Seker A, Kilic T, Gunel M, Demir N. Ultrastructural analysis of vascular features in cerebral cavernous malformations. *Clin Neurol Neurosurg* 2013;115:438-44.
- Choquet H, Trapani E, Goitre L, Tralbalzini L, Akers A, Fontanella M, et al. Cytochrome P450 and matrix metalloproteinase genetic modifiers of disease severity in Cerebral Cavernous Malformation type 1. *Free Radic Biol Med* 2016;92:100-9.
- Trapani E, Retta SF. Cerebral cavernous malformation (CCM) disease: from monogenic forms to genetic susceptibility factors. *J Neurosurg Sci* 2015;59:201-9.
- Gunel M, Awad IA, Finberg K, Anson JA, Steinberg GK, Batjer HH, Kopitnik TA, Morrison L, Giannotta SL, Nelson-Williams C, Lifton RP. A founder mutation as a cause of cerebral cavernous malformation in Hispanic Americans. *N Engl J Med* 1996;334:946-51.
- Denier C, Labauge P, Bergametti F, Marchelli F, Riant F, Arnoult M, Maciazek J, Vicaute E, Brunereau L, Tournier-Lasserre E; Société Française de Neurochirurgie. Genotype-phenotype correlations in cerebral cavernous malformations patients. *Ann Neurol* 2006;60:550-6.
- Liquori CL, Berg MJ, Squitieri F, Ottenbacher M, Sorlie M, Leedom TP, Cannella M, Maglione V, Ptacek L, Johnson EW, Marchuk DA. Low frequency of PDCD10 mutations in a panel of CCM3 probands: potential for a fourth CCM locus. *Hum Mutat* 2006;27:118.
- Liquori CL, Penco S, Gault J, Leedom TP, Tassi L, Esposito T, Awad IA, Frati L, Johnson EW, Squitieri F, Marchuk DA, Gianfrancesco F. Different spectra of genomic deletions within the CCM genes between Italian and American CCM patient cohorts. *Neurogenetics* 2008;9:25-31.
- Scimone C, Bramanti P, Alafaci C, Granata F, Piva F, Rinaldi C, Donato L, Greco F, Sidoti A, D'Angelo R. Update on Novel CCM Gene Mutations in Patients with Cerebral Cavernous Malformations. *J Mol Neurosci* 2017;61:189-98.
- Akers AL, Johnson E, Steinberg GK, Zabramski JM, Marchuk DA. Biallelic somatic and germline mutations in cerebral cavernous malformations (CCMs): evidence for a two-hit mechanism of CCM pathogenesis. *Hum Mol Genet* 2009;18:919-30.
- Pagenstecher A, Stahl S, Sure U, Felber U. A two-hit mechanism causes cerebral cavernous malformations: complete inactivation of CCM1, CCM2 or CCM3 in affected endothelial cells. *Hum Mol Genet* 2009;18:911-8.
- Batra S, Lin D, Recinos PF, Zhang J, Rigamonti D. Cavernous malformations: natural history, diagnosis and treatment. *Nat Rev Neurol* 2009;5:659-70.
- Zhang J, Basu S, Clatterbuck RE, Rigamonti D, Dietz HC. Pathogenesis of cerebral cavernous malformation: Depletion of Krit1 leads to perturbation of 1 integrin-mediated endothelial cell mobility and survival. *Am J Hum Genet* 2004;suppl:S222.

16. Hilder TL, Malone MH, Bencharit S, Colicelli J, Haystead TA, Johnson GL, Wu CC. Proteomic identification of the cerebral cavernous malformation signaling complex. *J Proteome Res* 2007;6:4343-55.
17. Zawistowski JS, Stalheim L, Uhlik MT, Abell AN, Ancrile BB, Johnson GL, Marchuk DA. CCM1 and CCM2 protein interactions in cell signaling: implications for cerebral cavernous malformations pathogenesis. *Hum Mol Genet* 2005;14:2521-31.
18. Zhang J, Rigamonti D, Dietz HC, Clatterbuck RE. Interaction between krit1 and malcavernin: implications for the pathogenesis of cerebral cavernous malformations. *Neurosurgery* 2007;60:353-9; discussion 9.
19. Zhang J, Dubey P, Padarti A, Zhang A, Patel R, Patel V, Cistola D, Badr A. Novel functions of CCM1 delimit the relationship of PTB/PH domains. *Biochim Biophys Acta* 2017;1865:1274-86.
20. Zhang J, Clatterbuck RE, Rigamonti D, Chang DD, Dietz HC. Interaction between krit1 and icap1alpha infers perturbation of integrin beta1-mediated angiogenesis in the pathogenesis of cerebral cavernous malformation. *Hum Mol Genet* 2001;10:2953-60.
21. Ma X, Zhao H, Shan J, Long F, Chen Y, Chen Y, Zhang Y, Han X, Ma D. PDCD10 interacts with Ste20-related kinase MST4 to promote cell growth and transformation via modulation of the ERK pathway. *Mol Biol Cell* 2007;18:1965-78.
22. Uhlik MT, Abell AN, Johnson NL, Sun W, Cuevas BD, Lobel-Rice KE, et al. Rac-MEKK3-MKK3 scaffolding for p38 MAPK activation during hyperosmotic shock. *Nat Cell Biol* 2003;5:1104-10.
23. Zhang J. Molecular biology of cerebral cavernous malformation. In: Rigamonti D, editor. *Cavernous Malformations of the Nervous System*. Cambridge: Cambridge University Press 2011 p. 31-40.
24. Zhang J, Basu S, Rigamonti D, Dietz HC, Clatterbuck RE. Depletion of KRIT1 leads to perturbation of beta 1 integrin-mediated endothelial cell angiogenesis in the pathogenesis of cerebral cavernous malformation. *Stroke* 2005;36:425.
25. Zhang J, Basu S, Rigamonti D, Dietz HC, Clatterbuck RE. Krit1 modulates beta 1-integrin-mediated endothelial cell proliferation. *Neurosurgery* 2008;63:571-8; discussion 8.
26. Richardson BT, Dibble CF, Borikova AL, Johnson GL. Cerebral cavernous malformation is a vascular disease associated with activated RhoA signaling. *Biol Chem* 2013;394:35-42.
27. Labauge P, Enjolras O, Bonerandi JJ, Laberge S, Dandurand M, Joujoux JM, Tournier-Lasserre E. An association between autosomal dominant cerebral cavernomas and a distinctive hyperkeratotic cutaneous vascular malformation in 4 families. *Ann Neurol* 1999;45:250-4.
28. Gianfrancesco F, Cannella M, Martino T, Maglione V, Esposito T, Innocenzi G, Vitale E, Liquori CL, Marchuk DA, Squitieri F. Highly variable penetrance in subjects affected with cavernous cerebral angiomas (CCM) carrying novel CCM1 and CCM2 mutations. *Am J Med Genet B Neuropsychiatr Genet* 2007;144B:691-5.
29. Grippaudo FR, Piane M, Amoroso M, Longo B, Penco S, Chessa L, Giubettini M, Santanelli F. Cutaneous venous malformations related to KRIT1 mutation: case report and literature review. *J Mol Neurosci* 2013;51:442-5.
30. Toldo I, Drigo P, Mammi I, Marini V, Carollo C. Vertebral and spinal cavernous angiomas associated with familial cerebral cavernous malformation. *Surg Neurol* 2009;71:167-71.
31. Riant F, Bergametti F, Fournier HD, Chapon F, Michalak-Provost S, Cecillon M, Lejeune P, Hosseini H, Choe C, Orth M, Bernreuther C, Boulday G, Denier C, Labauge P, Tournier-Lasserre E. CCM3 Mutations Are Associated with Early-Onset Cerebral Hemorrhage and Multiple Meningiomas. *Mol Syndromol* 2013;4:165-72.
32. Nikoubashman O, Wiesmann M, Tournier-Lasserre E, Mankad K, Bourgeois M, Brunelle F, Sainte-Rose C, Wiesmann M, Zerah M, Di Rocco F. Natural history of cerebral dot-like cavernomas. *Clin Radiol* 2013;68:e453-9.
33. Fauth C, Rostasy K, Rath M, Gizewski E, Lederer AG, Sure U, et al. Highly variable intrafamilial manifestations of a CCM3 mutation ranging from acute childhood cerebral haemorrhage to late-onset meningiomas. *Clin Neurol Neurosurg* 2015;128:41-3.
34. Sirvente J, Enjolras O, Wassef M, Tournier-Lasserre E, Labauge P. Frequency and phenotypes of cutaneous vascular malformations in a consecutive series of 417 patients with familial cerebral cavernous malformations. *J Eur Acad Dermatol Venereol* 2009;23:1066-72.
35. Shenkar R, Shi C, Rebeiz T, Stockton RA, McDonald DA, Mikati AG, Zhang L, Austin C, Akers AL, Gallione CJ, Rorrer A, Gunel M, Min W, De Souza JM, Lee C, Marchuk DA, Awad IA. Exceptional aggressiveness of cerebral cavernous malformation disease associated with PDCD10 mutations. *Genet Med*. 2015;17(3):188-96.
36. Choquet H, Pawlikowska L, Lawton MT, Kim H. Genetics of cerebral cavernous malformations: current status and future prospects. *J Neurosurg Sci* 2015;59:211-20.
37. Rath M, Jenssen SE, Schwefel K, Spiegler S, Kleimeier D, Sperling C, Kaderali L, Felbor U. High-throughput sequencing of the entire genomic regions of CCM1/KRIT1, CCM2 and CCM3/PDCD10 to search for pathogenic deep-intronic splice mutations in cerebral cavernous malformations. *Eur J Med Genet* 2017;60:479-84.
38. Zhang J, Clatterbuck RE, Rigamonti D, Dietz HC. Mutations in KRIT1 in familial cerebral cavernous malformations. *Neurosurgery* 2000;46:1272-7; discussion 7-9.
39. Riant F, Bergametti F, Aygnac X, Boulday G, Tournier-Lasserre E. Recent insights into cerebral cavernous malformations: the molecular genetics of CCM. *FEBS J* 2010;277:1070-5.
40. Haasdijk RA, Cheng C, Maat-Kievit AJ, Duckers HJ. Cerebral cavernous malformations: from molecular pathogenesis to genetic counselling and clinical management. *Eur J Hum Genet* 2012;20:134-40.
41. Petersen TA, Morrison LA, Schrader RM, Hart BL. Familial versus sporadic cavernous malformations: differences in developmental venous anomaly association and lesion phenotype. *AJNR Am J Neuroradiol* 2010;31:377-82.
42. Zhang J, Clatterbuck RE, Rigamonti D, Dietz HC. Cloning of the murine Krit1 cDNA reveals novel mammalian 5' coding exons. *Genomics* 2000;70:392-5.
43. Gingras AR, Puzon-McLaughlin W, Ginsberg MH. The structure of the ternary complex of Krev interaction trapped 1 (KRIT1) bound to both the Rap1 GTPase and the heart of glass (HEG1) cytoplasmic tail. *J Biol Chem* 2013;288:23639-49.

44. Li X, Zhang R, Draheim KM, Liu W, Calderwood DA, Boggon TJ. Structural basis for small G protein effector interaction of Ras-related protein 1 (Rap1) and adaptor protein Krev interaction trapped 1 (KRIT1). *J Biol Chem* 2012;287:22317-27.
45. Fisher OS, Boggon TJ. Signaling pathways and the cerebral cavernous malformations proteins: lessons from structural biology. *Cell Mol Life Sci* 2014;71:1881-92.
46. Faurobert E, Albiges-Rizo C. Recent insights into cerebral cavernous malformations: a complex jigsaw puzzle under construction. *FEBS J* 2010;277:1084-96.
47. Francalanci F, Avolio M, De Luca E, Longo D, Menchise V, Guazzi P, et al. Structural and functional differences between KRIT1A and KRIT1B isoforms: a framework for understanding CCM pathogenesis. *Exp Cell Res* 2009;315:285-303.
48. Beraud-Dufour S, Gautier R, Albiges-Rizo C, Chardin P, Faurobert E. Krit 1 interactions with microtubules and membranes are regulated by Rap1 and integrin cytoplasmic domain associated protein-1. *FEBS J* 2007;274:5518-32.
49. Goult BT, Bate N, Anthis NJ, Wegener KL, Gingras AR, Patel B, Barsukov IL, Campbell ID, Roberts GC, Critchley DR. The structure of an interdomain complex that regulates talin activity. *J Biol Chem* 2009;284:15097-106.
50. Goult BT, Bouaouina M, Elliott PR, Bate N, Patel B, Gingras AR, Grossmann JG, Roberts GC, Calderwood DA, Critchley DR, Barsukov IL. Structure of a double ubiquitin-like domain in the talin head: a role in integrin activation. *EMBO J* 2010;29:1069-80.
51. Pecqueur L, Duellberg C, Dreier B, Jiang Q, Wang C, Plückthun A, Surrey T, Gigant B, Knossow M. A designed ankyrin repeat protein selected to bind to tubulin caps the microtubule plus end. *Proc Natl Acad Sci U S A* 2012;109:12011-6.
52. Zhang R, Li X, Boggon TJ. Structural analysis of the KRIT1 ankyrin repeat and FERM domains reveals a conformationally stable ARD-FERM interface. *J Struct Biol* 2015;192:449-56.
53. Bessman MJ, Frick DN, O'Handley SF. The MutT proteins or "Nudix" hydrolases, a family of versatile, widely distributed, "housecleaning" enzymes. *J Biol Chem* 1996;271:25059-62.
54. Liu W, Draheim KM, Zhang R, Calderwood DA, Boggon TJ. Mechanism for KRIT1 release of ICAP1-mediated suppression of integrin activation. *Mol Cell* 2013;49:719-29.
55. Petit N, Bleon A, Denier C, Tournier-Lasserre E. Patterns of expression of the three cerebral cavernous malformation (CCM) genes during embryonic and postnatal brain development. *Gene Expr Patterns* 2006;6:495-503.
56. Seker A, Pricola KL, Guclu B, Ozturk AK, Louvi A, Gunel M. CCM2 expression parallels that of CCM1. *Stroke* 2006;37:518-23.
57. Zhang J, Carr C, Badr A. The cardiovascular triad of dysfunctional angiogenesis. *Transl Stroke Res* 2011;2:339-45.
58. Stockton RA, Shenkar R, Awad IA, Ginsberg MH. Cerebral cavernous malformations proteins inhibit Rho kinase to stabilize vascular integrity. *J Exp Med* 2010;207:881-96.
59. Fisher OS, Liu W, Zhang R, Stiegler AL, Ghedia S, Weber JL, Boggon TJ. Structural basis for the disruption of the cerebral cavernous malformations 2 (CCM2) interaction with Krev interaction trapped 1 (KRIT1) by disease-associated mutations. *J Biol Chem* 2015;290:2842-53.
60. Scimone C, Bramanti P, Ruggeri A, Donato L, Alafaci C, Crisafulli C, Mucciardi M, Rinaldi C, Sidoti A, D'Angelo R. CCM3/SERPINI1 bidirectional promoter variants in patients with cerebral cavernous malformations: a molecular and functional study. *BMC Med Genet* 2016;17:74.
61. Kean MJ, Ceccarelli DF, Goudreaux M, Sanches M, Tate S, Larsen B, Gibson LC, Derry WB, Scott IC, Pelletier L, Baillie GS, Sicheri F, Gingras AC. Structure-function analysis of core STRIPAK Proteins: a signaling complex implicated in Golgi polarization. *J Biol Chem* 2011;286(28):25065-75.
62. Dibble CF, Horst JA, Malone MH, Park K, Temple B, Cheeseman H, Barbaro JR, Johnson GL, Bencharit S. Defining the functional domain of programmed cell death 10 through its interactions with phosphatidylinositol-3,4,5-trisphosphate. *PLoS One* 2010;5:e11740.
63. Li X, Zhang R, Zhang H, He Y, Ji W, Min W, Boggon TJ. Crystal structure of CCM3, a cerebral cavernous malformation protein critical for vascular integrity. *J Biol Chem* 2010;285:24099-107.
64. Lant B, Yu B, Goudreaux M, Holmyard D, Knight JD, Xu P, Zhao L, Chin K, Wallace E, Zhen M, Gingras AC, Derry WB. CCM-3/STRIPAK promotes seamless tube extension through endocytic recycling. *Nat Commun* 2015;6:6449.
65. Rehain-Bell K, Love A, Werner ME, MacLeod I, Yates JR, 3rd, Maddox AS. A Sterile 20 Family Kinase and Its Co-factor CCM-3 Regulate Contractile Ring Proteins on Germline Intercellular Bridges. *Curr Biol* 2017;27:860-7.
66. Berman JR, Kenyon C. Germ-cell loss extends *C. elegans* life span through regulation of DAF-16 by kri-1 and lipophilic-hormone signaling. *Cell* 2006;124:1055-68.
67. Guzeloglu-Kayisli O, Amankulor NM, Voorhees J, Luleci G, Lifton RP, Gunel M. KRIT1/cerebral cavernous malformation 1 protein localizes to vascular endothelium, astrocytes, and pyramidal cells of the adult human cerebral cortex. *Neurosurgery* 2004;54:943-9; discussion 9.
68. Zhang J, Clatterbuck RE, Rigamonti D, Chang DD, Dietz HC. Novel insights regarding the pathogenesis of cerebral cavernous malformation (CCM). *American Journal of Human Genetics* 2001;69:178.
69. Serebriiskii I, Estoak J, Sonoda G, Testa JR, Golemis EA. Association of Krev-1/rap1a with Krit1, a novel ankyrin repeat-containing protein encoded by a gene mapping to 7q21-22. *Oncogene* 1997;15:1043-9.
70. Frische EW, Zwartkruis FJ. Rap1, a mercenary among the Ras-like GTPases. *Dev Biol* 2010;340:1-9.
71. Liu JJ, Stockton RA, Gingras AR, Ablooglu AJ, Han J, Bobkov AA, Ginsberg MH. A mechanism of Rap1-induced stabilization of endothelial cell-cell junctions. *Mol Biol Cell* 2011;22:2509-19.
72. Liu H, Rigamonti D, Badr A, Zhang J. Ccm1 regulates microvascular morphogenesis during angiogenesis. *J Vasc Res* 2011;48:130-40.
73. Lakshmikanthan S, Sobczak M, Chun C, Henschel A, Dargatz J, Ramchandran R, Chrzanowska-Wodnicka M. Rap1 promotes VEGFR2 activation and angiogenesis by a mechanism involving integrin alphavbeta(3). *Blood* 2011;118:2015-26.

74. Chrzanowska-Wodnicka M, White GC, 2nd, Quilliam LA, Whitehead KJ. Small GTPase Rap1 Is Essential for Mouse Development and Formation of Functional Vasculature. *PLoS One* 2015;10:e0145689.
75. Hamada K, Shimizu T, Matsui T, Tsukita S, Hakoshima T. Structural basis of the membrane-targeting and unmasking mechanisms of the radixin FERM domain. *EMBO J* 2000;19:4449-62.
76. Gingras AR, Liu JJ, Ginsberg MH. Structural basis of the junctional anchorage of the cerebral cavernous malformations complex. *J Cell Biol* 2012;199:39-48.
77. Brüttsch R, Liebler SS, Wüsthube J, Bartol A, Herberich SE, Adam MG, Telzerow A, Augustin HG, Fischer A. Integrin cytoplasmic domain-associated protein-1 attenuates sprouting angiogenesis. *Circ Res* 2010;107:592-601.
78. Fournier HN, Dupé-Manet S, Bouvard D, Luton F, Degani S, Block MR, Retta SF, Albiges-Rizo C. Nuclear translocation of integrin cytoplasmic domain-associated protein 1 stimulates cellular proliferation. *Mol Biol Cell* 2005;16:1859-71.
79. Calderwood DA, Fujioka Y, de Pereda JM, García-Alvarez B, Nakamoto T, Margolis B, McGlade CJ, Liddington RC, Ginsberg MH. Integrin beta cytoplasmic domain interactions with phosphotyrosine-binding domains: a structural prototype for diversity in integrin signaling. *Proc Natl Acad Sci U S A* 2003;100:2272-7.
80. Bouvard D, Aszodi A, Kostka G, Block MR, Albiges-Rizo C, Fassler R. Defective osteoblast function in ICAP-1-deficient mice. *Development* 2007;134:2615-25.
81. Brunner M, Millon-Frémillon A, Chevalier G, Nakchbandi IA, Mosher D, Block MR, Albigès-Rizo C, Bouvard D. Osteoblast mineralization requires beta1 integrin/ICAP-1-dependent fibronectin deposition. *J Cell Biol* 2011;194:307-22.
82. Liu W, Boggon TJ. Cocystal structure of the ICAP1 PTB domain in complex with a KRIT1 peptide. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 2013;69:494-8.
83. Draheim KM, Fisher OS, Boggon TJ, Calderwood DA. Cerebral cavernous malformation proteins at a glance. *J Cell Sci* 2014;127:701-7.
84. Stiegler AL, Zhang R, Liu W, Boggon TJ. Structural determinants for binding of sorting nexin 17 (SNX17) to the cytoplasmic adaptor protein Krev interaction trapped 1 (KRIT1). *J Biol Chem* 2014;289:25362-73.
85. Uhlik MT, Temple B, Bencharit S, Kimple AJ, Siderovski DP, Johnson GL. Structural and evolutionary division of phosphotyrosine binding (PTB) domains. *J Mol Biol* 2005;345:1-20.
86. Harel L, Costa B, Tcherpakov M, Zapatka M, Oberthuer A, Hansford LM, Vojvodic M, Levy Z, Chen ZY, Lee FS, Avigad S, Yaniv I, Shi L, Eils R, Fischer M, Brors B, Kaplan DR, Fainzilber M. CCM2 mediates death signaling by the TrkA receptor tyrosine kinase. *Neuron* 2009;63:585-91.
87. Costa B, Kean MJ, Ast V, Knight JD, Mett A, Levy Z, Ceccarelli DF, Badillo BG, Eils R, König R, Gingras AC, Fainzilber M. STK25 protein mediates TrkA and CCM2 protein-dependent death in pediatric tumor cells of neural origin. *J Biol Chem* 2012;287:29285-9.
88. Crose LE, Hilder TL, Sciaky N, Johnson GL. Cerebral cavernous malformation 2 protein promotes smad ubiquitin regulatory factor 1-mediated RhoA degradation in endothelial cells. *J Biol Chem* 2009;284:13301-5.
89. Whitehead KJ, Chan AC, Navankasattusas S, Koh W, London NR, Ling J, Mayo AH, Drakos SG, Jones CA, Zhu W, Marchuk DA, Davis GE, Li DY. The cerebral cavernous malformation signaling pathway promotes vascular integrity via Rho GTPases. *Nat Med* 2009;15:177-84.
90. Zhou Z, Tang AT, Wong WY, Bamezai S, Goddard LM, Shenkar R, Zhou S, Yang J, Wright AC, Foley M, Arthur JS, Whitehead KJ, Awad IA, Li DY, Zheng X, Kahn ML. Cerebral cavernous malformations arise from endothelial gain of MEKK3-KLF2/4 signalling. *Nature* 2016;532:122-6.
91. Draheim KM, Li X, Zhang R, Fisher OS, Villari G, Boggon TJ, Calderwood DA. CCM2-CCM3 interaction stabilizes their protein expression and permits endothelial network formation. *J Cell Biol* 2015;208:987-1001.
92. Zheng X, Xu C, Smith AO, Stratman AN, Zou Z, Kleaveland B, Yuan L, Didiku C, Sen A, Liu X, Skuli N, Zaslavsky A, Chen M, Cheng L, Davis GE, Kahn ML. Dynamic regulation of the cerebral cavernous malformation pathway controls vascular stability and growth. *Dev Cell* 2012;23:342-55.
93. Rosen JN, Sogah VM, Ye LY, Mably JD. ccm2-like is required for cardiovascular development as a novel component of the Heg-CCM pathway. *Dev Biol* 2013;376:74-85.
94. Ceccarelli DF, Laister RC, Mulligan VK, Kean MJ, Goudreaux M, Scott IC, Derry WB, Chakrabarty A, Gingras AC, Sicheri F. CCM3/PDCD10 heterodimerizes with germinal center kinase III (GCKIII) proteins using a mechanism analogous to CCM3 homodimerization. *J Biol Chem* 2011;286:25056-64.
95. Xu X, Wang X, Zhang Y, Wang DC, Ding J. Structural basis for the unique heterodimeric assembly between cerebral cavernous malformation 3 and germinal center kinase III. *Structure* 2013;21:1059-66.
96. Ding J, Wang X, Li DF, Hu Y, Zhang Y, Wang DC. Crystal structure of human programmed cell death 10 complexed with inositol-(1,3,4,5)-tetrakisphosphate: a novel adaptor protein involved in human cerebral cavernous malformation. *Biochem Biophys Res Commun*. 2010;399:587-92.
97. Voss K, Stahl S, Hogan BM, Reinders J, Schleider E, Schulte-Merker S, Felbor U. Functional analyses of human and zebrafish 18-amino acid in-frame deletion pave the way for domain mapping of the cerebral cavernous malformation 3 protein. *Hum Mutat* 2009;30:1003-11.
98. Ridley AJ, Hall A. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* 1992;70:389-99.
99. Zheng X, Xu C, Di Lorenzo A, Kleaveland B, Zou Z, Seiler C, Chen M, Cheng L, Xiao J, He J, Pack MA, Sessa WC, Kahn ML. CCM3 signaling through sterile 20-like kinases plays an essential role during zebrafish cardiovascular development and cerebral cavernous malformations. *J Clin Invest* 2010;120:2795-804.
100. Chan AC, Drakos SG, Ruiz OE, Smith AC, Gibson CC, Ling J, Passi SF, Stratman AN, Sacharidou A, Revelo MP, Grossmann AH,

- Diakos NA, Davis GE, Metzstein MM, Whitehead KJ, Li DY. Mutations in 2 distinct genetic pathways result in cerebral cavernous malformations in mice. *J Clin Invest* 2011;121:1871-81.
101. McDonald DA, Shenkar R, Shi C, Stockton RA, Akers AL, Kucherlapati MH, Kucherlapati R, Brainer J, Ginsberg MH, Awad IA, Marchuk DA. A novel mouse model of cerebral cavernous malformations based on the two-hit mutation hypothesis recapitulates the human disease. *Hum Mol Genet* 2011;20:211-22.
102. Li X, Ji W, Zhang R, Folta-Stogniew E, Min W, Boggon TJ. Molecular recognition of leucine-aspartate repeat (LD) motifs by the focal adhesion targeting homology domain of cerebral cavernous malformation 3 (CCM3). *J Biol Chem* 2011;286:26138-47.
103. Lu TJ, Lai WY, Huang CY, Hsieh WJ, Yu JS, Hsieh YJ, Chang WT, Leu TH, Chang WC, Chuang WJ, Tang MJ, Chen TY, Lu TL, Lai MD. Inhibition of cell migration by autophosphorylated mammalian sterile 20-like kinase 3 (MST3) involves paxillin and protein-tyrosine phosphatase-PEST. *J Biol Chem* 2006;281:38405-17.
104. Wüsthube J, Bartol A, Liebler SS, Brütsh R, Zhu Y, Felbor U, Sure U, Augustin HG, Fischer A. Cerebral cavernous malformation protein CCM1 inhibits sprouting angiogenesis by activating DELTA-NOTCH signaling. *Proc Natl Acad Sci U S A* 2010;107:12640-5.
105. Liu H, Rigamonti D, Badr A, Zhang J. Ccm1 assures microvascular integrity during angiogenesis. *Transl Stroke Res* 2010;1:146-53.
106. Goitre L, Balzac F, Degani S, Degan P, Marchi S, Pinton P, Retta SF. KRIT1 regulates the homeostasis of intracellular reactive oxygen species. *PLoS One* 2010;5:e11786.
107. Guazzi P, Goitre L, Ferro E, Cutano V, Martino C, Trabalzini L, Retta SF. Identification of the Kelch family protein Nd1-L as a novel molecular interactor of KRIT1. *PLoS One* 2012;7:e44705.
108. Kato Y, Kravchenko VV, Tapping RI, Han J, Ulevitch RJ, Lee JD. BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *EMBO J* 1997;16:7054-66.
109. Sohn SJ, Li D, Lee LK, Winoto A. Transcriptional regulation of tissue-specific genes by the ERK5 mitogen-activated protein kinase. *Mol Cell Biol* 2005;25:8553-66.
110. Dekker RJ, van Soest S, Fontijn RD, Salamanca S, de Groot PG, VanBavel E, Pannekoek H, Horrevoets AJ. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Kruppel-like factor (KLF2). *Blood* 2002;100:1689-98.
111. Ohnesorge N, Viemann D, Schmidt N, Czymai T, Spiering D, Schmolke M, Ludwig S, Roth J, Goebeler M, Schmidt M. Erk5 activation elicits a vasoprotective endothelial phenotype via induction of Kruppel-like factor 4 (KLF4). *J Biol Chem* 2010;285:26199-210.
112. Komaravolu RK, Adam C, Moonen JR, Harmsen MC, Goebeler M, Schmidt M. Erk5 inhibits endothelial migration via KLF2-dependent down-regulation of PAK1. *Cardiovasc Res* 2015;105:86-95.
113. Maddaluno L, Rudini N, Bravi L, Giampietro C, Corada M, Ferrarini L, Orsenigo F, Papa E, Boulday G, Tournier-Lasserre E, Chapon F, Richichi C, Retta SF, Lampugnani MG, Dejana E. EndMT contributes to the onset and progression of cerebral cavernous malformations. *Nature* 2013;498:492-6.
114. Cuttano R, Rudini N, Bravi L, Corada M, Giampietro C, Papa E, Morini MF, Maddaluno L, Baeyens N, Adams RH, Jain MK, Owens GK, Schwartz M, Lampugnani MG, Dejana E. KLF4 is a key determinant in the development and progression of cerebral cavernous malformations. *EMBO Mol Med* 2016;8:6-24.
115. Zhou Z, Rawnsley DR, Goddard LM, Pan W, Cao XJ, Jakus Z, Zheng H, Yang J, Arthur JS, Whitehead KJ, Li D, Zhou B, Garcia BA, Zheng X, Kahn ML. The cerebral cavernous malformation pathway controls cardiac development via regulation of endocardial MEKK3 signaling and KLF expression. *Dev Cell* 2015;32:168-80.
116. Renz M, Otten C, Faurobert E, Rudolph F, Zhu Y, Boulday G, Duchene J, Mickoleit M, Dietrich AC, Ramspacher C, Steed E, Manet-Dupé S, Benz A, Hassel D, Vermot J, Huysken J, Tournier-Lasserre E, Felbor U, Sure U, Albiges-Rizo C, Abdelilah-Seyfried S. Regulation of beta1 integrin-Klf2-mediated angiogenesis by CCM proteins. *Dev Cell* 2015;32:181-90.
117. Zhang X, Lawler J. Thrombospondin-based antiangiogenic therapy. *Microvasc Res* 2007;74:90-9.
118. Lopez-Ramirez MA, Fonseca G, Zeineddine HA, Girard R, Moore T, Pham A, Cao Y, Shenkar R, de Kreuk BJ, Lagarrigue F, Lawler J, Glass CK, Awad IA, Ginsberg MH. Thrombospondin1 (TSP1) replacement prevents cerebral cavernous malformations. *J Exp Med* 2017;214:3331-46.
119. Draheim KM, Huet-Calderwood C, Simon B, Calderwood DA. Nuclear Localization of Integrin Cytoplasmic Domain-associated Protein-1 (ICAP1) Influences beta1 Integrin Activation and Recruits Krev/Interaction Trapped-1 (KRIT1) to the Nucleus. *J Biol Chem* 2017;292:1884-98.
120. Piedra J, Miravet S, Castaño J, Pálmer HG, Heisterkamp N, García de Herreros A, Duñach M. p120 Catenin-associated Fer and Fyn tyrosine kinases regulate beta-catenin Tyr-142 phosphorylation and beta-catenin-alpha-catenin Interaction. *Mol Cell Biol* 2003;23:2287-97.
121. Potter MD, Barbero S, Cheresh DA. Tyrosine phosphorylation of VE-cadherin prevents binding of p120- and beta-catenin and maintains the cellular mesenchymal state. *J Biol Chem* 2005;280:31906-12.
122. DiStefano PV, Kuebel JM, Sarelus IH, Glading AJ. KRIT1 protein depletion modifies endothelial cell behavior via increased vascular endothelial growth factor (VEGF) signaling. *J Biol Chem* 2014;289:33054-65.
123. Hilder TL, Malone MH, Johnson GL. Hyperosmotic induction of mitogen-activated protein kinase scaffolding. *Methods Enzymol* 2007;428:297-312.
124. Zhou X, Izumi Y, Burg MB, Ferraris JD. Rac1/osmosensing scaffold for MEKK3 contributes via phospholipase C-gamma1 to activation of the osmoprotective transcription factor NFAT5. *Proc Natl Acad Sci U S A* 2011;108:12155-60.
125. Louvi A, Chen L, Two AM, Zhang H, Min W, Gunel M. Loss of cerebral cavernous malformation 3 (Ccm3) in neuroglia leads to CCM and vascular pathology. *Proc Natl Acad Sci U S A* 2011;108:3737-42.
126. You C, Sandalcioğlu IE, Dammann P, Felbor U, Sure U, Zhu Y. Loss of CCM3 impairs DLL4-Notch signalling: implication in endothelial angiogenesis and in inherited cerebral cavernous malformations. *J Cell Mol Med* 2013;17:407-18.

127. Zhu Y, Wu Q, Fass M, Xu JF, You C, Müller O, Sandalcioğlu IE, Zhang JM, Sure U. In vitro characterization of the angiogenic phenotype and genotype of the endothelia derived from sporadic cerebral cavernous malformations. *Neurosurgery* 2011;69:722-31; discussion 31-2.
128. Harrington LS, Sainson RC, Williams CK, Taylor JM, Shi W, Li JL, Harris AL. Regulation of multiple angiogenic pathways by Dll4 and Notch in human umbilical vein endothelial cells. *Microvasc Res* 2008;75:144-54.
129. Kume T. Novel insights into the differential functions of Notch ligands in vascular formation. *J Angiogenesis Res* 2009;1:8.
130. Hellstrom M, Phng LK, Gerhardt H. VEGF and Notch signaling: the yin and yang of angiogenic sprouting. *Cell Adh Migr* 2007;1:133-6.
131. Bheeshmachar G, Purushotaman D, Sade H, Gunasekharan V, Rangarajan A, Sarin A. Evidence for a role for notch signaling in the cytokine-dependent survival of activated T cells. *J Immunol* 2006;177:5041-50.
132. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998;282:2085-8.
133. Huang Q, Yang J, Lin Y, Walker C, Cheng J, Liu ZG, Su B. Differential regulation of interleukin 1 receptor and Toll-like receptor signaling by MEKK3. *Nat Immunol* 2004;5:98-103.
134. Tang AT, Choi JP, Kotzin JJ, Yang Y, Hong CC, Hobson N, Girard R, Zeineddine HA, Lightle R, Moore T, Cao Y, Shenkar R, Chen M, Mericko P, Yang J, Li L, Tanes C, Kobuley D, Vösa U, Whitehead KJ, Li DY, Franke L, Hart B, Schwaninger M, Henao-Mejia J, Morrison L, Kim H, Awad IA, Zheng X, Kahn ML. Endothelial TLR4 and the microbiome drive cerebral cavernous malformations. *Nature* 2017;545:305-10.
135. He Y, Zhang H, Yu L, Gunel M, Boggon TJ, Chen H, Min W. Stabilization of VEGFR2 signaling by cerebral cavernous malformation 3 is critical for vascular development. *Sci Signal* 2010;3:ra26.
136. Fischer A, Zalvide J, Faurobert E, Albiges-Rizo C, Tournier-Lasserre E. Cerebral cavernous malformations: from CCM genes to endothelial cell homeostasis. *Trends Mol Med* 2013;19:302-8.
137. Herberich SE, Klose R, Moll I, Yang WJ, Wustehube-Lausch J, Fischer A. ANKS1B Interacts with the Cerebral Cavernous Malformation Protein-1 and Controls Endothelial Permeability but Not Sprouting Angiogenesis. *PLoS One* 2015;10:e0145304.
138. Vestweber D, Winderlich M, Cagna G, Nottebaum AF. Cell adhesion dynamics at endothelial junctions: VE-cadherin as a major player. *Trends Cell Biol* 2009;19:8-15.
139. Glading A, Han J, Stockton RA, Ginsberg MH. KRIT-1/CCM1 is a Rap1 effector that regulates endothelial cell cell junctions. *J Cell Biol* 2007;179:247-54.
140. Goitre L, DiStefano PV, Moglia A, Nobiletti N, Baldini E, Trabalzini L, Keubel J, Trapani E, Shuvaev VV, Muzykantov VR, Sarelius IH, Retta SF, Glading AJ. Up-regulation of NADPH oxidase-mediated redox signaling contributes to the loss of barrier function in KRIT1 deficient endothelium. *Sci Rep* 2017;7:8296.
141. Goitre L, De Luca E, Braggion S, Trapani E, Guglielmotto M, Biasi F, Forni M, Moglia A, Trabalzini L, Retta SF. KRIT1 loss of function causes a ROS-dependent upregulation of c-Jun. *Free Radic Biol Med* 2014;68:134-47.
142. Hsieh HL, Lin CC, Chan HJ, Yang CM, Yang CM. c-Src-dependent EGF receptor transactivation contributes to ET-1-induced COX-2 expression in brain microvascular endothelial cells. *J Neuroinflammation* 2012;9:152.
143. Corr M, Lerman I, Keubel JM, Ronacher L, Misra R, Lund F, Sarelius IH, Glading AJ. Decreased Krev interaction-trapped 1 expression leads to increased vascular permeability and modifies inflammatory responses in vivo. *Arterioscler Thromb Vasc Biol* 2012;32:2702-10.
144. Retta SF, Glading AJ. Oxidative stress and inflammation in cerebral cavernous malformation disease pathogenesis: Two sides of the same coin. *Int J Biochem Cell Biol* 2016;81:254-70.
145. Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, Lamas S. Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol* 2015;6:183-97.
146. Dodson M, Redmann M, Rajasekaran NS, Darley-Usmar V, Zhang J. KEAP1-NRF2 signalling and autophagy in protection against oxidative and reductive proteotoxicity. *Biochem J* 2015;469:347-55.
147. Siow RC, Mann GE. Dietary isoflavones and vascular protection: activation of cellular antioxidant defenses by SERMs or hormesis? *Mol Aspects Med* 2010;31:468-77.
148. Bryan HK, Olayanju A, Goldring CE, Park BK. The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. *Biochem Pharmacol* 2013;85:705-17.
149. Yuan X, Xu C, Pan Z, Keum YS, Kim JH, Shen G, Yu S, Oo KT, Ma J, Kong AN. Butylated hydroxyanisole regulates ARE-mediated gene expression via Nrf2 coupled with ERK and JNK signaling pathway in HepG2 cells. *Mol Carcinog* 2006;45:841-50.
150. Schalkwijk CG, van Bezou J, van der Schors RC, Uchida K, Stehouwer CD, van Hinsbergh VW. Heat-shock protein 27 is a major methylglyoxal-modified protein in endothelial cells. *FEBS Lett* 2006;580:1565-70.
151. Nomura W, Inoue Y. Methylglyoxal activates the target of rapamycin complex 2-protein kinase C signaling pathway in *Saccharomyces cerevisiae*. *Mol Cell Biol* 2015;35:1269-80.
152. Antognelli C, Trapani E, Delle Monache S, Perrelli A, Daga M, Pizzimenti S, Barrera G, Cassoni P, Angelucci A, Trabalzini L, Talesa VN, Goitre L, Retta SF. KRIT1 loss-of-function induces a chronic Nrf2-mediated adaptive homeostasis that sensitizes cells to oxidative stress: Implication for Cerebral Cavernous Malformation disease. *Free Radic Biol Med* 2018;115:202-18.
153. Munch J, Grivas D, Gonzalez-Rajal A, Torregrosa-Carrion R, de la Pompa JL. Notch signalling restricts inflammation and serpentine expression in the dynamic endocardium of the regenerating zebrafish heart. *Development* 2017;144:1425-40.
154. Beis D, Bartman T, Jin SW, Scott IC, D'Amico LA, Ober EA, Verkade H, Frantsve J, Field HA, Wehman A, Baier H, Tallafuss A, Bally-Cuif L, Chen JN, Stainier DY, Jungblut B. Genetic and cellular analyses of zebrafish atrioventricular cushion and valve development.

- Development 2005;132:4193-204.
155. Donat S, Lourenco M, Paolini A, Otten C, Renz M, Abdelilah-Seyfried S. Hg1 and Ccm1/2 proteins control endocardial mechanosensitivity during zebrafish valvulogenesis. *Elife* 2018;7: pii: e28939.
156. Yaba A, Ordueri NE, Tanriover G, Sahin P, Demir N, Celik-Ozenci C. Expression of CCM2 and CCM3 during mouse gonadogenesis. *J Assist Reprod Genet* 2015;32:1497-507.
157. Guo S, Kempthues KJ. A non-muscle myosin required for embryonic polarity in *Caenorhabditis elegans*. *Nature* 1996;382:455-8.
158. Pal S, Lant B, Yu B, Tian R, Tong J, Krieger JR, Moran MF, Gingras AC, Derry WB. CCM-3 Promotes *C. elegans* Germline Development by Regulating Vesicle Trafficking Cytokinesis and Polarity. *Curr Biol* 2017;27:868-76.
159. Fidalgo M, Guerrero A, Fraile M, Iglesias C, Pombo CM, Zalvide J. Adaptor protein cerebral cavernous malformation 3 (CCM3) mediates phosphorylation of the cytoskeletal proteins ezrin/radixin/moesin by mammalian Ste20-4 to protect cells from oxidative stress. *J Biol Chem* 2012;287:11556-65.
160. Pivot-Pajot C, Varoqueaux F, de Saint Basile G, Bourgoin SG. Munc13-4 regulates granule secretion in human neutrophils. *J Immunol* 2008;180:6786-97.
161. Boswell KL, James DJ, Esquibel JM, Bruinsma S, Shirakawa R, Horiuchi H, Martin TF. Munc13-4 reconstitutes calcium-dependent SNARE-mediated membrane fusion. *J Cell Biol* 2012;197:301-12.
162. Zhang M, Dong L, Shi Z, Jiao S, Zhang Z, Zhang W, Liu G, Chen C, Feng M, Hao Q, Wang W, Yin M, Zhao Y, Zhang L, Zhou Z. Structural mechanism of CCM3 heterodimerization with GCKIII kinases. *Structure* 2013;21:680-8.
163. Fiedler U, Reiss Y, Scharpfenecker M, Grunow V, Koidl S, Thurston G, Gale NW, Witzernath M, Rosseau S, Suttorp N, Sobke A, Herrmann M, Preissner KT, Vajkoczy P, Augustin HG. Angiopoietin-2 sensitizes endothelial cells to TNF- α and has a crucial role in the induction of inflammation. *Nat Med* 2006;12:235-9.
164. Lowenstein CJ, Morrell CN, Yamakuchi M. Regulation of Weibel-Palade body exocytosis. *Trends Cardiovasc Med* 2005;15:302-8.
165. Sugden PH, McGuffin LJ, Clerk A. SOcK, MiSTs, MASK and STicKs: the GCKIII (germline centre kinase III) kinases and their heterologous protein-protein interactions. *Biochem J* 2013;454:13-30.
166. Fidalgo M, Fraile M, Pires A, Force T, Pombo C, Zalvide J. CCM3/PDCD10 stabilizes GCKIII proteins to promote Golgi assembly and cell orientation. *J Cell Sci* 2010;123:1274-84.
167. Maddox AS, Habermann B, Desai A, Oegema K. Distinct roles for two *C. elegans* anillins in the gonad and early embryo. *Development* 2005;132:2837-48.
168. Yamamoto S, Bayat V, Bellen HJ, Tan C. Protein phosphatase 1ss limits ring canal constriction during *Drosophila* germline cyst formation. *PLoS One* 2013;8:e70502.
169. Marchi S, Corricelli M, Trapani E, Bravi L, Pittaro A, Delle Monache S, Ferroni L, Patergnani S, Missiroli S, Goitre L, Trabalzini L, Rimessi A, Giorgi C, Zavan B, Cassoni P, Dejana E, Retta SF, Pinton P. Defective autophagy is a key feature of cerebral cavernous malformations. *EMBO Mol Med* 2015;7:1403-17.
170. Yogev O, Shaulian E. Jun proteins inhibit autophagy and induce cell death. *Autophagy* 2010;6:566-7.
171. Liu J, Bi X, Chen T, Zhang Q, Wang SX, Chiu JJ, Liu GS, Zhang Y, Bu P, Jiang F. Shear stress regulates endothelial cell autophagy via redox regulation and Sirt1 expression. *Cell Death Dis* 2015;6:e1827.
172. Kar S, Bali KK, Baisanthy A, Geffers R, Samii A, Bertalanffy H. Genome-Wide Sequencing Reveals MicroRNAs Downregulated in Cerebral Cavernous Malformations. *J Mol Neurosci*. 2017;61:178-88.
173. Clark VE, Erson-Omay EZ, Serin A, Yin J, Cotney J, Ozduman K, Avşar T, Li J, Murray PB, Henegariu O, Yilmaz S, Günel JM, Carrión-Grant G, Yilmaz B, Grady C, Tanrikulu B, Bakircioğlu M, Kaymakçalan H, Caglayan AO, Sencar L, Ceyhan E, Atik AF, Bayri Y, Bai H, Kolb LE, Hebert RM, Omay SB, Mishra-Gorur K, Choi M, Overton JD, Holland EC, Mane S, State MW, Bilgüvar K, Baehring JM, Gutin PH, Piepmeyer JM, Vortmeyer A, Brennan CW, Pamir MN, Kiliç T, Lifton RP, Noonan JP, Yasuno K, Günel M. Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. *Science* 2013;339:1077-80.
174. Labauge P, Fontaine B, Neau JP, Bergametti F, Riant F, Blecon A, Marchelli F, Arnoult M, Lannuzel A, Clanet M, Olschwang S, Denier C, Tournier-Lasserre E. Multiple dural lesions mimicking meningiomas in patients with CCM3/PDCD10 mutations. *Neurology* 2009;72:2044-6.
175. Guerrero A, Iglesias C, Raguz S, Florida E, Gil J, Pombo CM, Zalvide J. The cerebral cavernous malformation 3 gene is necessary for senescence induction. *Aging Cell* 2015;14:274-83.
176. Fu X, Zhang W, Su Y, Lu L, Wang D, Wang H. MicroRNA-103 suppresses tumor cell proliferation by targeting PDCD10 in prostate cancer. *Prostate* 2016;76:543-51.

Review

Open Access



The endothelial progenitor cell dysfunction in hypertension: the diagnostic and predictive values

Alexander E Berezin

Department of Internal Medicine, State Medical University, Zaporozhye 69035, Ukraine.

Correspondence to: Prof. Alexander E Berezin, Department of Internal Medicine, State Medical University, Mayakovsky av., Zaporozhye 69035, Ukraine. E-mail: aeberezin@gmail.com; dr_berezin@mail.ru

How to cite this article: Berezin AE. The endothelial progenitor cell dysfunction in hypertension: the diagnostic and predictive values. *Vessel Plus* 2018;2:22. <http://dx.doi.org/10.20517/2574-1209.2018.23>

Received: 28 Apr 2018 **First Decision:** 23 Jul 2018 **Revised:** 25 Jul 2018 **Accepted:** 2 Aug 2018 **Published:** 13 Sep 2018

Science Editor: Alexander D. Verin **Copy Editor:** Huan-Liang Wu **Production Editor:** Zhong-Yu Guo

Abstract

Hypertension remains a leading risk factor of cardiovascular (CV) events and disease in the general population. The prevalence of hypertension is present in developed and developing countries and according to various assessments may fluctuate between 30% to 90% with considerable regional differences. Hypertension influences CV risk and mortality rate through target organ damages that affect vasculature particularly endothelium. Endothelial dysfunction is an independent risk factor of CV complications. Recent studies have shown that a decreased number and altered function of circulating endothelial progenitor cells (EPCs) may be a powerful marker of endothelial dysfunction with possible predictive value. The aim of this review is to update the current evidence of the role of endothelial progenitor cell dysfunction in impaired vascular reparation and CV risk in hypertension. The review discusses the interrelation between EPC dysfunction and traditional CV risk factors, such as hypertension, dyslipidemia, obesity, prediabetes/diabetes mellitus. It has been speculated that EPC dysfunction could appear prior to hypertension and represents an appropriate hypertensive phenotype with exaggerated CV risk. However, the predictive value of EPC dysfunction in hypertensive patients is not established and requires to be investigated in large clinical controlled trials.

Keywords: Hypertension, endothelial dysfunction, endothelial progenitor cells, vascular reparation, biomarkers

INTRODUCTION

Hypertension is now recognized as an established powerful risk factor for cardiovascular disease (CVD) that is a primary cause of mortality and disability worldwide^[1]. The prevalence of hypertension relates closely to age, sex, urban/rural location, lowered income in certain countries, affordability of insurance, type of nutrition, and comorbidities, such as diabetes mellitus, obesity, and chronic kidney disease^[2,3]. The prevalence of the disease in developing and developed countries varies and fluctuates from 30% to 90% confirming



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



considerable regional differences in the prevalence of hypertension^[4,5]. Moreover, there is evidence that up to 90% of all hypertensive patients have uncontrolled blood pressure (BP) determined as > 140 mmHg and > 90 mmHg for office systolic and diastolic BP respectively^[5,6]. Recent observational and clinical studies have shown that target organ damages were more frequently detected in uncontrolled hypertensive individuals and resistant hypertensive patients than those who had fully controlled hypertension^[5,7]. Nevertheless, it was found that the endothelial dysfunction may be a result of direct vasculature damage due to the effect of cardiovascular (CV) risk factors and the natural evolution of hypertension^[7,8]. Additionally, endothelial dysfunction is strongly associated with not only a very high CV risk profile, but a higher rate of CV events in hypertensive individuals^[8]. Although endothelial dysfunction emerges as a primary cause of essential hypertension and its evolution understanding the whole components involving in the pathophysiology of regulation of vascular structure and function become of great importance for the prediction of target organ damage and risk stratification^[9]. The investigation of clinical significance of the role of endothelial dysfunction in developing and progression of essential hypertension could open novel perspectives for targeting the treatment of hypertension.

The pathophysiological mechanisms of endothelial dysfunction development involve several factors contributing to various causes of loss of normal vascular function and structure. The experimental models of essential hypertension have confirmed that genetic/epigenetic factors interacting with traditional (smoking, dyslipidemia, insulin resistance, diabetes mellitus) and specific (hyperuricemia, vitamin D deficiency, elevated homocysteine levels, inflammation, oxidative stress, hypercoagulation) CV risk factors and surrounding environment may regulate vascular reparation through synthesizing and release of various spectra of vasoactive substances including nitric oxide (NO), involving cell mechanisms and attenuation of synthesis of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS)^[10-14]. Consequently, vascular resident cells and circulating progenitor cells with different origin and phenotypes (mononuclear and endothelial progenitor cells) may play a pivotal role in vascular repair, improving vascular integrity and function^[15]. Finally, the imbalance between vasculature damage and vascular reparation capability could be considered as an independent factor contributing to endothelial function and vasculature structure that are central players in vascular tone regulation and target organ damage prevention^[16-19]. The aim of this review is to update the current evidence of the role of endothelial progenitor cell dysfunction in impaired vascular reparation and CV risk in hypertension.

DEFINITION OF ENDOTHELIAL PROGENITOR CELLS

The pioneer work provided by Asahara *et al.*^[20] (1997) first reported about existing populations of circulating cells with impressive angiogenic capacities. Using animal models of ischemia the authors found sites of active angiogenesis with incorporation into its putative progenitor endothelial cells or angioblasts, which were further isolated from peripheral blood of animals and humans by magnetic bead selection on the basis of cell surface antigen expression^[20,21]. *In vitro* these cells expanded and committed to form an endothelial lineage in colonies in culture, and *in vivo* after transplantation these cells have been incorporated into cores of active neovascularization demonstrating an ability to attenuate angiogenesis and vascular function through differentiation into mature microvascular endothelial cells^[22,23]. It has been postulated that these cells called endothelial progenitor cells (EPCs) may origin from bone marrow stem cells and human umbilical cord blood and that they may mobilize and migrate from bone marrow, differentiate into mature endothelial cells and probably smooth muscle cells of vessels, as well as synthesize and release a wide range of active molecules and growth factors [vascular endothelial growth factor (VEGF), fibroblast growth factor, granulocyte-macrophage colony-stimulating factor] that modulate vasculogenesis and improve vascular integrity^[24,25].

DETERMINATION OF IMMUNE PHENOTYPES AND FUNCTIONALITIES OF EPCS

Early attempts to verify the population of EPCs were based on determination of molecular characteristics especially when flow cytometry technique was being implemented. To improve the understanding in im-

mune phenotypes of EPCs, a consensus on common strong endothelial markers was initiated, which are expressed on the surface of these precursors. The cells may express cell markers of endothelial cells and their precursors, such as CD31, CD144, KDR (CD309, VEGF receptor-2), and CD133, but in absence of CD45^[26,27]. The CD45(-) cells, which are detected and isolated based on single or combined expression of CD34, CD133 and CD309, were referred to as EPCs^[26]. Three antigens, i.e., CD133, CD34, and CD309, are constitutively determined on the surfaces of EPCs, while after differentiation EPCs lose CD133 antigen and begin to be positively present on CD31, vascular endothelial cadherin, endothelial NO synthase (eNOS) and von Willebrand factor. However, EPCs expressed sufficient distinction in self-renewal ability that was determined using colony-forming method^[28]. It has been speculated that a specific property may allow a more precise definition of EPCs, because the majority of antigens used as molecular markers were commonly shared with the various cells from hematopoietic lineage^[29]. Indeed, some fractions of EPCs with high proliferating capacity could contain non-hematopoietic population of CD45(-), CD31(+) cells with additional presentation CD117(+). Taken together, the combination of CD45(-), CD31(+), CD 133(+), CD34(+) and CD309(+) or von Willebrand factor (+) probably allows for getting reliable purification of progenitor endothelial subsets from mononuclear cells with presentation of endothelial markers.

Depending on the ability to appear in fibronectin coated dish all EPCs were divided into early outgrowth (5-7 days after fibronectin plating) or late outgrowth endothelial cells (7-10 days after fibronectin plating). Interestingly, the late outgrowth precursors originated from peripheral blood mononuclea cells and ex vivo demonstrated immune phenotypes (CD31⁺, CD146⁺, CD105⁺, and CD309⁺) and functional properties suitable as mature endothelial cells. Indeed, two distinguished populations of late outgrowth progenitor cells based on differential expression of the cell surface marker CD34 have been identified. The population of EPCs with co-expression of CD34 antigen additionally to CD31(+), CD146(+), CD105(+), and CD309(+) exhibited higher proliferative capability to CD34(-) EPCs. Therefore, CD34(+) EPCs had reproduced tubes and colony shaping in the single-cell colony-formation investigation as well as they responded to angiogenic growth factors^[28]. In contrast, CD34(-) cells had limited capability to reproduce colonies or even had none of these properties *in vitro*. There is evidence that the absence of CD34(+) EPCs in the colony leads to cultures collapsing within one or two passage and confirming a strong hierarchy in self-renewal of EPCs, which may be an extremely important functional feature of precursors^[28]. Thus, late outgrowth precursors may differentiate into functionally mature endothelial cells and progenitor-like angiogenesis-promoting cells (CD34⁺ EPCs)^[29]. In fact, the colonies are shaped by heterogeneous populations of EPCs that express different markers suitable for endothelial and monocyte lineage. Finally, CD34(+) EPCs mediate marginally to angiogenesis and neovascularization by differentiation, although they are potent triggers and powerful regulators of pro-inflammatory response and remodeling of vascular wall^[29,30]. Other candidates for cell surface molecule markers and genes' signatures to a functional determination based surely on self-renewal hierarchy of EPCs are actively investigated. Thus, there are several populations of endothelial progenitors with different proliferative activity and angiopoietic capabilities that can represent markers of endothelial reparation/injury and endothelial dysfunction.

THE MECHANISMS OF ANGIOPOETIC ACTIVITY OF EPCS

Previous preclinical and clinical studies have shown that several factors, such as VEGF, stromal cell-derived factor-1 (SDF-1) may enhance differentiation, proliferation, adhesion, and incorporation into endothelial cell monolayers of EPCs through VEGF/eNOS-related and chemokine SDF-1 Akt-related pathways^[31-33]. Moreover, there is evidence that the blockade of VEGF or eNOS prevented all VEGF-induced and SDF-1 α -induced effects toward EPCs including ischemia-induced proliferation, vasculogenesis and angiogenesis^[33,34]. In this context, the transfer of genes (cdkn1c and il33) that are coding p57 and an endothelial cell cycle inhibitor may regulate self-renewal capacity of EPCs. Recent studies confirmed that increased expression of these target genes of Notch signaling play a pivotal role in regulation of cell cycling with EPCs and stem cells^[35,36]. Thus, EPCs contribute their angiopoietic effect through direct differentiation into mature endothelial cells and probably into cells with other phenotypes like smooth muscle cells^[37].

Additionally, there are data that confirmed that circulating EPCs originated from bone-marrow stem cells and peripheral mononuclear cells may directly contact to the injury sites of endothelium and influence them to induce local proliferation of residence cells, as well as promote auto- and paracrine effects. Indeed, acting as circulating EPCs enables to exhibit auto- and paracrine influences of hematopoietic cells including mononuclear cells, as well as local residence cells with high proliferative capacity^[37-39]. In this way, EPCs release micro vesicles with regulatory proteins, peptides, micro-RNAs, growth factors (VEGF), and hormones (aldosterone) and secrete a wide spectrum of active molecules (E-selectin, P-selectin) directly into the circulation^[40,41]. Both secretome and proteome of EPCs were recognized as central players in the reparation of the endothelial layer and restoring of vascular function^[42]. Indeed, EPCs turn over mature endothelial cells and immediately become a target for inflammatory cytokines, factors of coagulation, hormones (catecholamines, aldosterone, angiotensin-II, endothelin-1), active molecules that have the ability to induce apoptosis. Apoptotic endothelial cells switch over to the secretion of micro vesicles to nanoparticles, which contain chromatin. Apoptotic-related nanoparticles are produced by mature endothelial cells and directly lead to injury of the endothelium and mediate inflammation and coagulation, but activated mature endothelial cells realize micro vesicles with angiopoietic properties that enhance vascular reparation and attenuate endothelial function^[42,43]. Thus, EPCs with angiopoietic phenotypes may interact with vasculature in direct and indirect ways depending on the pre-existing ability of EPCs to proliferation, differentiation and survival as well as the spectrum of co-regulatory factors, which mainly alter maturation and commitment of stem cells/progenitor precursors and impair EPC mobilization.

THE EPCS DYSFUNCTION: RELATION TO CARDIOVASCULAR RISK FACTORS

EPC dysfunction was described as a phenomenon strongly associated with decreased number and/or weak function of circulating precursors^[42]. Deficiency of circulating number of EPCs and weak function of them are found in senescence, atherosclerosis, stable coronary artery disease, myocardial infarction/acute coronary syndrome, heart failure, atrial fibrillation/flutter, chronic kidney disease, morbid obesity, diabetes mellitus, hyperthyroidism, insulin resistance^[15,19]. Recent clinical studies have shown that EPCs strongly related to metabolic comorbidities (hyperuricemia, dyslipidemia, hyperglycemia) and appeared to result in epigenetic modification of these cells^[43-46]. Although simple count of circulating number of EPCs is not superior to the assay of colony-shaping ability of EPCs in the context of association with CV mortality rate, the number of circulating EPC has shown to be negatively correlated with CV risk factors and vascular function and to predict CV disease/events independently of both conventional and non-traditional CV risk factors^[47-49]. Moreover, the loss of the ability to release micro vesicles from EPCs can be a mechanism of worsening of glomerular function due to microvascular inflammation and endothelial dysfunction in chronic kidney disease^[50].

There is a suggestion that EPC dysfunction may appear prior to CVD without close association with CV risk factors, although conflicting data were obtained by numerous investigators. It has been found that the metabolic memory phenomenon in diabetics and pre-diabetics could be a result of EPC dysfunction^[44]. Variability of glycated hemoglobin levels at early stages of diabetes mellitus development and insulin resistance are well established factors contributing to lowered numbers and poor function of circulating EPCs^[19,40,51]. In contrast, there are studies that reported unchanged or increased numbers of circulating EPCs in diabetics, in patients with increased serum uric acid and in individuals with hypertriglyceridemia in comparison with healthy age-matched volunteers^[52-54]. Previous studies reported that in healthy individuals the gender had no essential effect on the number of EPCs and that there was no effect on the number of EPCs factors such as: smoking, physical activity and alcohol consumption^[55]. Kulwas *et al.*^[56] reported that an increased number of circulating EPCs was found in patients with diabetic foot syndrome (DFS), but in diabetics without foot complications and healthy volunteers, the circulating number of EPCs was similar. In contrast, subjects with DFS, even with healed ulceration, had fewer EPCs and more CD45-CD29(+)CD90(+) mesenchymal stem cells when compared with the T2DM without DFS^[57]. However, numerous metabolic

risk factors and diseases including diabetes mellitus themselves did not affect bone-marrow mobilization of precursors, but they altered the EPC profile and decreased the circulating number of late outgrowth EPCs with high proliferative capability that sufficiently worsened the reparative ability of vasculature^[54]. On the other hand, local tissue ischemia is thought to be the strongest inductor of EPC mobilization, angiogenesis and reparation, but data mentioned above did not confirm that several conditions, such as DFS, obligatory accompany intensive angiogenesis are associated with increased circulating number of angiogenic EPCs and high concentration of VEGF and fibroblast growth factor. Additionally, it has been found that decreased soluble receptors for VEGF due to inactivation of VEGF, may correspond to decrease in the number of EPCs in the circulation^[56-58]. Although EPCs are crucial to vasculogenesis and angiogenesis during ischemic neovascularization the controversial findings regarding both number and function of EPCs in peripheral blood require to be explained in details. Probably, EPCs are under tight control of several epigenetic mechanisms, i.e., DNA methylation, DNA phosphorylation, micro-RNA-related translation, etc., which mediate a modification of EPC function, decrease the number of EPCs and suppress their survival. Moreover, it has been postulated that the worsening of EPCs function is attributed to the time period during which EPCs have been in contact with CV risk factors and epigenetic stimuli^[59]. As a result, the ability of the vasculature to repair itself is dramatically lowered. All these facts explain that even newly differentiated mature endothelial cells from precursors did not completely restore vascular integrity and function. Finally, endothelial dysfunction tends to persist and damage target organs leading to increased CV risk.

The next controversies affect the fact of unpredictable changes of number of EPCs in the peripheral blood in acute situations, such as acute heart failure (AHF) or acute coronary syndrome (ACS), or cardiac/vascular surgery procedures^[60,61]. Some investigators reported that the number of circulating EPCs in AHF or ACS/myocardial infarction was increased, but on the other hand there were reports of a lowered or an unchanged number of EPCs in humans within hours or days after manifestation of the events^[62-66]. Also, the number of urgently recruited bone-marrow precursors can be limited due to previous expenditures for tissue reparations, which occurred prior to CV events, i.e., traumas and infections. On the other hand, ischemia/hypoxia, several spectra of pro-inflammatory cytokines [tumor necrosis factor-alpha, interleukin (IL)-2, IL-4, IL-6, C-reactive protein], chemokines (E selectin), active molecules (intercellular adhesion molecule-1), matrix metalloproteinases (MMPs) and ROS that accompany atherothrombotic states, AHF, and release due progression of them are established factors for mobilization, proliferation and differentiation of EPC^[66-69]. These factors impair hypoxia inducible factor-1 alpha (HIF)/p-Akt/p-eNOS/MMP-9 signaling system in stem cells and circulating precursors that lead to delayed and reduced EPC mobilization from bone-marrow and probably from peripheral tissues^[70,71]. The final result for changes of the number of circulating of EPCs depends on a balance between the ability of stimuli to immediately mobilize precursors from bone-marrow/tissues and the pool of putative precursor cells^[72-74]. Interestingly, improved functions of EPCs in hypertensive patients can be attained with the implementation of renin-angiotensin system blockers, such as angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, direct renin inhibitors, and probably mineralocorticoid antagonists acting via intracellular signaling mechanisms related to SDF-1/CXC chemokine receptor four (CXCR4) and Janus kinase-2/CXCR4 axis^[75-78]. Thus, EPC dysfunction is a novel pathophysiological mechanism underlying defective neovascularization and vascular/tissues reparation in CVD.

THE DIAGNOSTIC AND PROGNOSTIC VALUES OF EPCS IN HYPERTENSION

Endothelial dysfunction is expressed by an increase of the endothelin-1 level and decreased bioavailability of nitric oxide associated with altered pro-coagulative, pro-inflammatory, and pro-vasoconstrictive phenotypes, and it is an early event in CVD that frequently precedes CV complications^[79]. It has been reported that EPC colony number was significantly and inversely correlated with systolic and diastolic BP in subjects with hypertension^[78]. A lowered number of EPCs in circulation was found at an early stage and represents a reliable predictor of endothelial dysfunction as well as a marker of target organ damages^[80-82]. Indeed,

decreased EPC levels may contribute to the pathophysiology of albuminuria or proteinuria in hypertensive patients with nephropathy. In patients with essential hypertension with ECG evidence of left ventricular hypertrophy (LVH), the circulating levels of EPCs were lowered to those who did not have LVH^[81]. In hypertensive individuals with end-stage renal disease, EPC number and function were significantly reduced and inversely associated with CV risk^[82]. Moreover, the levels of EPCs in the peripheral blood of women with pregnancy-induced hypertension were significantly lower compared with those of control pregnant women with normal BP level^[83]. Numerous investigators reported that a lowered number of EPC and an altered EPC function related strongly not only with brachial BP levels, but the increased central aortic systolic pressure, aortic augmentation index, and pulse wave velocity as a marker of arterial stiffness. It altered brachial artery flow-mediated dilatation as a marker of endothelial dysfunction and left ventricular (LV) twisting^[77,84-87]. Additionally, patients with newly diagnosed essential hypertension had increased proportions of various CD34(+) populations of EPCs and CD34(+)VEGFR2(+) c-Kit(+) EPCs^[88]. Moreover, CD34(+) EPCs seem to be influenced by angiotensin II and KLOTHO encoding gene polymorphism^[88,89]. It has been suggested that the increased proportions of CD34⁺ EPCs in the circulation may be a compensatory mechanism for increased endothelial damage and microvasculature inflammation in hypertension^[88].

However, the predictive role of EPC dysfunction in hypertensive individuals remains controversial. Although there is a relation between both lowered level of circulating EPCs and weak EPC function *in vitro* and an increased CV risk, there is not quite enough evidence regarding independent prognostication of EPC dysfunction in the hypertensive population^[90]. In contrast, in patients with established CAD, myocardial infarction, heart failure, cardiomyopathies, the EPC dysfunction was determined to be a predictor of fatal clinical outcomes^[91,92]. In fact, EPC dysfunction associates with CV risk and frequently associates with a number of CV risk factors including hypertension, dyslipidemia, abdominal obesity, prediabetes/diabetes mellitus. Whether EPC dysfunction appears prior to hypertension or it shapes the hypertensive phenotype without corresponding to other CV risk factors is not fully clear^[93]. In this context, the independent predictive value of EPC dysfunction in hypertensive patients requires to be investigated in details in large clinical controlled trials.

CONCLUSION

The altered endothelial function in hypertensive patients is strongly associated with a decreased number and a reduced function of EPCs and may be determined even at a pre-hypertensive stage of the evolution of the disease. There is much evidence that EPCs play a pivotal role in maintaining the vascular integrity and reparation preventing alteration of the endothelium and manifestation of endothelial dysfunction. The EPCs dysfunction is established independent of CV risk factors in the general population and in patients with known CV disease. In this context, a number of circulating EPCs can be recognized as potential diagnostic and prognostic biomarkers in hypertensive individuals, while there is not quite enough evidence from large clinical trials.

DECLARATIONS

Authors' contributions

Berezin AE solely responsible for the paper

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

The author declares that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Diederichs C, Neuhauser H. Regional variations in hypertension prevalence and management in Germany: results from the German Health Interview and Examination Survey (DEGS1). *J Hypertens* 2014;32:1405-13.
2. de Burgos-Lunar C, Jiménez-García R, Salinero-Fort MA, Gómez-Campelo P, Gil A, Abánades-Herranz JC, Cárdenas-Valladolid J, del Cura-González I. Trends in hypertension prevalence, awareness, treatment and control in an adult type 2 diabetes Spanish population between 2003 and 2009. *PLoS One* 2014;9:e86713.
3. Pereira M, Lunet N, Azevedo A, Barros H. Differences in prevalence, awareness, treatment and control of hypertension between developing and developed countries. *J Hypertens* 2009;27:963-75.
4. Basu S, Millett C. Social epidemiology of hypertension in middle-income countries: determinants of prevalence, diagnosis, treatment, and control in the WHO SAGE study. *Hypertension* 2013;62:18-26.
5. Brambilla G, Bombelli M, Seravalle G, Cifkova R, Laurent S, Narkiewicz K, Facchetti R, Redon J, Mancia G, Grassi G. Prevalence and clinical characteristics of patients with true resistant hypertension in central and Eastern Europe: data from the BP-CARE study. *J Hypertens* 2013;31:2018-24.
6. Egan BM, Zhao Y, Axon RN. US trends in prevalence, awareness, treatment, and control of hypertension, 1988-2008. *JAMA* 2010;303:2043-50.
7. Gkaliagkousi E, Gavrilaki E, Triantafyllou A, Douma S. Clinical significance of endothelial dysfunction in essential hypertension. *Curr Hypertens Rep* 2015;17:85.
8. Bernatova I. Endothelial dysfunction in experimental models of arterial hypertension: cause or consequence? *Biomed Res Int* 2014;2014:598271.
9. Brandes RP. Endothelial dysfunction and hypertension. *Hypertension* 2014;64:924-8.
10. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. *Biomed Res Int* 2014;2014:406960.
11. de Faria AP, Fontana V, Modolo R, Barbaro NR, Sabbatini AR, Pansani IF, Ferreira-Melo SE, Moreno H. Plasma 8-isoprostane levels are associated with endothelial dysfunction in resistant hypertension. *Clin Chim Acta* 2014;433:179-83.
12. Choi YJ, Yoon Y, Lee KY, Hien TT, Kang KW, Kim KC, Lee J, Lee MY, Lee SM, Kang DH, Lee BH. Uric acid induces endothelial dysfunction by vascular insulin resistance associated with the impairment of nitric oxide synthesis. *FASEB J* 2014;28:3197-204.
13. Wong WT, Tian XY, Huang Y. Endothelial dysfunction in diabetes and hypertension: cross talk in RAS, BMP4, and ROS-dependent COX-2-derived prostanoids. *J Cardiovasc Pharmacol* 2013;61:204-14.
14. Martynowicz H, Janus A, Nowacki D, Mazur G. The role of chemokines in hypertension. *Adv Clin Exp Med* 2014;23:319-25.
15. Berezin AE. Preconditioned endothelial progenitor cells as biomarker of vascular repair? *Insights in Biomed* 2017;2:4-7.
16. Shan Y, Lin J, Xu P, Zeng M, Lin H, Yan H. The combined effect of hypertension and type 2 diabetes mellitus on aortic stiffness and endothelial dysfunction: an integrated study with high-resolution MRI. *Magn Reson Imaging* 2014;32:211-6.
17. Jamwal S, Sharma S. Vascular endothelium dysfunction: a conservative target in metabolic disorders. *Inflamm Res* 2018;67:391-405.
18. Triches CB, Mayer S, Quinto BMR, Batista MC, Zanella MT. Association of endothelial dysfunction with cardiovascular risk factors and new-onset diabetes mellitus in patients with hypertension. *J Clin Hypertens (Greenwich)* 2018; doi: 10.1111/jch.13269.
19. Berezin AE. Endothelial progenitor cells dysfunction and impaired tissue repair: the missed link in diabetes mellitus development. *Diabetes Metab Syndr* 2017;11:215-20.
20. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964-6.
21. Asahara T. Endothelial progenitor cells for vascular medicine. *Yakugaku Zasshi* 2007;127:841-5. [in Japanese]
22. Patel J, Donovan P, Khosrotehrani K. Concise review: functional definition of endothelial progenitor cells: a molecular perspective. *Stem Cells Transl Med* 2016;5:1302-6.
23. Yi C, Xia W, Zheng Y, Zhang L, Shu M, Liang J, Han Y, Guo S. Transplantation of endothelial progenitor cells transferred by vascular endothelial growth factor gene for vascular regeneration of ischemic flaps. *J Surg Res* 2006;135:100-6.

24. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999;85:221-8.
25. Murohara T. Angiogenesis and vasculogenesis for therapeutic neovascularization. *Nagoya J Med Sci* 2003;66:1-7.
26. Charlene A. McQueen, editor-in-chief. *Comprehensive toxicology*. Amsterdam: Elsevier Science; 2018. p. 130-82.
27. Böldicke T, Tesar M, Griesel C, Rohde M, Gröne HJ, Waltenberger J, Kollet O, Lapidot T, Yayon A, Weich H. Anti-VEGFR-2 scFvs for cell isolation. Single-chain antibodies recognizing the human vascular endothelial growth factor receptor-2 (VEGFR-2/flk-1) on the surface of primary endothelial cells and preselected CD34+ cells from cord blood. *Stem Cells* 2001;19:24-36.
28. Ferreras C, Cole CL, Urban K, Jayson GC, Avizienyte E. Segregation of late outgrowth endothelial cells into functional endothelial CD34- and progenitor-like CD34+ cell populations. *Angiogenesis* 2015;18:47-68.
29. Popa ER, Harmsen MC, Tio RA, van der Strate BW, Brouwer LA, Schipper M, Koerts J, De Jongste MJ, Hazenberg A, Hendriks M, van Luyn MJ. Circulating CD34+ progenitor cells modulate host angiogenesis and inflammation in vivo. *J Mol Cell Cardiol* 2006;41:86-96.
30. Xu QB. Endothelial progenitor cells in angiogenesis. *Sheng Li Xue Bao* 2005;57:1-6.
31. Hristov M, Erl W, Weber PC. Endothelial progenitor cells: mobilization, differentiation, and homing. *Arterioscler Thromb Vasc Biol* 2003;23:1185-9.
32. Iwaguro H, Yamaguchi J, Kalka C, Murasawa S, Masuda H, Hayashi S, Silver M, Li T, Isner JM, Asahara T. Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. *Circulation* 2002;105:732-8.
33. Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, Murasawa S, Bosch-Marce M, Masuda H, Losordo DW, Isner JM, Asahara T. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation* 2003;107:1322-8.
34. Hiasa K, Ishibashi M, Ohtani K, Inoue S, Zhao Q, Kitamoto S, Sata M, Ichiki T, Takeshita A, Egashira K. Gene transfer of stromal cell-derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: next-generation chemokine therapy for therapeutic neovascularization. *Circulation* 2004;109:2454-61.
35. Pasut A, Chang NC, Gurriaran-Rodriguez U, Faulkes S, Yin H, Lacaria M, Ming H, Rudnicki MA. Notch signaling rescues loss of satellite cells lacking Pax7 and promotes brown adipogenic differentiation. *Cell Rep* 2016;16:333-43.
36. Kachamakova-Trojanowska N, Bukowska-Strakova K, Zukowska M, Dulak J, Jozkowicz A. The real face of endothelial progenitor cells - circulating angiogenic cells as endothelial prognostic marker? *Pharmacol Rep* 2015;67:793-802.
37. Fang S, Wei J, Pentimikko N, Leinonen H, Salven P. Generation of functional blood vessels from a single c-kit+ adult vascular endothelial stem cell. *PLoS Biol* 2012;10:e1001407.
38. Ingram DA, Mead LE, Moore DB, Woodard W, Fenoglio A, Yoder MC. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood* 2005;105:2783-6.
39. Yoder MC. Is endothelium the origin of endothelial progenitor cells? *Arterioscler Thromb Vasc Biol* 2010;30:1094-103.
40. Berezin AE. Biomarkers for cardiovascular risk in patients with diabetes. *Heart* 2016;102:1939-41.
41. Qu K, Wang Z, Lin XL, Zhang K, He XL, Zhang H. MicroRNAs: key regulators of endothelial progenitor cell functions. *Clin Chim Acta* 2015;448:65-73.
42. Berezin AE, Kremzer AA, Berezina TA, Martovitskaya YV. The pattern of circulating microparticles in patients with diabetes mellitus with asymptomatic atherosclerosis. *Acta Clin Belg* 2016;71:38-45.
43. Berezin AE, Kremzer AA, Berezina TA, Martovitskaya YV, Gronenko EA. Data regarding association between serum osteoprotegerin level, numerous of circulating endothelial-derived and mononuclear-derived progenitor cells in patients with metabolic syndrome. *Data Brief* 2016;8:717-22.
44. Berezin AE, Samura TA, Kremzer AA, Berezina TA, Martovitskaya YV, Gromenko EA. An association of serum vistafin level and number of circulating endothelial progenitor cells in type 2 diabetes mellitus patients. *Diabetes Metab Syndr* 2016;10:205-12.
45. Berezin AE, Kremzer AA. Relationship between circulating endothelial progenitor cells and insulin resistance in non-diabetic patients with ischemic chronic heart failure. *Diabetes Metab Syndr* 2014;8:138-44.
46. Berezin AE, Kremzer AA, Samura TA, Berezina TA, Kruzliak P. Impaired immune phenotype of circulating endothelial-derived microparticles in patients with metabolic syndrome and diabetes mellitus. *J Endocrinol Invest* 2015;38:865-74.
47. Bakogiannis C, Tousoulis D, Androulakis E, Briasoulis A, Papageorgiou N, Vogiatzi G, Kampoli AM, Charakida M, Siasos G, Latsios G, Antoniadis C, Stefanadis C. Circulating endothelial progenitor cells as biomarkers for prediction of cardiovascular outcomes. *Curr Med Chem* 2012;19:2597-604.
48. Berezin AE, Kremzer AA. Circulating endothelial progenitor cells as markers for severity of ischemic chronic heart failure. *J Card Fail* 2014;20:438-47.
49. Berezin AE, Kremzer AA, Samura TA, Berezina TA, Martovitskaya YV. Serum uric Acid predicts declining of circulating proangiogenic mononuclear progenitor cells in chronic heart failure patients. *J Cardiovasc Thorac Res* 2014;6:153-62.
50. Mohandas R, Segal MS. Endothelial progenitor cells and endothelial vesicles - what is the significance for patients with chronic kidney disease? *Blood Purif* 2010;29:158-62.
51. Fadini GP, Sartore S, Baesso I, Lenzi M, Agostini C, Tiengo A, Avogaro A. Endothelial progenitor cells and the diabetic paradox. *Diabetes Care* 2006;29:714-6.
52. Tan K, Lessieur E, Cutler A, Nerone P, Vasanthi A, Asosingh K, Erzurum S, Anand-Apte B. Impaired function of circulating CD34(+) CD45(-) cells in patients with proliferative diabetic retinopathy. *Exp Eye Res* 2010;91:229-37.
53. Lombardo MF, Iacopino P, Cuzzola M, Spiniello E, Garreffa C, Ferrelli F, Coppola A, Saccardi R, Piaggese A, Piro R, Mannino D, Grossi G, Lauro D, Irrera G. Type 2 diabetes mellitus impairs the maturation of endothelial progenitor cells and increases the number of

- circulating endothelial cells in peripheral blood. *Cytometry A* 2012;81:856-64.
54. Fadini GP, Sartore S, Agostini C, Avogaro A. Significance of endothelial progenitor cells in subjects with diabetes. *Diabetes Care* 2007;30:1305-13.
55. Ruszkowska-Ciastek B, Sokup A, Leszcz M, Drela E, Stankowska K, Boinska J, Haor B, Ślusarz R, Lisewska B, Gadomska G, Kubica J, Roś D. The number of circulating endothelial progenitor cells in healthy individuals--effect of some anthropometric and environmental factors (a pilot study). *Adv Med Sci* 2015;60:58-63.
56. Kulwas A, Drela E, Jundziłł W, Góralczyk B, Ruszkowska-Ciastek B, Roś D. Circulating endothelial progenitor cells and angiogenic factors in diabetes complicated diabetic foot and without foot complications. *J Diabetes Complications* 2015;29:686-90.
57. Nowak WN, Borys S, Kusińska K, Bukowska-Strakova K, Witek P, Koblik T, Józkowicz A, Małecki MT, Dulak J. Number of circulating pro-angiogenic cells, growth factor and anti-oxidative gene profiles might be altered in type 2 diabetes with and without diabetic foot syndrome. *J Diabetes Investig* 2014;5:99-107.
58. Drela E, Stankowska K, Kulwas A, Roś D. Endothelial progenitor cells in diabetic foot syndrome. *Adv Clin Exp Med* 2012;21:249-54.
59. Berezin A. Epigenetics in heart failure phenotypes. *BBA Clin* 2016;6:31-7.
60. Chong AY, Lip GYH, Freestone B, Blann AD. Increased circulating endothelial cells in acute heart failure: comparison with von Willebrand factor and soluble E-selectin. *Eur J Heart Fail* 2006;8:167-72.
61. Boos CJ, Lip GYH, Blann AD. Circulating endothelial cells in cardiovascular disease. *J Am Coll Cardiol* 2006;48:1538-47.
62. Dignat-George F, Sampol J. Circulating endothelial cells in vascular disorders: new insights into an old concept. *Eur J Haematol* 2000;65:215-20.
63. Wang C, Li H, Fu P, Zhang S, Xiu R. Serum C-reactive protein and circulating endothelial cells in patients with acute myocardial infarction. *Clin Hemorheol Microcirc* 2005;32:287-96.
64. Makin AJ, Blann AD, Chung NAY, Silverman SH, Lip GYH. Assessment of endothelial 1018 damage in atherosclerotic vascular disease by quantification of circulating endothelial cells. Relationship with von Willebrand factor and tissue factor. *Eur Heart J* 2004;25:371-6.
65. Mutin M, Canavy I, Blann A, Bory M, Sampol J, Dignat-George F. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. *Blood* 1999;93:2951-8.
66. Grochot-Przeczek A, Kotlinowski J, Kozakowska M, Starowicz K, Jagodzinska J, Stachurska A, et al. Heme oxygenase-1 is required for angiogenic function of bone marrow-derived progenitor cells: role in therapeutic revascularization. *Antioxid Redox Signal* 2014;20:1677-92.
67. Yoon C-H, Hur J, Park K-W, Kim J-H, Lee C-S, Oh I-Y, Kim TY, Cho HJ, Kang HJ, Chae IH, Yang HK, Oh BH, Park YB, Kim HS. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. *Circulation* 2005;112:1618-27.
68. Oh IY, Yoon CH, Hur J, Kim JH, Kim TY, Lee CS, Park KW, Chae IH, Oh BH, Park YB, Kim HS. Involvement of E-selectin in recruitment of endothelial progenitor cells and angiogenesis in ischemic muscle. *Blood* 2007;110:3891-9.
69. Yoon CH, Hur J, Oh IY, Park KW, Kim TY, Shin JH, Kim JH, Lee CS, Chung JK, Park YB, Kim HS. Intercellular adhesion molecule-1 is upregulated in ischemic muscle, which mediates trafficking of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2006;26:1066-72.
70. Ling L, Shen Y, Wang K, Jiang C, Fang C, Ferro A, Kang L, Xu B. Worse clinical outcomes in acute myocardial infarction patients with type 2 diabetes mellitus: relevance to impaired endothelial progenitor cells mobilization. *PLoS One* 2012;7:e50739.
71. Hoenig MR, Bianchi C, Sellke FW. Hypoxia inducible factor-1 alpha, endothelial progenitor cells, monocytes, cardiovascular risk, wound healing, cobalt and hydralazine: a unifying hypothesis. *Curr Drug Targets* 2008;9:422-35.
72. Hexum MK, Tian X, Kaufman DS. In vivo evaluation of putative hematopoietic stem cells derived from human pluripotent stem cells. *Methods Mol Biol* 2011;767:433-47.
73. Li B, Cohen A, Hudson TE, Motlagh D, Amrani DL, Duffield JS. Mobilized human hematopoietic stem/progenitor cells promote kidney repair after ischemia/reperfusion injury. *Circulation* 2010;121:2211-20.
74. Kang L, Chen Q, Wang L, Gao L, Meng K, Chen J, Ferro A, Xu B. Decreased mobilization of endothelial progenitor cells contributes to impaired neovascularization in diabetes. *Clin Exp Pharmacol Physiol* 2009;36:e47-56.
75. Li Y, Alatan G, Ge Z, Liu D. Effects of benazepril on functional activity of endothelial progenitor cells from hypertension patients. *Clin Exp Hypertens* 2014;36:545-9.
76. Berezin AE, Kremzer AA, Martovitskaya YV, Samura TA. The effect of angiotensin-2 receptor blocker valsartan on circulating level of endothelial progenitor cells in diabetic patients with asymptomatic coronary artery disease. *Diabetes Metab Syndr* 2015;9:305-9.
77. Raptis AE, Markakis KP, Mazioti MC, Ikonomidis I, Maratou EP, Vlahakos DV, Kotsifaki EE, Voumvourakis AN, Tsirogianni AG, Lambadiari VA, Lekakis JP, Raptis SA, Dimitriadis GD. Effect of aliskiren on circulating endothelial progenitor cells and vascular function in patients with type 2 diabetes and essential hypertension. *Am J Hypertens* 2015;28:22-9.
78. Suzuki R, Fukuda N, Katakawa M, Tsunemi A, Tahira Y, Matsumoto T, Ueno T, Soma M. Effects of an angiotensin II receptor blocker on the impaired function of endothelial progenitor cells in patients with essential hypertension. *Am J Hypertens* 2014;27:695-701.
79. Burger D, Touyz RM. Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. *J Am Soc Hypertens* 2012;6:85-99.
80. Mehta JL, Szwedo J. Circulating endothelial progenitor cells, microparticles and vascular disease. *J Hypertens* 2010;28:1611-3.
81. Lee CW, Huang PH, Huang SS, Leu HB, Huang CC, Wu TC, Chen JW, Lin SJ. Decreased circulating endothelial progenitor cell levels and function in essential hypertensive patients with electrocardiographic left ventricular hypertrophy. *Hypertens Res* 2011;34:999-1003.
82. Lineen JR, Kuliszewski M, Dacouris N, Liao C, Rudenko D, Deva DP, Goldstein M, Leong-Poi H, Wald R, Yan AT, Yuen DA. Early

- outgrowth pro-angiogenic cell number and function do not correlate with left ventricular structure and function in conventional hemodialysis patients: a cross-sectional study. *Can J Kidney Health Dis* 2015;2:25.
83. Heimrath J, Paprocka M, Czekanski A, Ledwozyw A, Kantor A, Dus D. Pregnancy-induced hypertension is accompanied by decreased number of circulating endothelial cells and circulating endothelial progenitor cells. *Arch Immunol Ther Exp (Warsz)* 2014;62:353-6.
 84. Grundmann M, Woywodt A, Kirsch T, Hollwitz B, Oehler K, Erdbruegger U, Haller H, Haubitz M.. Circulating endothelial cells: a marker of vascular damage in patients with preeclampsia. *Am J Obstet Gynecol* 2008;198:e1-5.
 85. Hunting CB, Noort WA, Zwaginga JJ. Circulating endothelial progenitor cells reflect the state of endothelium: vascular injury, repair and neovascularization. *Vox Sang* 2005;88:1-9.
 86. Eirin A, Zhu XY, Ebrahimi B, Krier JD, Riester SM, van Wijnen AJ, Lerman A, Lerman LO. Intrarenal delivery of mesenchymal stem cells and endothelial progenitor cells attenuates hypertensive cardiomyopathy in experimental renovascular hypertension. *Cell Transplant* 2015;24:2041-53.
 87. Marketou ME, Kalyva A, Parthenakis FI, Pontikoglou C, Maragkoudakis S, Kontaraki JE, Chlouverakis G, Zacharis EA, Patrianakos A, Papadaki HA, Vardas PE. Circulating endothelial progenitor cells in hypertensive patients with increased arterial stiffness. *J Clin Hypertens (Greenwich)* 2014;16:295-300.
 88. Skrzypkowska MW, Ryba-Stanisławowska ME, Słomiński B, Gutknecht PG, Siebert J, Myśliwska JM. Association of circulating progenitor cells with angiotensin II in newly diagnosed hypertensive patients. *J Hum Hypertens* 2017;32:46-53.
 89. Skrzypkowska M, Słomiński B, Ryba-Stanisławowska M, Gutknecht P, Siebert J. Circulating CD34+ and CD34+VEGFR2+ progenitor cells are associated with KLOTHO KL-VS polymorphism. *Microvasc Res* 2018; doi: 10.1016/j.mvr.2018.03.014.
 90. King TF, McDermott JH. Endothelial progenitor cells and cardiovascular disease. *J Stem Cells* 2014;9:93-106.
 91. Berezin AE, Kremzer AA, Martovitskaya YV, Samura TA, Berezina TA, Zulli A, Klimas J, Kruzliak P. The utility of biomarker risk prediction score in patients with chronic heart failure. *Int J Clin Exp Med* 2015;8:18255-64.
 92. Berezin AE, Kremzer AA. Analysis of various subsets of circulating mononuclear cells in asymptomatic coronary artery disease. *J Clin Med* 2013;2:32-44.
 93. Giles TD, Sander GE. Endothelial progenitor cells as a biomarker for transitional phenotypes in hypertension. *J Clin Hypertens (Greenwich)* 2015;17:580-1.

Review

Open Access



Vascular approaches and its potential implications in transcatheter aortic valve implantation

Alessandro Sticchi, Edoardo Bressi, Annunziata Nusca, Germano Di Sciascio

Unit of Cardiovascular Science, Campus Bio-Medico University, Rome 00128, Italy.

Correspondence to: Dr. Alessandro Sticchi, Unit of Cardiovascular Science, Campus Bio-Medico University, Rome 00128, Italy. E-mails: sticchialessandro@gmail.com; a.sticchi@unicampus.it

How to cite this article: Sticchi A, Bressi E, Nusca A, Di Sciascio G. Vascular approaches and its potential implications in transcatheter aortic valve implantation. *Vessel Plus* 2018;2:23. <http://dx.doi.org/10.20517/2574-1209.2018.47>

Received: 20 Jun 2018 **First Decision:** 17 Jul 2018 **Revised:** 15 Aug 2018 **Accepted:** 17 Aug 2018 **Published:** 13 Sep 2018

Science Editors: Mario F. L. Gaudino, Cristiano Spadaccio **Copy Editor:** Yuan-Li Wang **Production Editor:** Huan-Liang Wu

Abstract

Transcatheter aortic valve implantation (TAVI) has become in the last years a primary therapeutic tool in order to treat percutaneously severe aortic stenosis in frail patients with multiple comorbidity and a high surgical risk. In almost all cases, the complexity of patients who are candidates for TAVI is also reflected in challenging access sites. This vascular issue addresses the invasive play of constantly evolving devices and resulting complications have a considerable impact on patient morbidity and mortality. For this reason, the study and the choice of the different access site require the attention and experience of the operators to reach the most reliable and feasible vascular approach for a real procedural success.

Keywords: Transcatheter aortic valve implantation, vascular access site, access complications

INTRODUCTION

The transcatheter implantation of the aortic valve is confirmed as a rapidly expanding treatment for severe aortic stenosis in patients with a great risk in terms of mortality to undergo cardiac surgery^[1]. At the beginning of this experience, a considerable size of sheaths and catheters required a real surgical access for the device insertion, later with the advancement of the technique, the percutaneous approach became more and more common because of its less invasive characteristics^[2]. However, these procedures are burdened by a significant risk of vascular complications that represent an element of criticality in transcatheter aortic valve implantation (TAVI) performance and are related with adverse events and mortality^[3]. In fact, these patients are predisposed to high procedural and bleeding risk due to challenging interventions, such as vascular access damage for larger devices but also vessels morbidities and patient frailty^[3]. The TAVI through femoral



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



access is recognized as the least invasive and consequently it makes best use of the sheaths currently available. Other access options include transapical access, transaxillary, transcarotid access or a direct aortic approach. These have specific advantages and disadvantages and are used in those cases where there is no fitting anatomy to ensure safer trans-femoral access (TF)^[4]. In order to achieve procedural success and avoid foreseeable complications, careful planning of the TAVI access site and route is essential. This is achieved through the pre-procedural study of the vessels involved in terms of both caliber and severity of tortuosity and calcification. Therefore, an appropriate high-quality computed tomography (CT) scan with contrast injection is required while arteriography and intravascular ultrasound (IVUS) furnish additional data^[5]. In this review we describe the different vascular access characteristics as well as the most relevant vascular complications.

TF ACCESS

Current experts' consensus strongly supports the use of femoral artery as preferred access site for TAVI^[6,7]. In consideration of the feasibility of using both a surgical and percutaneous approach through the femoral artery, besides the chosen option, the operator's attention must be paid to preserve the vessel from possible damage in its use^[6,7]. Although, reducing the size of the sheath with the new generation of devices, a small proportion of patients still exhibits unfavourable iliofemoral arteries that compel them to adopt different approaches^[4]. The techniques of choice for vessel closure, including percutaneous puncture and preliminary suture, are performed under loco-regional anaesthesia and require open surgical access up to 20% of cases^[6,7]. These conversions from percutaneous insertions into open or hybrid repairs apply vascular surgery closure techniques or percutaneous closure devices that reproduce them^[6,7].

TF access using a surgical cutdown

Since the sheaths of the first devices show large sizes of about 22-French (Fr) to 24-Fr, early TAVI experiences required surgical access to isolate the artery and access it^[7]. Surgical approach with tissue cutting and artery exposure is the first step in such a planned procedure and it allows to examine the vessel by checking the quality of the wall identifying optimal puncture site and at the same time potential vascular injuries can easily be controlled and repaired during one procedure. Alternatively, the procedure is performed by percutaneous puncture and the artery is treated surgically only to provide for closure of the vessel^[8,9]. Reported predictors of vascular complications in TF TAVI include moderate to severe iliofemoral calcified vessels as well as low femoral artery sheath to artery ratio and in this situation performing a surgical approach is preferable^[8,9]. Moreover, there are cases that deserve particular indications to come along surgically as an excessive depth of the vessel, as in individuals suffering from obesity, the presence of grafts or femoral stents or an anatomy that requires a higher puncture than the standard as in the case of high femoral bifurcation^[8,9]. However, the surgical approach is associated with wound complications such as lymphoceles, paraesthesia, and potential wound infection which may delay early patient mobilization, a crucial component of the recovery process for elderly patients who are prime candidates for this procedure^[10,11]. Even if the use of surgery becomes unsustainable due to the incompetence to accomplish a safe femoral access, it is recommended to consider other possible different accesses in order to avoid predictable complications, arising from the hostility of the route, that inevitably impact on morbidity and mortality of the patient^[8,9].

Percutaneous TF Access

The incidence of major vascular complications have decreased significantly from 15.3% in the TF cohort of the Placement of AoRTic TraNscatheter Valve Trial (PARTNER) trial utilizing 22 Fr and 24 Fr introducer sheaths to 4.2% in the Transcatheter Valve Therapy (TVT) Registry. Among them, life-threatening scenarios are the thoracic aortic dissection, access-related vascular injury leading to death or significant blood transfusions, distal embolization from a vascular source requiring surgery or resulting in irreversible end-organ damage^[12]. Thanks to reduced sheath size as well as the improvements in delivery systems and patient selection based on vascular assessment through the CT angiography, TAVI procedure can be done completely

percutaneously in most of the situations^[5]. The puncture for this access must be achieved on the common femoral artery tract bounded by the inferior epigastric artery and the femoral bifurcation, this is pivotal for functioning of the potential suture-mediated closure devices. The most recent and widespread sheaths have a size between 14 Fr and 20 Fr and they require femoral arteries of at least 6-6.5 mm of diameter and deposits of calcium and curves of mild-moderate but not severe degrees. After gaining access, the procedure continues by sliding the device upwards through the entire aorta until the valve plane is reached as previously programmed. Once the position is verified intraoperatively, the deployment of the valve, that is crimped inside the catheter, takes place either by inflating a balloon inside it or by slipping out of the catheter with the self-expanding valve structure^[13]. To optimize this placement, the ventricle is stimulated at a frequency of 180-200 bpm to lower the cardiac motion. After positioning the valve, the device is carefully pulled out, the anticoagulant is reversed and the entry breach is closed. In patients with severe atherosclerotic disease characterized by multiple critical stenoses, large calcium plaques and forbidding angles, the use of femoral access is unnecessarily risky and harmful, and we can affirm the same for aneurysmal pathology. In these cases, it is important to study solutions using other possible ways to accomplish the TAVI^[8]. The advantages of achieving the implantation in a totally percutaneous way include the use of local anaesthesia with the awake and collaborating patient as well as a rapid mobilization of the patient and a reduction in hospitalization time^[14,15]. Closing tools and techniques seem to have favourable outcomes and have been increasingly used; however due to the large caliber of the sheaths, vigilant closure is necessary to be effective^[14,15]. After removing the sheath, the suture threads with the guidance of additional devices such as ProGlide or ProStar as a “pre-closure” technique are definitely bound. In details, with the ProGlide system the femoral artery is punctured and dilated with a standard arterial sheath. Then, the ProGlide device is advanced over the guidewire, and the first suture is deployed slightly angulated at the 10 o'clock position. Guidewire access is maintained, and a second ProGlide device is inserted and deployed at the 2 o'clock position^[16]. Instead, the ProStar device is advanced over the guidewire until the dedicated marker lumen shows blood marking, indicating that the sutures and needles are within the vessel lumen. The needles are pulled back while maintaining the position and entry angle of the ProStar device. The device is retracted, and the guidewire is reinserted through its gate. Then the device is removed, and a dilator is inserted^[17]. At this point, similarly for both the systems, a regular J wire can be exchanged for a stiffer wire, and the large sheath is advanced under fluoroscopy. After conclusion of the procedure the introducer sheath is removed, the guidewire is left in position and then slowly withdrawn until haemostasis is achieved since it represents the possibility to access to the artery in the event of bleeding due to the lack of effectiveness of the closure^[16,17]. If successful, the suture knot is well tight and consolidated. Both the ProStar and ProGlide devices require experience and a check angiography is usually performed by contralateral access and it verifies the absence of injuries in the iliac and femoral vascular structures. The closure of all percutaneous endovascular access is related to a lower incidence of late groin complications and a diminished procedural time^[14,15].

Percutaneous vs. surgical cut-down in TF TAVI

Earlier studies concerning percutaneous endovascular repair of aortic aneurysm indicated comparable feasibility and safety of the complete percutaneous femoral approach compared to surgical cut-down and repair^[18]. More recently, several studies have reported complete percutaneous access and closure using the cross-over technique on the access for device deployment in TF TAVI. They suggest comparable expediency and security of this novel technique as a potential alternative to the surgical approach on arterial access of TF TAVI^[19]. Compared to the surgical approach, the complete percutaneous approach using pre-closure technique for arterial access/closure of large caliber sheaths (22 Fr or 24 Fr) for TAVI is suitable with acceptable safety and potential clinical benefit including reduction in wound infection, lowering bleeding complication, and reduced hospital stay while maintaining similar major vascular event rate^[19]. Nevertheless, the incidence of access site vascular events is still higher in the complete percutaneous group even though this did not appear to affect in-hospital mortality or length of hospital stay if it is recognized in timely manner and appropriately managed^[19,20].

TRANSAPICAL ACCESS

The transapical access must be performed under general anaesthesia and preferably in a hybrid operating room in order to have the surgical and transcatheter equipment ready for use and to the best of the possibilities^[21]. Briefly, a small left anterior mini-thoracotomy is performed in the fifth-sixth intercostal space to expose the apex of the left ventricle after opening the pericardium. In order to visualize and fix the operating window, the pericardium is anchored with several points to the skin. Polypropylene sutures forming a purse concentrically, usually in the number of two, are positioned catching wide portions of cardiac apex tissue. So, via the above-mentioned threads, the operator introduces the sheath into the left ventricle^[22]. After transcatheter placement of the valve, the sheath is retracted and the sutures are tightened during rapid ventricular pacing to maintain low pressure in the final repair time. Transapical access approach is a “surgical” procedure without impairing of the thoracic cage and has the theoretical advantages of stroke prevention avoiding to cross the aortic arch with the device during the delivery^[21,22]. Contrary to the direct aortic approach, transapical approach can be used only with the SAPIEN valve that can be assembled backwards^[23]. This access is advantageous especially in patients in whom the pre-procedure evaluation shows characteristics that determine a high risk of stroke and embolism as in the case of extensive calcifications, porcelain aorta and thrombus finding in the aortic arch^[22].

DIRECT AORTIC ACCESS

Direct transaortic access is one of the possibilities of a transcatheter implantation using a surgical access and it is either performed by a J-shaped mini-sternotomy or a right thoracotomy. The purpose of the operating window is to visualize an initial portion of the ascending aorta for the introduction of the valve delivery device. Depending on the anatomical position of the aorta, one of the two windows is suggested. The right thoracotomy is recommended in cases where the aorta runs in the right hemithorax and superficially near the rib cage, while the mini-sternotomy is chosen in those cases in which the vessel is in a central and deep position^[24]. Once the window is realized, a suture bag is obtained on the designated aorta section for the final closure and, in the center of the managed area, the access to the vessel is first performed with a needle puncture and then with the device. The insertion of the device into the aorta must take into account the type of valve that is implanted, for example for Medtronic CoreValve a distance of at least 6-7 cm from the valve plane is necessary in order to ensure the complete deployment of the skeleton from the sheath. Using this wire as a rail, the device slides up to the aortic valve. In this approach, the position of the valve prosthesis is promoted by the shortness of the path between the access and the valve and by the absence of stress on the device as it happens in the femoral access for the passage in the aortic arch. Favourably, these conditions allow a short learning curve by the operator^[25,26].

After the valve implantation, the device is withdrawn while the previously prepared suture threads are tightened to close the vessel wall, the technique is comparable to decannulation after cardiopulmonary bypass. Finally, the chest wall is restored as usually occurs in cardio-thoracic surgery. The hemisternotomy allows to protect the pleura and to visualize and handle a large portion of aorta in which to select the access point. In case of patients with coronary bypass graft, it is advisable to use the thoracotomy to avoid its course^[25,26]. Finally, the direct aortic approach is suitable if the patient has a horizontal valvular plane or a particularly straight ascending aorta^[25,26].

TRANSUBCLAVIAN

The approach through the subclavian artery takes advantage of a light sedation and local anaesthetic. In order to adequately display the artery, an incision is made between the deltopectoral groove and the pectoralis major. This technique is less invasive than a real surgical support and, at the same time, it overcomes a possible impairment of the peripheral accesses^[27]. The brachial plexus, one of the most important nerve bundles of our body, is located above the subclavian artery and for this reason it is fundamental to protect it from

complications such as artery dissection or pseudoaneurysm^[28]. The artery can be highlighted by a band if it is accomplished through a surgical isolation and, as seen in other accesses, a suture purse is created with a 5-0 polypropylene thread on the front side of the vessel, both for safety and for the final closure. Considering a vessel without calcific disease, a minimum of 6 mm is required for the introduction of a 18Fr sheath while in the case of the presence of a patent's left internal mammary artery graft, the diameter must be at least 7.5 mm to maintain a sustainable downstream flow^[27,28]. The artery is pierced with a standard needle which is inserted in the center of the prepared sutured area, on that insertion point a soft 0.035 wire with a J-tip is introduced and a 6 Fr sheath is inserted on it. A catheter slides over the wire in the ascending aorta, then the soft wire is replaced with a super-stiff Amplatz wire. So, in order to get the 18 Fr sheath through the subclavian artery in the proximal ascending aorta, a series of increasing size of dilators, from 10 Fr, 12 Fr, 14 Fr up to 18 Fr, follow each other at the insertion point^[27,28]. The succeeding steps for the valve deployment adhere to the standard protocol of the procedure. After the sheath is removed, the purse-string suture is tied under direct visualization that would determine if additional sutures are needed. Continuous advances of this technique have recently led to publications of fully percutaneous procedures without surgical cut-down^[27,28]. However, these improvements are not yet able to displace surgical exposure as the routine practice^[29]. Performing a transsubclavian approach may be particularly challenging if the aortic plane forms an angle greater than 30 degrees with the horizontal plane. This complexity derives from the curving that the device must perform and from the consequent tension on it, increasing the difficulty in the proper deployment of the prosthesis^[28,29].

Furthermore, the occlusion of the vessel by the sheath or any damage to neighbouring vascular structures may result in dangerous ischemia, especially when it involves the flow of an internal mammary artery graft with possible myocardial infarction in the most severe conditions^[28,29].

TRANSCAROTID

Among the possible strategies, an approach with a direct and short road to reach the planned position at the aortic valve plane is always recommended. This can also be achieved with the carotid access as well as with the transaortic and transapical approach^[30]. A short path also allows a better support and greater precision in valve distribution than the femoral approach^[30]. The patient is evaluated preliminarily to define the possibility of performing the procedure with the necessary occlusion of one carotid and therefore the maintenance of an adequate cerebral flow through the Willis circle from the contralateral carotid^[31]. This is assessed by placing a shunt in the vessel to measure passive antegrade pressure in the common carotid artery, then the procedure is performed using a small cut as access under local anaesthesia^[31]. The first series with this approach, through the proximal left common carotid artery, is reported by Modine *et al.*^[32] and it counts 12 consecutive cases performed under general anaesthesia. The study does not report peri-procedural events, vascular complications or bleedings, the only adverse event recorded is an embolic transient ischemic attack starting from the contralateral carotid access^[32]. Later, a study publishes a series of cases under local anaesthesia using both balloon expandable and self-expandable valve. Even if the study does not report access site complications, major adverse cardiac event or stroke, two patients died, one during valvuloplasty and one for multiple organ failure. Finally, three patients developed a third-degree atrioventricular block resulting in a definitive pacemaker implantation^[30]. Therefore, we can conclude that this type of approach requires further studies and technical evolutions in order to be able to enter currently as an access option, considering also the possible aesthetic issue that could derive from it.

TRANSCAVAL

Another recently developed approach is the transcaval-aortic access which can be a useful alternative in cases of severe peripheral artery disease. The access puncture is performed at the femoral vein site, reaching the abdominal aorta and creating an artificial cava-aorta fistula. The characteristics of the venous wall allow

an easy insertion of the oversized TAVI sheath. Furthermore, retroperitoneal anatomical structures guarantee a low risk of bleeding that may be due primarily to the arterio-venous shunt, which is repaired with an occluder device at the end of the procedure^[33].

The main challenge of this approach is the making of the cava-aorta connection through the intersection of the two vessel walls at the abdominal level. This can require multiple attempts by less experienced operators to achieve the entry in aorta, the use of haemostatic techniques, transfusions and the treating of leaks due to ineffective repairs. However, the learning curve appears to be short and procedural times are similar to those of femoral access^[34].

Considering the importance of the vascular roadmap to reach the aortic annulus for the valve implantation, the evaluation of the pre-procedure CT study becomes fundamental for the assessment of the calcifications, the diameters and the tortuosity of the vessels. Specifically, the crossing of the cava-aorta requires careful evaluation of the calcification and of secondary branches in order to allow a successful closure of the iatrogenic fistula with an occluder device. Porcelain aorta, previous abdominal endograft and other abnormalities of the aortic wall, represent a contraindication to this access, like an iliac severe tortuosity for the femoral access^[35].

So, this approach is a valid option only in patients with a precise vascular anatomy and for its hidden tricks it should be performed by skilled operators in experienced centers. Finally, it needs further studies, especially about the safety of the method^[36].

COMPARISON AMONG ACCESS SITE

The safety and performance of the TF approach, that have been achieved thanks to the extensive TAVI experience, are the main model of comparison for other access options. The TF approach is to be considered the only completely percutaneous access with the use of the femoral crossover technique and vascular closure devices. Alternative routes are subject to the inability to perform the procedure through the femoral access and they are identified as independent predictors of overall and cardiovascular mortality at 5 years in the real-world population^[37].

TA and transubclavian access are the most used routes in presence of contraindications to the femoral approach and they both show high rates of procedural success. The only difference between them and the TF, although it is not statistically significant, is represented by a greater incidence of potentially fatal bleeding for the trans-apical approach^[38].

Studies reveal a reduction in vascular complication rate using transapical and trans-subclavian access compared to a fully percutaneous femoral access. While this is easily understood for the transapical access due to its inherent features, data for the trans-subclavian are difficult to explain^[39].

A comparison between TF versus transubclavian access produces no considerable differences in 30 days mortality, stroke and new pacemaker implantation. Similarly to the transubclavian access, the trans-axillary compared to the TF approach shows no relevant differences in 30 days mortality but it needs general anaesthesia more frequently and leads to a greater tendency of vascular complications^[40].

On the other hand, the trans-apical approach is a more invasive procedure and involves a higher rate of surgical conversion, longer hospitalization, a higher rate of renal failure and higher mortality rates than the TF route^[41]. Furthermore, no statistically significant differences are reported in stroke incidence and new pacemaker implantation using the trans-apical compared to the femoral access^[39-41].

VASCULAR ACCESS SITE COMPLICATIONS

The most used access for TAVI is the femoral one, which represents 70% of the procedures^[42].

The most common complications performing TAVI are the dissection and rupture of the vessels that represent the vascular route of the valve device, so the vessels of the hip, pelvis, the aorta at the abdominal level and the aortic arch. Moreover, complications on the puncture site such as hematoma and pseudoaneurysm are recurrent^[42]. The importance of complications derives from their proven impact on mortality and hospitalization which disposes to further complications^[43,44]. From the standardization of the Valve Academic Research Consortium (VARC) definitions, the assessment of complications frequency is more accurate. The incidence of major vascular complications is ranged between 10% and 20% while minor vascular complications are around 10.2%^[44].

Since the valve is squeezed into the catheter for the deployment, the greater the diameter of the valve, the greater will be the diameter of the catheter and, therefore, the greater the complications for the trauma of the passage of the device inside vessels^[45]. In particular, a ratio between the diameter of the sheath and that of the femoral artery above 1.05 is considered risky, as well as unskilled operator, center with limited experience, severe calcifications and a sheath size greater than 19 Fr^[44-46].

In fact, in a large European multicentric registry, the prevalent use of the 18 Fr device brings to a considerable decrease of vascular complications, with a 2.9% for the trans-femoral access^[47]. The study shows no difference in vascular complications between the two main devices Medtronic Core Valve versus Edwards SAPIEN XT valves with a rate of 2.8% and 3.3% respectively ($P = 0.66$)^[47]. We are dealing with a further reduction of complications with the introduction of the new 14 Fr systems.

ACCESS AND BLEEDING

The formation of hematomas and the presence of bleeding at the TAVI access site are common complications, estimated between 11% and 18% of patients^[48]. As previously stated, complications and therefore also haemorrhage and hematoma of the access site result in longer hospitalization for their management or for the treatment of further complications such as infections^[47-49].

Following the reduction of the diameter of the devices and the improvement of the technique and experience of the operators and centers, there was a decrease in bleeding rates. A prompt management of bleedings and hematomas is essential, the less severe cases can be solved with adequate compression, possibly under ultrasound guidance. Hematomas of larger size, due to diagnosed late or creating compression of the surrounding tissue, must be treated surgically^[50]. Finally, in case of adequately early diagnosis and after failure of a compressive first treatment, endovascular techniques are used through a contralateral femoral access. These consist in prolonged inflation of a balloon at the level of the hole of vessel wall and in case of failure of this approach, angioplasty is performed with the placement of a covered stent^[51].

CONCLUSION

The indications for TAVI are expanding and the always more skilled operators must guarantee the best approach in terms of safety and procedural success. On the other hand, the devices make important progress on technical aspects, becoming smaller, with easy delivery, accurate positioning and thus realizing the valve implantation in a more feasible and effective way.

Access site is one of the main topic to assure the TAVI safety and in order to choose the best approach for every patient and plan successful strategy of implantation, the preprocedural CT angiography study results are fundamental. Especially in patients with difficult access, the evaluation of risk factors and the experience

in endovascular techniques allow the operators to predict, face promptly and overcome vascular access complications.

Whatever the access and the chosen strategy, a discerning management of the access site in structural procedures, such as TAVI, allows to protect the patient from a high morbidity and mortality.

DECLARATIONS

Authors' contributions

Planning and writing: Sticchi A

Writing: Bressi E

Revision of the manuscript: Nusca A, Di Sciascio G

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Baumgartner H, Falk V, Bax JJ, De Bonis M, Hamm C, Holm PJ, Iung B, Lancellotti P, Lansac E, Rodriguez Muñoz D, Rosenhek R, Sjögren J, Tornos Mas P, Vahanian A, Walther T, Wendler O, Windecker S, Zamorano JL; ESC Scientific Document Group. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. *Eur Heart J*. 2017;38:2739-91.
2. Reardon MJ, Van Mieghem NM, Popma JJ, Kleiman NS, Søndergaard L, Mumtaz M, Adams DH, Deeb GM, Maini B, Gada H, Chetcuti S, Gleason T, Heiser J, Lange R, Merhi W, Oh JK, Olsen PS, Piazza N, Williams M, Windecker S, Yakubov SJ, Grube E, Makkar R, Lee JS, Conte J, Vang E, Nguyen H, Chang Y, Mugglin AS, Serruys PW, Kappetein AP; SURTAVI Investigators. Surgical or transcatheter aortic-valve replacement in intermediate-risk patients. *N Engl J Med* 2017;376:1321-31.
3. Zajarias A, Cribier AG. Outcomes and safety of percutaneous aortic valve replacement. *J Am Coll Cardiol* 2009;53:1829-36.
4. Holmes DR, Jr, Mack MJ. Transcatheter valve therapy: a professional society overview from the American College of Cardiology Foundation and the Society of Thoracic Surgeons. *Ann Thorac Surg* 2011;92:380-9.
5. Willson AB, Webb JG, Labounty TM, Achenbach S, Moss R, Wheeler M, Thompson C, Min JK, Gurvitch R, Norgaard BL, Hague CJ, Toggweiler S, Binder R, Freeman M, Poulter R, Poulsen S, Wood DA, Leipsic J. 3-dimensional aortic annular assessment by multidetector computed tomography predicts moderate or severe paravalvular regurgitation after transcatheter aortic valve replacement: a multicenter retrospective analysis. *J Am Coll Cardiol* 2012;59:1287-94.
6. Gilard M1, Eltchaninoff H, Iung B, Donzeau-Gouge P, Chevreul K, Fajadet J, Leprince P, Leguerrier A, Lievre M, Prat A, Teiger E, Lefevre T, Himbert D, Tchetché D, Carrié D, Albat B, Cribier A, Rioufol G, Sudre A, Blanchard D, Collet F, Dos Santos P, Meneveau N, Tirouvanziam A, Caussin C, Guyon P, Bosch J, Le Breton H, Collart F, Houel R, Delpine S, Souteyrand G, Favereau X, Ohlmann P, Doisy V, Grollier G, Gommeaux A, Claudel JP, Bournon F, Bertrand B, Van Belle E, Laskar M; FRANCE 2 Investigators. Registry of transcatheter aortic-valve implantation in high-risk patients. *N Engl J Med* 2012;366:1705-15.
7. Webb JG, Altwegg L, Boone RH, Cheung A, Ye J, Lichtenstein S, Lee M, Masson JB, Thompson C, Moss R, Carere R, Munt B,

- Nietlispach F, Humphries K. Transcatheter aortic valve implantation: impact on clinical and valve-related outcomes. *Circulation* 2009;119:3009-16.
8. Toggweiler S, Webb JG. Challenges in transcatheter aortic valve implantation. *Swiss Med Wkly* 2012;142:w13735.
 9. Thomas M, Schymik G, Walther T, Himbert D, Lefèvre T, Treede H, Eggebrecht H, Rubino P, Colombo A, Lange R, Schwarz RR, Wendler O. One-year outcomes of cohort 1 in the Edwards SAPIEN Aortic Bioprosthesis European Outcome (SOURCE) registry: the European registry of transcatheter aortic valve implantation using the Edwards SAPIEN valve. *Circulation* 2011;124:425-33.
 10. Tirado-Conte G, Freitas-Ferraz AB, Nombela-Franco L, Jimenez-Quevedo P, Biagioni C, Cuadrado A, Nuñez-Gil I, Salinas P, Gonzalo N, Ferrera C, Vivas D, Higuera J, Viana-Tejedor A, Perez-Vizcayno MJ, Vilacosta I, Escaned J, Fernandez-Ortiz A, Macaya C. Incidence, causes, and impact of in-hospital infections after transcatheter aortic valve implantation. *Am J Cardiol* 2016;118:403-9.
 11. Al-Rashid F, Kahlert P, Selge F, Hildebrandt H, Patsalis PC, Totzeck M, Mummel P, Rassaf T, Jánosi RA. Risk assessment of patients undergoing transfemoral aortic valve implantation upon admission for post-interventional intensive care and surveillance: implications on short- and midterm outcomes. *PLoS One* 2016;11:e0167072.
 12. Leon MB, Smith CR, Mack MJ, Makkar RR, Svensson LG, Kodali SK, Thourani VH, Tuzcu EM, Miller DC, Herrmann HC, Doshi D, Cohen DJ, Pichard AD, Kapadia S, Dewey T, Babaliaros V, Szeto WY, Williams MR, Kereiakes D, Zajarias A, Greason KL, Whisenant BK, Hodson RW, Moses JW, Trento A, Brown DL, Fearon WF, Pibarot P, Hahn RT, Jaber WA, Anderson WN, Alu MC, Webb JG; PARTNER 2 Investigators. Transcatheter or surgical aortic-valve replacement in intermediate-risk patients. *N Engl J Med* 2016;374:1609-20.
 13. van der Boon RM, Van Mieghem NM, Theuns DA, Nuis RJ, Nauta ST, Serruys PW, Jordaens L, van Domburg RT, de Jaegere PP. Pacemaker dependency after transcatheter aortic valve implantation with the self-expanding Medtronic CoreValve System. *Int J Cardiol* 2013;168:1269-73.
 14. Hayashida K, Lefèvre T, Chevalier B, Hovasse T, Romano M, Garot P, Mylotte D, Uribe J, Farge A, Donzeau-Gouge P, Bouvier E, Cormier B, Morice MC. True percutaneous approach for transfemoral aortic valve implantation using the Prostar XL device: impact of learning curve on vascular complications. *JACC Cardiovasc Interv* 2012;5:207-14.
 15. Sharp AS, Michev I, Maisano F, Taramasso M, Godino C, Latib A, Denti P, Dorigo E, Giacomini A, Iaci G, Manca M, Ielasi A, Montorfano M, Alfieri O, Colombo A. A new technique for vascular access management in transcatheter aortic valve implantation. *Catheter Cardiovasc Interv* 2010;75:784-93.
 16. Dimitriadis Z, Scholtz W, Ensminger SM, Piper C, Bitter T, Wiemer M, Vlachoianis M, Börgermann J, Faber L, Horstkotte D, Gummert J, Scholtz S. Impact of sheath diameter of different sheath types on vascular complications and mortality in transfemoral TAVI approaches using the ProGlide closure device. *PLoS One* 2017;12:e0183658.
 17. Hayashida K, Lefèvre T, Chevalier B, Hovasse T, Romano M, Garot P, Mylotte D, Uribe J, Farge A, Donzeau-Gouge P, Bouvier E, Cormier B, Morice MC. True percutaneous approach for transfemoral aortic valve implantation using the Prostar XL device: impact of learning curve on vascular complications. *JACC Cardiovasc Interv* 2012;5:207-14.
 18. Jean-Baptiste E, Hassen-Khodja R, Haudebourg P, Bouillanne PJ, Declémy S, Batt M. Percutaneous closure devices for endovascular repair of infrarenal abdominal aortic aneurysms: a prospective, non-randomized comparative study. *Eur J Vasc Endovasc Surg* 2008; 35: 422-8.
 19. Sharp AS, Michev I, Maisano F, Taramasso M, Godino C, Latib A, Denti P, Dorigo E, Giacomini A, Iaci G, Manca M, Ielasi A, Montorfano M, Alfieri O, Colombo A. A new technique for vascular access management in transcatheter aortic valve implantation. *Catheter Cardiovasc Interv* 2010;75:784-93.
 20. Nakamura M1, Chakravarty T, Jilani H, Doctor N, Dohad S, Fontana G, Cheng W, Makkar RR. Complete percutaneous approach for arterial access in transfemoral transcatheter aortic valve replacement: a comparison with surgical cut-down and closure. *Catheter Cardiovasc Interv* 2014;84:293-300.
 21. Lichtenstein SV, Cheung A, Ye J, Thompson CR, Carere RG, Pasupati S, Webb JG. Transapical transcatheter aortic valve implantation in humans: initial clinical experience. *Circulation* 2006;114:591-6.
 22. Walther T, Möllmann H, van Linden A, Kempfert J. Transcatheter aortic valve implantation transapical: step by step. *Semin Thorac Cardiovasc Surg* 2011;23:55-61.
 23. Walther T, Kempfert J, Dewey T. Transapical aortic valve implantation - procedural steps. In: Serruys PW, Piazza N, Cribier A, Webb JG, Laborde JC, de Jaegere P, editors. *Transcatheter aortic valve implantation. Tips and tricks to avoid failure*. New York: Informa Healthcare USA, Inc.; 2010. p. 207-17.
 24. Bruschi G, De Marco F, Fratto P, Oreglia J, Colombo P, Paino R, Klugmann S, Martinelli L. Direct aortic access through right minithoracotomy for implantation of self-expanding aortic bioprosthetic valves. *J Thorac Cardiovasc Surg* 2010;140:715-7.
 25. Bapat VN, Bruschi G. Transaortic access is the key to success. *EuroIntervention* 2013;9 Suppl:S25-32.
 26. Lardizabal JA, O'Neill BP, Desai HV, Macon CJ, Rodriguez AP, Martinez CA, Alfonso CE, Bilsker MS, Carillo RG, Cohen MG, Heldman AW, O'Neill WW, Williams DB. The transaortic approach for transcatheter aortic valve replacement: initial clinical experience in the United States. *J Am Coll Cardiol* 2013;61:2341-5.
 27. Caceres M, Braud R, Roselli EE. The axillary/subclavian artery access route for transcatheter aortic valve replacement: a systematic review of the literature. *Ann Thorac Surg* 2012;93:1013-8.
 28. van Mieghem NM, Lüthen C, Oei F, Schultz C, Ligthart J, Kappetein AP, de Jaegere PP. Completely percutaneous transcatheter aortic valve implantation through transaxillary route: an evolving concept. *EuroIntervention* 2012;7:1340-2.
 29. Schäfer U, Ho Y, Frerker C, Schewel D, Sanchez-Quintana D, Schofer J, Bjuklic K, Meincke F, Thielsen T, Kreidel F, Kuck KH. Direct percutaneous access technique for transaxillary transcatheter aortic valve implantation: "the Hamburg Sankt Georg approach". *JACC*

- Cardiovasc Interv 2012;5:477-86.
30. Azmoun A, Amabile N, Ramadan R, Ghostine S, Caussin C, Fradi S, Raoux F, Brenot P, Nottin R, Deleuze P. Transcatheter aortic valve implantation through carotid artery access under local anaesthesia. *Eur J Cardiothorac Surg* 2014;46:693-8.
 31. Guyton RA, Block PC, Thourani VH, Lerakis S, Babaliaros V. Carotid artery access for transcatheter aortic valve replacement. *Catheter Cardiovasc Interv* 2013;82:E583-6.
 32. Modine T, Sudre A, Delhay C, Fayad G, Lemesle G, Collet F, Koussa M. Transcatheter aortic valve implantation using the left carotid access: feasibility and early clinical outcomes. *Ann Thorac Surg* 2012;93:1489-94.
 33. Halabi M, Ratnayaka K, Faranesh AZ, Chen MY, Schenke WH, Lederman RJ. Aortic access from the vena cava for large caliber transcatheter cardiovascular interventions: pre-clinical validation. *J Am Coll Cardiol* 2013;61:1745-6.
 34. Greenbaum AB, O'Neill WW, Paone G, Guerrero ME, Wyman JF, Cooper RL, Lederman RJ. Caval-aortic access to allow transcatheter aortic valve replacement in otherwise ineligible patients: initial human experience. *J Am Coll Cardiol* 2014;63:2795-804.
 35. Pascual I, Carro A, Avanzas P, Hernández-Vaquero D, Díaz R, Rozado J, Lorca R, Martín M, Silva J, Moris C. Vascular approaches for transcatheter aortic valve implantation. *J Thorac Dis* 2017;9:S478-87.
 36. Lederman RJ, Babaliaros VC, Greenbaum AB. How to perform transcaval access and closure for transcatheter aortic valve implantation. *Catheter Cardiovasc Interv* 2015;86:1242-54.
 37. Ruparel N, Latib A, Buzzatti N, Giannini F, Figini F, Mangieri A, Regazzoli D, Stella S, Sticchi A, Kawamoto H, Tanaka A, Agricola E, Monaco F, Castiglioni A, Ancona M, Cioni M, Spagnolo P, Chieffo A, Montorfano M, Alfieri O, Colombo A. Long-term outcomes after transcatheter aortic valve implantation from a single high-volume center (The Milan Experience). *Am J Cardiol* 2016;117:813-9.
 38. Ciuca C, Tarantini G, Latib A, Gasparetto V, Savini C, Di Eusanio M, Napodano M, Maisano F, Gerosa G, Sticchi A, Marzocchi A, Alfieri O, Colombo A, Saia F. Trans-subclavian versus transapical access for transcatheter aortic valve implantation: a multicenter study. *Catheter Cardiovasc Interv* 2016;87:332-8.
 39. Garcia DC, Benjo A, Cardoso RN, Macedo FY, Chavez P, Aziz EF, Herzog E, Alam M, de Marchena E. Device stratified comparison among transfemoral, transapical and transsubclavian access for transcatheter aortic valve replacement (TAVR): a meta-analysis. *Int J Cardiol* 2014;172:e318-21.
 40. Chandrasekhar J, Hibbert B, Ruel M, Lam BK, Labinaz M, Glover C. Transfemoral vs non-transfemoral access for transcatheter aortic valve implantation: a systematic review and meta-analysis. *Can J Cardiol* 2015;31:1427-38.
 41. Shults C, Gunter R, Thourani VH. The versatility of transapical access: will it lead to a completely new approach to valvular therapy? *Ann Cardiothorac Surg* 2012;1:220-3.
 42. Stortecky S, Buellesfeld L, Wenaweser P, Windecker S. Transcatheter aortic valve implantation: the procedure. *Heart* 2012;98:iv44-51.
 43. G  n  reux P, Webb JG, Svensson LG, Kodali SK, Satler LF, Fearon WF, Davidson CJ, Eisenhauer AC, Makkar RR, Bergman GW, Babaliaros V, Bavaria JE, Velazquez OC, Williams MR, Hueter I, Xu K, Leon MB; PARTNER Trial Investigators. Vascular complications after transcatheter aortic valve replacement: insights from the PARTNER (Placement of AoRTic TraNscathetER Valve) trial. *J Am Coll Cardiol* 2012;60:1043-52.
 44. Hayashida K, Lef  vre T, Chevalier B, Hovasse T, Romano M, Garot P, Mylotte D, Uribe J, Farge A, Donzeau-Gouge P, Bouvier E, Cormier B, Morice MC. Transfemoral aortic valve implantation new criteria to predict vascular complications. *JACC Cardiovasc Interv* 2011;4:851-8.
 45. Reidy C, Sophocles A, Ramakrishna H, Ghadimi K, Patel PA, Augoustides JG. Challenges after the first decade of transcatheter aortic valve replacement: focus on vascular complications, stroke, and paravalvular leak. *J Cardiothorac Vasc Anesth* 2013;27:184-9.
 46. Toggweiler S, Gurvitch R, Leipsic J, Wood DA, Willson AB, Binder RK, Cheung A, Ye J, Webb JG. Percutaneous aortic valve replacement: vascular outcomes with a fully percutaneous procedure. *J Am Coll Cardiol* 2012;59:113-8.
 47. Di Mario C, Eltchaninoff H, Moat N, Goicolea J, Ussia GP, Kala P, Wenaweser P, Zembala M, Nickenig G, Alegria Barrero E, Snow T, Iung B, Zamorano P, Schuler G, Corti R, Alfieri O, Prendergast B, Ludman P, Windecker S, Sabate M, Gilard M, Witowski A, Danenberg H, Schroeder E, Romeo F, Macaya C, Derumeaux G, Maggioni A, Tavazzi L; Transcatheter Valve Treatment Sentinel Registry (TCVT) Investigators of the EURObservational Research Programme (EORP) of the European Society of Cardiology. The 2011-12 pilot European Sentinel Registry of Transcatheter Aortic Valve Implantation: in-hospital results in 4,571 patients. *EuroIntervention* 2013;8:1362-71.
 48. Van Mieghem NM, Tchetch   D, Chieffo A, Dumonteil N, Messika-Zeitoun D, van der Boon RM, Vahdat O, Buchanan GL, Marcheix B, Himbert D, Serruys PW, Fajadet J, Colombo A, Carri   D, Vahanian A, de Jaegere PP. Incidence, predictors, and implications of access site complications with transfemoral transcatheter aortic valve implantation. *Am J Cardiol* 2012;110:1361-7.
 49. Mussardo M, Latib A, Chieffo A, Godino C, Ielasi A, Cioni M, Takagi K, Davidavicius G, Montorfano M, Maisano F, Carlino M, Franco A, Covello RD, Spagnolo P, Grimaldi A, Alfieri O, Colombo A. Periprocedural and short-term outcomes of transfemoral transcatheter aortic valve implantation with the Sapien XT as compared with the Edwards Sapien valve. *JACC Cardiovasc Interv* 2011;4:743-50.
 50. De Backer O, Arnous S, Sandholt B, Brooks M, Biasco L, Franzen O, L  nn L, Bech B, S  ndergaard L. Safety and efficacy of using the Viabahn endoprosthesis for percutaneous treatment of vascular access complications after transfemoral aortic valve implantation. *Am J Cardiol* 2015;115:1123-9.
 51. Starnes BW, Arthurs ZM. Endovascular management of vascular trauma. *Perspect Vasc Surg Endovasc Ther* 2006;18:114-29.

Original Article

Open Access



Is tacrolimus more likely to induce diabetes mellitus than ciclosporin in heart transplant patients?

Anisha Jagpal¹, Sudeep Das De², Sanjeet Singh Avtaar Singh², Alan Kirk²

¹College of Medical, Veterinary and Life Sciences, University Avenue, University of Glasgow, Glasgow G12 8QQ, UK.

²Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK.

Correspondence to: Mr. Sanjeet Singh Avtaar Singh, Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK. E-mail: sanjeetsingh@nhs.net

How to cite this article: Jagpal A, Das De S, Avtaar Singh SS, Kirk A. Is tacrolimus more likely to induce diabetes mellitus than ciclosporin in heart transplant patients? *Vessel Plus* 2018;2:24. <http://dx.doi.org/10.20517/2574-1209.2018.27>

Received: 28 Apr 2018 **First Decision:** 23 Jul 2018 **Revised:** 25 Jul 2018 **Accepted:** 2 Aug 2018 **Published:** 13 Sep 2018

Science Editors: Mario F. L. Gaudino, Cristiano Spadaccio **Copy Editor:** Huan-Liang Wu **Production Editor:** Zhong-Yu Guo

Abstract

Aim: Immunosuppression has evolved since the first successful orthotopic heart transplant 50 years ago. Currently, calcineurin inhibitors lie at the focal point of the immunosuppressive regimen. However, these drugs exhibit a variety of side effects, including hyperglycaemia. This in turn compounds the risk of cardiovascular disease. There is conflict around which calcineurin inhibitor, tacrolimus or ciclosporin, is more likely to induce diabetes.

Methods: A retrospective analysis of data from 52 patients who had received a heart transplantation at the Scottish heart transplant unit between January 2011 and August 2017. All patients received a combination immunosuppressive regimen consisting of mycophenolate mofetil, corticosteroids and either tacrolimus or ciclosporin. Fasting glucose levels were compared every 3 months after transplantation for a year. HbA1c was collected and compared at one interval during follow-up postoperatively. Statistical analysis was achieved using Students t-test for continuous variables and Chi-squared test for categorical variables.

Results: The drug regimens remained unchanged in the two cohorts over the study period. The fasting glucose of tacrolimus treated patients was higher over the 12-month period compared to ciclosporin treated patients (7.3 ± 1 vs. 5.9 ± 0.5 , $P = 0.017$). The results were significantly higher in the tacrolimus group at 9 months ($P = 0.013$). In contrast to these findings, HbA1c of the tacrolimus group was lower than the ciclosporin group, although there was no significant difference (38 ± 11.4 vs. 43 ± 1.3 , $P = 0.104$).

Conclusion: This study suggests a relationship between tacrolimus and rising fasting glucose among heart transplant population. However, a longer follow-up and control of confounding variables is required to denote the long-term impact of immunosuppression related diabetes in heart transplant patients.

Keywords: Immunosuppression, heart transplantation, tacrolimus, ciclosporin, diabetes



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



INTRODUCTION

Over the past 50 years, heart transplantation has evolved to become an acceptable treatment for end-stage heart failure. Immunosuppression has been pivotal to the success of this procedure. Many drugs have been involved in post-transplant immunosuppressive regimens. However, current guidelines recommend the use of calcineurin inhibitors, tacrolimus and ciclosporin. Due to the potency of these drugs, patients are prone to a variety of side effects including diabetes. Preventing post-transplant diabetes mellitus (PTDM) is an important element of successful immunosuppression in cardiac transplantation, as uncontrolled diabetes is associated with cardiovascular morbidity and mortality. This can be detrimental to heart transplant recipients as they are already at risk of cardiac allograft vasculopathy (CAV).

The aim of this paper is to retrospectively analyse the possibility of developing diabetes mellitus in heart transplant patients who have been treated with either a ciclosporin or tacrolimus-based regimen. This will be done by focusing on fasting glucose and HbA1c levels in 52 patients up to a year after transplantation.

History of heart transplantation

In 1967, Barnard^[1] performed the first successful human-to-human heart transplant in Cape Town, South Africa. The techniques used during this operation were created by Dr. Norman Shumway several years earlier and are still used in modern times^[1]. Although a breakthrough in science at the time, the patient died of *Pseudomonas pneumonia* on the 18th day postoperatively^[1]. This highlighted concerns surrounding immunosuppressive regimen, an issue which would remain prevalent for subsequent years.

A year on from Barnard^[1]'s success, more than 100 cardiac transplants were performed at transplant centres around the world. But due to the high risk of complications, the procedure soon declined with only 9 taking place in 1971. During this time, one-year survival was 30%^[2]. A key advancement in rejection monitoring was the introduction of endomyocardial biopsy^[3] and the classification of histological rejection^[4]. This allowed clinicians to combine clinical and histological findings to make the diagnosis of rejection more accurate. Worldwide interest in heart transplantation was revived in the 1980s after an immunological milestone, the introduction of ciclosporin A.

Between April 2016 and March 2017, 197 heart transplants were performed in the UK^[5] with one-year survival over 80%^[6]. This is a testament to the progress made since the 1970's. Nevertheless, there are still serious challenges facing the field, limiting the success of heart transplantation. Such barriers include CAV, a type of chronic rejection that results in hardened arteries. This can compromise long term survival of the cardiac allograft and ultimately lead to cardiac arrest. Another major barrier is created by chronic immunosuppression, which can result in drug induced complications including diabetes mellitus^[7].

Overview of rejection immunology

Complex mechanisms of immunity pose a significant barrier to successful transplantation. In cardiac transplantation, the response to alloantigens is mediated by host T-cells. Peptide antigens are presented to T-cell receptors in the context of major histocompatibility complex, by antigen-presenting cells^[8]. This binding leads to an increase in cytoplasmic calcium ions, which in turn activates the protein phosphatase, calcineurin. When activated, calcineurin dephosphorylates nuclear factor of activated T-cells (NFAT)^[9]. Following this, NFAT triggers the upregulation IL-2 expression, leading to T-cell stimulation.

The adaptive response is efficiently suppressed by conventional drugs. But, when unsuccessful, alloimmune response can lead to destruction of the allograft (rejection). Rejection is categorised into three major types: hyperacute, acute and chronic. Due to effective screening (HLA and ABO blood-group cross-matching) on transplant recipients, hyperacute rejection is rare^[10]. Although acute and chronic rejection are more common, the mechanisms involved are incompletely understood. There is, however, a variety of immune-system components involved, including the T-cell response mentioned above^[11].

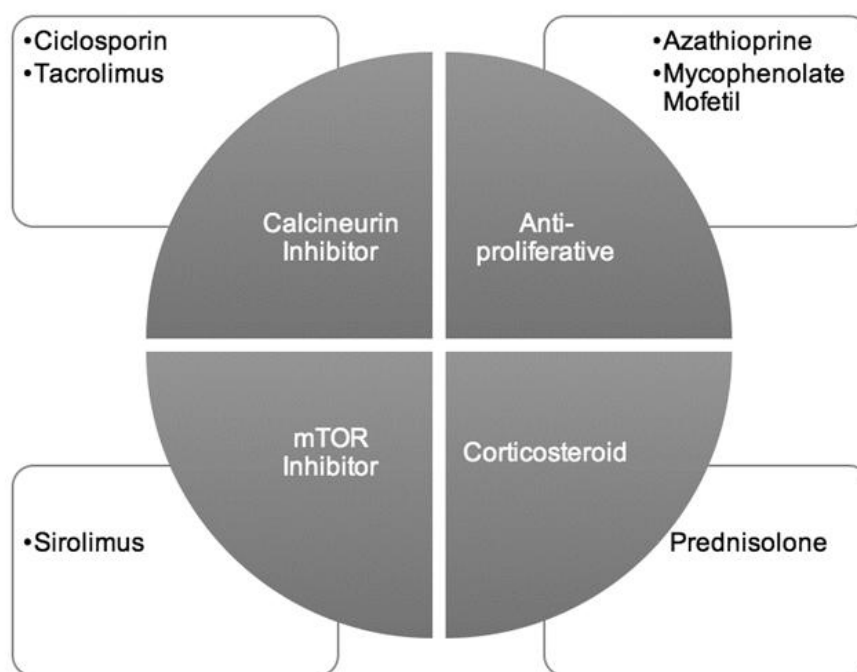


Figure 1. Four drug groups that make up the foundation of maintenance immunosuppression in heart transplantation

Despite the combination drug regimen targeting different stages of the immune response, illustrated in [Figure 1](#), patients are still at risk of rejection. From 2010, 24% of heart transplant recipients have experienced rejection^[12]. This suggests there is room for more pharmacological discovery in this area.

Immunosuppression regimens in transplantation

Immunosuppression aims to dampen the immune response in order to sufficiently permit engraftment of the transplant, while simultaneously being suitably specific such that other protective immune responses remain intact. This requires a complementary combination of medication that optimise immunosuppression while decreasing toxicity. To create this balance in regimen, drugs are provided at different doses and time-points so their effects are maximised.

Immunosuppressive treatment consists of induction and maintenance regimens. Induction therapy is intense and occurs before, during and after transplantation in aim to markedly reduce rejection in early postoperative period. Rabbit derived lympholytic agent, anti-thymocyte globulin (rATG) is a standard induction drug given postoperatively. Immediate postoperative rATG therapy allows CNIs to be introduced later. CNIs are nephrotoxic, so this action avoids exacerbation of renal dysfunction. Batches of rATG vary in potency, thus in order to assess the effectiveness, patients T cells should be monitored with flow cytometry^[13].

Maintenance therapy consists of a combination of drugs including: an antiproliferative, a calcineurin inhibitor (CNI) and steroids. Although maintenance regimens are continually evolving, corticosteroids have remained at the core since the first heart transplantation. However, due to their significant side effects, the duration and dosage of corticosteroids has decreased with time^[14]. To combat this issue, combination therapy has moved towards targeting steps in T-cell activation, permitting lower doses for each individual drug.

Antiproliferative agents, Mycophenolate Mofetil (MMF) and Azathioprine, operate alongside other drugs to target the proliferation of T- and B-cells, resulting in diminished cytotoxic T-cell response^[15,16]. MMF is recommended over due to its decreased incidence of rejection and CAV^[17,18]. mTor inhibitors, such as Sirolimus, also target T-cell proliferation and are an effective alternative for CNI in patients with CNI

induced nephrotoxicity^[19].

Ciclosporin

The discovery of ciclosporin in the early 1970s revolutionised the field of transplantation. Initially developed as an anti-fungal, the potent immunosuppressive properties of ciclosporin were first recognised in 1986 (Borel, 1986). Ciclosporin was approved for use in kidney transplantation in 1979. This dramatically improved the mortality of transplant patients and provided sufficient evidence to permit approval for use in heart transplantation in 1980. Five years after introducing the drug to cardiac transplant recipients, Shumway's group reported a 1-year survival rate of 83%^[20]. In 1994, the original oil-based drug was replaced by a new micro-emulsion formulation (Neoral, Novartis). This provided greater bioavailability and more predictable pharmacokinetics, enhancing the role of ciclosporin in immunosuppression^[21]. The use of ciclosporin in transplantation has led to a steady increase in survival, with worldwide 1-year survival now approaching 90%^[22].

The calcineurin inhibitor, ciclosporin inhibits T-cell proliferation by blocking its activation. When entering lymphocytes, ciclosporin binds to cyclophilin, a type of immunophilin. This ciclosporin-cyclophilin complex binds to and inhibits the action of calcineurin. As a result, the IL-2 transcription cascade is halted. This ultimately blocks T-cell activation, preventing allograft rejection process.

Tacrolimus

Tacrolimus, a macrolide antibiotic, was found to possess potent immunosuppressive qualities in 1984. The compound was first approved for use in transplantation in Japan, 1993^[23]. Due to its decreased incidence of rejection when combined with MMF, tacrolimus has surpassed ciclosporin to become the cornerstone of immunosuppressant therapy today^[24]. Over 70% of patients worldwide are on a tacrolimus and MMF combination therapy, further highlighting the superiority of tacrolimus^[12].

Tacrolimus exhibits similar immunosuppressive activity to that of ciclosporin. However, tacrolimus inhibits calcineurin through binding to a different immunophilin, FK506 binding protein (FKBP12). Tacrolimus is also effective as a rescue rejection therapy^[25].

Although CNIs play a critical role in the preservation of allograft function, these drugs cause a variety of side effects, including hyperglycaemia. This in turn can result in significant morbidity and reduced quality of life^[26].

Post-transplant diabetes mellitus

Diabetes is a metabolic disorder characterised by chronic hyperglycaemia, resulting from defects in insulin secretion, insulin action, or both^[27]. There are several diagnostic tests for diabetes shown in Table 1, each test is used alongside any symptoms a patient is experiencing^[28]. Although it has been proposed that PTDM could be a distinct entity, the natural history of diabetes after transplantation resembles type 2 diabetes^[29]. Since the onset of both PTDM and type 2 diabetes can be insidious and patients may be asymptomatic for years before symptoms present. Accordingly, PTDM management follows the conventional approach used for patients with type 2 diabetes^[30]. PTDM is not always permanent and may stabilise within weeks or months^[31].

PTDM is a major problem among this population of patients as impaired glucose metabolism can contribute to increased risk of cardiovascular disease, subsequently reducing mortality in these patients.

CNI induced diabetes

The exact mechanism by which CNIs induce diabetes is unknown. One mechanism that has gained popularity involves calcineurin expression in pancreatic insulin-secreting β -cells. Here, calcineurin

Table 1. Diagnostic criteria for diabetes and pre-diabetes (WHO, 2006)

Type of diagnostic test	Normal	Pre-diabetes	Diabetes
Fasting glucose (mmol/L)	< 5.5	5.5-7.0	> 7.0
Oral glucose tolerance test (mmol/L)	< 7.8	7.8-11.1	> 11.1
HbA1c (mmol/L)	< 42	42-47	> 47

Table 2. Target CNI levels of heart transplant patients in the Golden Jubilee National Hospital, Glasgow

Time after transplantation	Tacrolimus ($\mu\text{g/L}$) target therapeutic range	Time after transplantation	Ciclosporin ($\mu\text{g/L}$) target therapeutic range
0-3 months	10-15	0-4 weeks	240-300
3-6 months	8-12	1-6 months	160-200
6-12 months	7-10	6-12 months	130-160
> 12 months	5-7	> 12 months	64-96

undergoes a similar action to its role in T-cell activation. In pancreatic β -cells, the activation of calcineurin leads to the dephosphorisation and translocation of a different family of NFAT transcription factors. Following this, NFAT induces the expression of genes critical for multiple factors that control growth and hallmark β -cell functions, including insulin production and expression^[19]. Therefore, calcineurin inhibition impairs β -cell proliferation and decreases β -cell mass resulting in reduced insulin expression. This ultimately leads to diabetes.

In heart transplantation, diabetes is a well-recognised complication. More than 22% of heart transplant patients develop diabetes 1 year after transplantation^[12]. Immunosuppressive agents contribute to this morbidity. Nevertheless, evidence surrounding which CNI is more likely to negatively affect glucose metabolism is contradictory. It is understood that both tacrolimus and ciclosporin are equivalent in inducing diabetes but it is suggested that, in clinical practice, tacrolimus has greater diabetogenic potential^[32].

METHODS

Study population

A retrospective cohort study of 52 patients who underwent a first-time heart transplant at the Golden Jubilee National Hospital (Glasgow, Scotland) between January 2011 and August 2017. In January 2014, tacrolimus was made the primary CNI at the unit. As a result, 33 patients were on a tacrolimus-based maintenance regimen postoperatively. A comparable cohort of 19 patients on a ciclosporin-based maintenance regimen were selected. Prior to the investigation, one of the patients in the study population was diagnosed with diabetes. That patient was on a ciclosporin based regimen and included in the study. Patients who received changes in their drug regimens and patients who died were excluded. All patients were over the age of 18.

Post-transplant Management

Each patient received immunosuppression according to the unit's protocol. Immediate post-operative induction therapy consisted of rATG. This was given for up to 4 days after transplantation, until the patients kidney function is sufficient. Thereafter, patients received a combination regimen of CNI, MMF and a steroid. Patients remain on a CNI and MMF for their lifetime but steroids are removed 6 months after transplantation. Drug level monitoring was performed during routine follow-up visits at regular time intervals. Target CNI levels depend on time after transplantation, as illustrated in Table 2. These levels were collected and analysed in a laboratory at the Queen Elizabeth University Hospital, Glasgow, using tandem mass spectrometry.

Data collection and outcomes measured

Data was collected from the transplant unit's database. Clinical notes provided all the information necessary for analysis, including demographic information. Glucose metabolism was studied via fasting glucose levels

Table 3. Selected demographic and baseline characteristics of the two patient groups

	All (<i>n</i> = 52)	Tacrolimus & MMF (<i>n</i> = 33)	Ciclosporin & MMF (<i>n</i> = 19)	<i>P</i> -value
Age	50 ± 13	49 ± 12	51 ± 14	0.561
Male:female	37:15	24:9	13:6	0.741
Body Mass Index (kg/m ²)	26.8 ± 4.6	25.4 ± 4.5	26.8 ± 4.8	0.103
Preoperative Diabetics	5	4 (12)	1 (5)	0.641
Creatinine	115.7 ± 40.3	119 ± 46.2	109.9 ± 27.3	0.440
Pre-transplant diagnosis				
DCM	32	18 (55)	14 (74)	
HCM	4	3 (9)	1 (5)	
IHD	9	6 (18)	3 (16)	
Other	7	6 (18)	1 (5)	0.479

Table 4. Fasting glucose levels of the two cohorts over time

Fasting glucose (mmol/L)	Tacrolimus & MMF (<i>n</i> = 33) (mean ± SD)	Ciclosporin & MMF (<i>n</i> = 19) (Mean ± SD)	<i>P</i> -value
BASELINE	5.9 ± 1.3	5.4 ± 0.9	0.726
1 month	6.4 ± 1.7	5.5 ± 1.3	0.064
3 months	7.8 ± 6.3	6.1 ± 1.6	0.275
6 months	8.8 ± 7.5	5.9 ± 1.3	0.102
9 months	6.9 ± 2.0	5.4 ± 1.4	0.013
12 months	6.7 ± 3.7	6.5 ± 1.9	0.836

and HbA_{1c}. Fasting glucose levels were collected from five points in time; 1, 3, 6, 9 and 12 months post-operatively. HbA_{1c} was collected at one follow-up visit. The time points were chosen according to the post-operative follow-up protocol used by the unit, this was consistent between patients.

Statistical analysis

Data was extracted, collated and stored on a Microsoft Excel 2017 spreadsheet. This data was then imported into Prism 7.0c software application for statistical analysis. Independent *t*-test was used to analyse any differences in continuous data and the Chi-squared test was used for categorical data. The level of significance was set at *P*-value < 0.05.

RESULTS

Patient demographics

Patient characteristics are listed in Table 3. The study groups were not matched, but all had appropriate clinical records and information that was accessible, allowing comparisons to be made. There was no statistical significant difference in the patient baseline characteristics, including age, gender and pre-transplant diagnosis. Of the 52 patients enrolled, 5 had a diagnosis of diabetes, 4 of which were on a tacrolimus-based regimen and diagnosed after transplantation.

Laboratory Blood Glucose measurements

As mentioned previously, fasting glucose of the study population was collected at five different points of time postoperatively. This data was collated into a mean fasting glucose for each time-period, the results of which are presented in Table 4. At one month postoperatively, there was no statistical difference in fasting glucose between the two cohorts. Here, fasting glucose for the TAC cohort was 6.4 ± 1.7 mmol/L and 5.5 ± 1.3 mmol/L for the CyA cohort (*P* = 0.064). A fasting glucose of 6.4 mmol/L suggests TAC patients were pre-diabetic at 1 month [Table 1].

In the first six months, there was a rise in fasting glucose among the TAC cohort, with levels peaking at the 6-month follow-up. The fasting glucose of the TAC group at this stage was 8.8 mmol/L. This greatly exceeds

Fasting glucose of patients on tacrolimus or ciclosporin

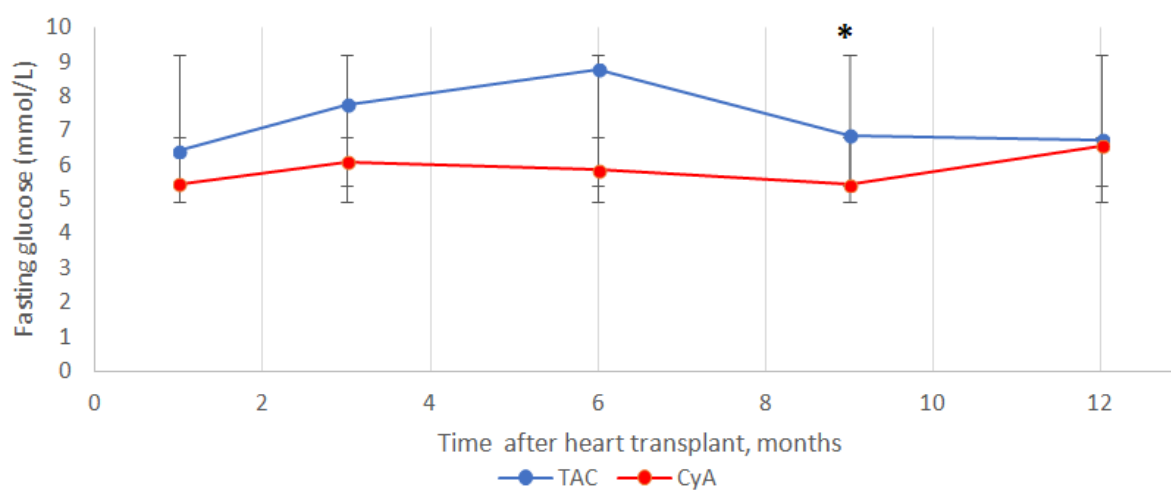


Figure 2. Fasting blood glucose of heart transplant patients on tacrolimus or ciclosporin maintenance immunosuppression over time postoperatively (mean \pm SD). At 9 months $P = 0.013$

the diagnostic range for diabetes. The 6-month point also marks the largest difference between the two cohorts across the 12-month period, 8.8 ± 7.5 mmol/L vs. 5.9 ± 1.3 mmol/L ($P = 0.102$), this is highlighted in Figure 2.

During the first year after transplantation, drug dosage is monitored and adjusted cautiously according to the transplant unit's target levels, incidence of transplant rejection and any side effects which may present, such as hyperglycaemia. Patients experiencing hyperglycaemia are followed up and managed according to the unit's protocol. This includes reducing drug target levels for CNIs and providing appropriate management for the unbalanced glucose metabolism.

Moreover, after 6-months fasting glucose declines across the population; this is especially seen in the TAC group. The difference in mean between the two cohorts shows significance at 9 months postoperatively, with the TAC cohort showing higher values. The mean fasting glucose was 6.9 ± 2.0 mmol/L in the TAC group and 5.4 ± 1.4 mmol/L in the CyA group ($P = 0.013$).

One year after transplantation, mean fasting glucose of TAC cohort continued to decline. However, levels are still considered pre-diabetic and values are above the CyA group, 6.7 ± 3.7 mmol/L vs. 6.5 ± 1.9 mmol/L ($P = 0.836$). Nevertheless, the 12-month follow-up marks the highest mean fasting glucose levels for the CyA cohort. Consequently, CyA could possess a diabetogenic potential 12 months after transplantation.

Overall, the mean fasting glucose over the 12-month period indicated significance. TAC treated patients had a higher mean fasting glucose, 7.3 ± 1.0 mmol/L, compared to the CyA cohort, 5.9 ± 0.5 mmol/L ($P = 0.017$).

In contrast to the fasting glucose results, mean HbA1c of the TAC group was lower than the CyA group, as shown in Figure 3. These results however, did not reach statistical significance ($P = 0.104$). The mean HbA1c was collated from information at one interval for patients on both regimens. According to the diagnostic criteria [Table 1], CyA treated patients are pre-diabetic whilst TAC treated patients are not at risk of diabetes.

HbA1c of patients on tacrolimus or ciclosporin

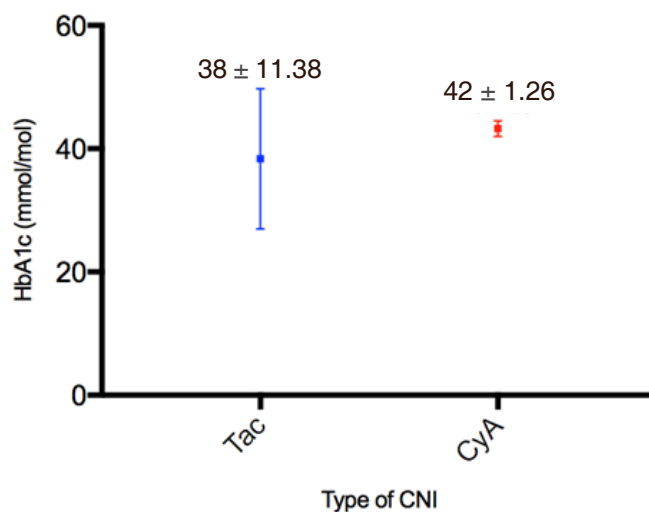


Figure 3. HbA1c of heart transplant patients on tacrolimus or ciclosporin at one time interval (mean \pm SD)

DISCUSSION

Principle findings

This study confirmed the risk of post-transplant diabetes is worse in heart transplant patients treated with tacrolimus-based maintenance immunosuppression compared with those treated with ciclosporin, when using mean fasting glucose as an indicator. The difference was significant at 9 months post-operatively. This timing fits with changes in the unit's drug regimen, especially the removal of corticosteroids which also contribute to altered glucose metabolism. In both cohorts, fasting glucose normalises at 12 months, further emphasising the role of corticosteroids in the results produced.

The mean HbA1c was greater in the ciclosporin treated cohort. At the beginning of the study, the two cohorts were comparable, with many relevant contributors to post-transplant diabetes considered- including age, sex, weight, creatinine, pretransplant diagnosis and diagnosis of diabetes.

Context of research

Much evidence involving the effectiveness of both tacrolimus and ciclosporin is based on data from kidney and liver transplantation. For that reason, Reichart *et al.*^[33] (2001) conducted a large European multicentre study to investigate the effectiveness of both CNIs in heart transplant recipients. With a study population of 82 patients across 5 centres, they found that when combined with azathioprine and corticosteroids, tacrolimus was just as effective as ciclosporin. While this three-year follow up confirmed tacrolimus a viable alternative for ciclosporin in heart transplantation, Reichart and colleagues also observed a higher proportion of patients treated with tacrolimus requiring insulin for PTDM, a consequence that would affect the morbidity and mortality of patients in years to come^[33].

Another multicentre study published a few months later Taylor *et al.*^[34] (1999) compared tacrolimus and ciclosporin to find both equally safe and effective maintenance immunosuppression in heart transplantation. They did observe a higher rate of hypertension and hyperlipidaemia in patients receiving tacrolimus. This was a significant finding as both hypertension and hyperlipidaemia negatively impact renal function. There was also no difference in the incidence of diabetes or hyperglycaemia between the two groups. This, at the time, differed from liver and kidney trials where diabetes was significantly more common in tacrolimus

treated patients. The results of this study confirmed the advantages of tacrolimus over ciclosporin.

Early studies used a combination of azathioprine and corticosteroids with either tacrolimus or ciclosporin. Nevertheless, since its use in the first heart transplant operation, treatment with azathioprine has been replaced in clinical practice by MMF due to its increased toxicity. An early investigation into the substitution of MMF for azathioprine found that in 650 heart transplant patients, MMF was more effective in reducing mortality and rejection and was associated with decreased toxicity^[18]. In the 2000's, Kobashigawa and colleagues further investigated this area. They found that a combination of tacrolimus and MMF offered more advantages than ciclosporin and MMF in cardiac transplant patients, including fewer rejections and an improved side effect profile. Post-transplant diabetic rates were greater among the tacrolimus and MMF treated group, although this difference was not statistically significant^[35]. The PTDM results Kobashigawa produced reflect similarity with the current study.

In the studies mentioned above, all patients are followed for a short time interval. Trials comparing triple immunosuppressive strategies involving tacrolimus or ciclosporin with MMF and steroids over a long term are rarely published. Guethoff *et al.*^[36] (2013) used prospective randomised trial to follow-up heart transplant patients over 10 years. Long term analysis found a lower incidence of rejection in the tacrolimus group, but there was no difference between groups in long-term survival^[36].

Clinical trials have been principle to the success of heart transplantation. Despite the vast number of trials, there is no single validated immunosuppression regimen. Nevertheless, protocols used worldwide in 2017 are strongly influenced by the results of SYMPHONY study which demonstrated that a low dose of tacrolimus, MMF and corticosteroids had the best allograft outcomes^[37].

Since the superior rejection profile of tacrolimus has been established, research has been directed towards the side effects produced by the two CNIs and the consequences of this. However, there is limited data surrounding the onset of diabetes after tacrolimus or ciclosporin treatment in cardiac transplant patients. A large European multicentre trial published by Grimm *et al.*^[38] (2006) revealed the incidence of PTDM in heart transplantation was significantly higher in the tacrolimus group. Accordingly, more tacrolimus patients required insulin therapy^[38].

Grimm *et al.*^[38]'s results highlighting the diabetogenic potential of tacrolimus, it should be noted that published studies in this area have often failed to reach significance to due low patient numbers. A study by Teebken *et al.*^[39] (2002) reflects this, as out of 32 heart transplant recipients, 4 patients treated with tacrolimus developed PTDM compared with 1 patient treated with ciclosporin. The small sample size makes these results difficult to statistically analyse and compare.

Corticosteroids are also associated with a greater risk of developing diabetes after heart transplantation. Heart transplant recipients developing diabetes were found to be receiving higher mean doses of prednisolone compared to those without the condition^[40]. Consequently, an increased number of studies are considering the effect of corticosteroid-sparing and corticosteroid-free regimens on the development of PTDM. Baran *et al.*^[41] (2002) have shown that tapering of and weaning patients from corticosteroid treatment considerably lowered the incidence of diabetes.

Impact of PTDM on heart transplantation

PTDM has been a recognised complication of transplantation for several years, despite this, the importance of the condition has been underestimated. In the general population, it is putative that diabetes increases risk of cardiovascular disease (CVD). Nonetheless, the complications associated with diabetes were not thought to be a concern for patients who had undergone heart transplantation. Initial studies into the effect of PTDM

on transplant patients focused on kidney transplantation. Lindholm *et al.*^[42] (1995) demonstrated that PTDM increased the risk of CVD in transplant recipients. The relationship between PTDM and CVD is a concern in heart transplant patients, as these patients are already at risk of CAV. As previously mentioned, CAV is an accelerated form of coronary artery disease, it is also a major cause of death in patients surviving over a year after heart transplantation^[43].

A study by Kato *et al.*^[44] (2004) looked at the relationship between glucose intolerance and CVD in 151 heart transplant recipients. The researchers used HbA1c as an indicator of glucose metabolism and found that increased HbA1c levels were associated with occurrence of CVD and could play a pivotal role in its pathogenesis^[44]. A limitation of this study and many others in this area is the lack of information surrounding the outcome of CVD in this population of patients. While it is important for clinicians to recognise the link between PTDM and CVD, the management and consequences of this need to be addressed.

Individuals with prediabetes are also at risk of CVD. Elevated fasting glucose among this group results from both impaired insulin action and secretion^[45]. A meta-analysis comprising 1,611,399 individuals conducted by Huang *et al.*^[46] (2016) found that prediabetes was associated with increased risk of composite cardiovascular events which all lead to mortality. They also observed increased risk occurring when fasting glucose was as low as 5.6 mmol/L.

In this study, it has been shown that patients on tacrolimus were prediabetic throughout the duration of the trial, with levels considerably higher than 5.6 mmol/L. It is therefore essential to not only focus on a blood test for diagnosis of diabetes but also any symptoms a patient may be experiencing. However, the study published by Huang *et al.*^[46] consisted of data that was not specific to the transplant field. It is therefore difficult to generalise Huang's results to the current study's population of patients. There is scope for research in this area as data regarding the cardiovascular implications of prediabetes in heart transplant patients is lacking.

Limitations of the study

Retrospective studies are prone to limitations which can affect the reliability of any results and conclusions produced. This study is prone to selection bias as data used was collected from patient files, as a result there was no participation selection.

The data for this study was collected from patient files where information was transplant specific. Therefore, data that could influence the risk of diabetes was not measured, such as family history of diabetes and ethnicity. Ideally, HbA1c would be collected at different time points after transplantation. However, there was only one measurement available for both cohorts and so it is difficult to compare the results of the mean fasting glucose and HbA1c.

The use of a steroid in immunosuppressive maintenance therapy is a significant confounding factor in this study. As mentioned previously, a common side effect of steroid use is hyperglycaemia. Patients were followed up for a 12-month period, so fluctuating fasting glucose levels seen may be a result of steroid use. As steroids are removed from the regimen at 6 months, following up patients for a longer period could eliminate this confounding variable.

As well as a short follow up, this was a single centre study consisting of 52 patients. These factors can create lack of external validity as results are difficult to generalise. Nonetheless, because the study was carried out in one unit, all patients received similar care, similar selection for surgery, drug regimens and follow-up. These factors give validity to the findings of this study.

Due to the nature of this study, follow-up times were not exactly on time for each patient, but for this study, flexibility of two weeks was allowed in data collection. All information was readily available and there were no technical difficulties faced during this study.

Implications

Understanding the risk of hyperglycaemia with different maintenance immunosuppressant regimens is very important as chronic hyperglycaemia can negatively affect a patient's quality of life and can result in high morbidity and mortality. A major modifiable risk factor for the development of PTDM is immunosuppression, but risk versus benefit analysis is needed to balance the risk of developing PTDM versus rejection. This study does not support the view of switching patients from tacrolimus to ciclosporin. Rejection episodes and survival data collection were not included in the study; therefore, this study cannot comment on the superiority of one drug over the other.

Instead, implications lie in informing clinicians about the possibility of developing diabetes after tacrolimus use and the complications of this on patients. Therefore, greater attention should be paid to recognising diabetes through different glycaemic parameters. As a result of the findings of this study, the heart transplant unit at the Golden Jubilee National Hospital has introduced regular HbA1c testing in all heart transplant patients. A HbA1c diagnosis of diabetes is already endorsed in the general population and should be used to recognise PTDM, especially due to its ability to predict diabetic complications.

Hyperglycaemia is extremely common in the early postoperative period. It can also occur because of critical conditions such as infections or as a consequence of rejection therapy. In this study, there is no data on patient glucose metabolism before transplantation. This would be helpful when making a formal diagnosis of PTDM, as the patient's pre-transplant glucose function can be compared to after transplantation when they are stable on their maintenance immunosuppression, have stable cardiac allograft function and no acute infections.

Further studies should consider collaborating and combining data that links fasting glucose and HbA1c with end points, including microvascular complications, cardiovascular events, patient and graft survival. There is also a need to facilitate clinical trials into the prevention of PTDM, one way by which this can be done is to identify patients at risk. A limitation of this study, as mentioned previously, is the lack of data. Patient risk factors for PTDM are well established and encompass general information such as family history of diabetes. There is a room for possibly preventing PTDM and its complications when such factors are collected.

The future of pharmacological management in transplant recipients is beyond calcineurin inhibitors. Based on this research and personal recommendation, more advanced immunological methods could reduce side-effects produced currently. This could also help reach the ultimate goal of organ transplantation, which is the development of safe and effective regimens that manipulate host immune system into accepting transplanting organs in absence of immunosuppressive management or 'tolerance'. This would prevent the morbidity of chronic immunosuppression including CVD. Nevertheless, CNIs have dominated transplantation for decades through targeting T-cells, a key player in rejection. Research surrounding the function and clinical effects of both tacrolimus and ciclosporin will be valuable for the manipulation of immune cells in the future.

In conclusion, both tacrolimus and ciclosporin continue to remain at the backbone of pharmacological management in heart transplant patients. Even though tacrolimus is a newer agent with a greater ability to prevent allograft rejection, patients are at increased risk of diabetes. Pharmacological development in this area is required as the gap between the need for cardiac transplantations worldwide and the development of effective immunosuppressant regimens is growing. A heart transplantation can transform a patient's life,

but this significant procedure can be jeopardised by co-morbidities associated with its management. As a result, a new heart may fail for the same reasons the original heart failed.

DECLARATIONS

Authors' contributions

Data collection, data analysis, manuscript writing: Jagpal A

Statistical analysis, manuscript review: Das De S

Manuscript editing and reviewing: Avtaar Singh SS

Study supervisor, manuscript reviewing: Kirk A

Availability of data and materials

At request. Kindly email corresponding author.

Financial support and sponsorship

None.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

This report is not considered research by the NHS, as defined by the UK Policy Framework for Health and Social Care Research. Therefore, this study did not require approval from an NHS Research Ethics Committee (REC).

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Barnard CN. The operation. A human cardiac transplant: an interim report of a successful operation performed at Groote Schuur Hospital, Cape Town. *S Afr Med J* 1967;41:1271-4.
2. Stehlik J, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Hertz MI; International Society of Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report--2012. *J Heart Lung Transplant* 2012;31:1052-64.
3. Caves PK, Stinson EB, Billingham M, Shumway NE. Percutaneous transvenous endomyocardial biopsy in human heart recipients. *Ann Thorac Surg* 1973;16:325-36.
4. Billingham ME. Endomyocardial biopsy detection of acute rejection in cardiac allograft recipients. *Heart Vessels Suppl* 1985;1:86-90.
5. NHS. Organ donation and transplantation- activity (1)s for the UK as at April 2017. NHS blood ant transplantation activity 2017. Available from: <https://www.organdonation.nhs.uk/supporting-my-decision/statistics-about-organ-donation/transplant-activity-report/>. [Last accessed on 12 Sep 2018]
6. NHS. Annual report on cardiothoracic transplantation. NHS Blood ant Transplantation Activity 2015. Available from: <https://www.organdonation.nhs.uk/supporting-my-decision/statistics-about-organ-donation/transplant-activity-report/>. [Last accessed on 12 Sep 2018]
7. Tonsho M, Michel S, Ahmed Z, Alessandrini A, Madsen JC. Heart transplantation: challenges facing the field. *Cold Spring Harb Perspect Med* 2014;4: pii: a015636.
8. Ayala Garcia MA, Gonzalez Yebra B, Lopez Flores AL, Guani Guerra E. The major histocompatibility complex in transplantation. *J Transplant* 2012;2012:842141.
9. Heit JJ, Apelqvist AA, Gu X, Winslow MM, Neilson JR, Crabtree GR, Kim SK. Calcineurin/NFAT signalling regulates pancreatic beta-cell growth and function. *Nature* 2006;443:345-9.

10. Friend PJ. Rejection reactions to different organ transplants. *Eye* 1995;9:190.
11. Azuma H, Tilney NL. Chronic graft rejection. *Curr Opin Immunol* 1994;6:770-6.
12. Lund LH, Khush KK, Cherikh WS, Goldfarb S, Kucheryavaya AY, Levvey BJ, Meiser B, Rossano JW, Chambers DC, Yusen RD, Stehlik J; International Society for Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: thirty-fourth adult heart transplantation report-2017; focus theme: allograft ischemic time. *J Heart Lung Transplant* 2017;36:1037-46.
13. Lindenfeld J, Miller GG, Shakar SF, Zolty R, Lowes BD, Wolfel EE, Mestroni L, Page RL 2nd, Kobashigawa J. Drug therapy in the heart transplant recipient: part II: immunosuppressive drugs. *Circulation* 2004;110:3858-65.
14. Steiner RW, Awdishu L. Steroids in kidney transplant patients. *Semin Immunopathol* 2011;33:157-67.
15. Elion GB. The George Hitchings and Gertrude Elion Lecture. The pharmacology of azathioprine. *Ann N Y Acad Sci* 1993;685:400-7.
16. Wiseman AC. Immunosuppressive medications. *Clin J Am Soc Nephrol* 2016;11:332-43.
17. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, Fedson S, Fisher P, Gonzales-Stawinski G, Martinelli L, McGiffin D, Smith J, Taylor D, Meiser B, Webber S, Baran D, Carboni M, Dengler T, Feldman D, Frigerio M, Kfoury A, Kim D, Kobashigawa J, Shullo M, Stehlik J, Teuteberg J, Uber P, Zuckermann A, Hunt S, Burch M, Bhat G, Canter C, Chinnock R, Crespo-Leiro M, Delgado R, Dobbels F, Grady K, Kao W, Lamour J, Parry G, Patel J, Pini D, Towbin J, Wolfel G, Delgado D, Eisen H, Goldberg L, Hosenpud J, Johnson M, Keogh A, Lewis C, O'Connell J, Rogers J, Ross H, Russell S, Vanhaecke J; International Society of Heart and Lung Transplantation Guidelines. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant* 2010;29:914-56.
18. Kobashigawa J, Miller L, Renlund D, Mentzer R, Alderman E, Bourge R, Costanzo M, Eisen H, Dureau G, Ratkovec R, Hummel M, Ipe D, Johnson J, Keogh A, Mamelok R, Mancini D, Smart F, Valentine H. A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients. Mycophenolate mofetil investigators. *Transplantation* 1998;66:507-15.
19. Snell GI, Levvey BJ, Chin W, Kotsimbos T, Whitford H, Waters KN, Richardson M, Williams TJ. Sirolimus allows renal recovery in lung and heart transplant recipients with chronic renal impairment. *J Heart Lung Transplant* 2002;21:540-6.
20. Colombo D, Ammirati E. Cyclosporine in transplantation - a history of converging timelines. *J Biol Regul Homeost Agents* 2011;25:493-504.
21. Eisen HJ, Hobbs RE, Davis SF, Carrier M, Mancini DM, Smith A, Valentine H, Ventura H, Mehra M, Vachieri JL, Rayburn BK, Canver CC, Laufer G, Costanzo MR, Copeland J, Dureau G, Frazier OH, Dorent R, Hauptman PJ, Kells C, Masters R, Michaud JL, Paradis I, Renlund DG, Vanhaecke J, Mellein B, Mueller EA. Safety, tolerability, and efficacy of cyclosporine microemulsion in heart transplant recipients: a randomized, multicenter, double-blind comparison with the oil-based formulation of cyclosporine--results at 24 months after transplantation. *Transplantation* 2001;71:70-8.
22. Watson CJ, Dark JH. Organ transplantation: historical perspective and current practice. *Br J Anaesth* 2012;108 Suppl 1:i29-42.
23. Fung JJ. Tacrolimus and transplantation: a decade in review. *Transplantation* 2004;77:S41-3.
24. Meiser BM, Groetzner J, Kaczmarek I, Landwehr P, Müller M, Jung S, Uberfuhr P, Fraunberger P, Stempfle HU, Weis M, Reichart B. Tacrolimus or cyclosporine: which is the better partner for mycophenolate mofetil in heart transplant recipients? *Transplantation* 2004;78:591-8.
25. Armitage JM, Kormos RL, Griffith BP, Hardesty RL, Fricker FJ, Stuart RS, Marrone GC, Todo S, Fung J, Starzl TE. A clinical trial of FK 506 as primary and rescue immunosuppression in cardiac transplantation. *Transplant Proc* 1991;23:1149-52.
26. Mukherjee S, Mukherjee U. A comprehensive review of immunosuppression used for liver transplantation. *J Transplant* 2009;2009:701464.
27. WHO. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. WHO: Geneva, Switzerland 2006.
28. Excellence NifHaC. Type 2 diabetes: prevention in people at high risk. NICE guideline (PH38), 2012.
29. Sharif A, Cohn S. Post-transplantation diabetes-state of the art. *Lancet Diabetes Endocrinol* 2016;4:337-49.
30. Pham PT, Pham PM, Pham SV, Pham PA, Pham PC. New onset diabetes after transplantation (NODAT): an overview. *Diabetes Metab Syndr Obes* 2011;4:175-86.
31. Davidson J, Wilkinson A, Dantal J, Dotta F, Haller H, Hernández D, Kasiske BL, Kiberd B, Krentz A, Legendre C, Marchetti P, Markell M, van der Woude FJ, Wheeler DC; International Expert Panel. New-onset diabetes after transplantation: 2003 international consensus guidelines. Proceedings of an international expert panel meeting. Barcelona, Spain, 19 February 2003. *Transplantation* 2003;75:SS3-24.
32. Velleca A, Kittleson M, Patel J, Rafiei M, Osborne A, Ngan A, Hage A, Chang DH, Czer L, Kobashigawa J. Tacrolimus-versus cyclosporine-induced diabetes leads to more diabetic complications after heart transplantation. *J Heart Lung Transplant* 2013;32:S202.
33. Reichart B, Meiser B, Viganò M, Rinaldi M, Yacoub M, Banner NR, Gandjbakhch I, Dorent R, Hetzer R, Hummel M. European multicenter tacrolimus heart pilot study: three year follow-up. *J Heart Lung Transplant* 2001;20:249-50.
34. Taylor DO, Barr ML, Radovancevic B, Renlund DG, Mentzer RM Jr, Smart FW, Tolman DE, Frazier OH, Young JB, VanVeldhuisen P. A randomized, multicenter comparison of tacrolimus and cyclosporine immunosuppressive regimens in cardiac transplantation: decreased hyperlipidemia and hypertension with tacrolimus. *J Heart Lung Transplant* 1999;18:336-45.
35. Kobashigawa JA, Miller LW, Russell SD, Ewald GA, Zucker MJ, Goldberg LR, Eisen HJ, Salm K, Tolzman D, Gao J, Fitzsimmons W, First R; the Study Investigators. Tacrolimus with mycophenolate mofetil (MMF) or sirolimus vs. cyclosporine with MMF in cardiac transplant patients: 1-year report. *Am J Transplant* 2006;6:1377-86.
36. Guethoff S, Meiser BM, Groetzner J, Eifert S, Grinninger C, Ueberfuhr P, Reichart B, Hagl C, Kaczmarek I. Ten-year results of a randomized trial comparing tacrolimus versus cyclosporine in a combination with mycophenolate mofetil after heart transplantation. *Transplantation* 2013;95:629-34.

37. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, Margreiter R, Hugo C, Grinyo JM, Frei U, Vanrenterghem Y, Daloze P, Halloran PF. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; 357:2562-75.
38. Grimm M, Rinaldi M, Yonan NA, Arpesella G, Arizón Del Prado JM, Pulpón LA, Villemot JP, Frigerio M, Rodriguez Lambert JL, Crespo-Leiro MG, Almenar L, Duveau D, Ordonez-Fernandez A, Gandjbakhch J, Maccherini M, Laufer G. Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients--a large European trial. *Am J Transplant* 2006;6:1387-97.
39. Teebken OE, Strüber M, Harringer W, Pichlmaier MA, Haverich A. Primary immunosuppression with tacrolimus and mycophenolate mofetil versus cyclosporine and azathioprine in hearttransplant recipients. *Transplant Proc* 2002;34:1265-8.
40. Depczynski B, Daly B, Campbell LV, Chisholm DJ, Keogh A. Predicting the occurrence of diabetes mellitus in recipients of heart transplants. *Diabet Med* 2000;17:15-9.
41. Baran DA, Ashkar J, Galin ID, Sandler D, Segura L, Courtney MC, Correa R, Chan M, Lansman SL, Spielvogel D, Cheng J, Gass AL. Tacrolimus and new onset diabetes mellitus: the effect of steroid weaning. *Transplant Proc* 2002;34:1711-2.
42. Lindholm A, Albrechtsen D, Frodin L, Tufveson G, Persson NH, Lundgren G. Ischemic heart disease--major cause of death and graft loss after renal transplantation in Scandinavia. *Transplantation* 1995;60:451-7.
43. Prada-Delgado O, Estevez-Loureiro R, Paniagua-Martin MJ, Lopez-Sainz A, Crespo-Leiro MG. Prevalence and prognostic value of cardiac allograft vasculopathy 1 year after heart transplantation according to the ISHLT recommended nomenclature. *J Heart Lung Transplant* 2012;31:332-3.
44. Kato T, Chan MC, Gao SZ, Schroeder JS, Yokota M, Murohara T, Iwase M, Noda A, Hunt SA, Valentine HA. Glucose intolerance, as reflected by hemoglobin A1c level, is associated with the incidence and severity of transplant coronary artery disease. *J Am Coll Cardiol* 2004;43:1034-41.
45. Laakso M. Cardiovascular disease in type 2 diabetes from population to man to mechanisms: the Kelly West Award Lecture 2008. *Diabetes Care* 2010;33:442-9.
46. Huang Y, Cai X, Mai W, Li M, Hu Y. Association between prediabetes and risk of cardiovascular disease and all cause mortality: systematic review and meta-analysis. *BMJ* 2016;355:i5953.

Review

Open Access



Fetal programming and its effects on vascular pulmonary circulation

Carmela Rita Balistreri

Department of Pathobiology and Medical Biotechnologies, University of Palermo, Palermo 90134, Italy.

Correspondence to: Dr. Carmela Rita Balistreri, Department of Pathobiology and Medical Biotechnologies, University of Palermo, Palermo 90134, Italy. E-mail: carmelarita.balistreri@unipa.it

How to cite this article: Balistreri CR. Fetal programming and its effects on vascular pulmonary circulation. *Vessel Plus* 2018;2:25. <http://dx.doi.org/10.20517/2574-1209.2018.35>

Received: 17 May 2018 **First Decision:** 8 Aug 2018 **Revised:** 17 Aug 2018 **Accepted:** 21 Aug 2018 **Published:** 19 Sep 2018

Science Editor: Alexander D. Verin **Copy Editor:** Yuan-Li Wang **Production Editor:** Zhong-Yu Guo

Abstract

Into the scientific community, consensus about the emerging concept of “the fetal origin of adult diseases” is growing. It sustains that the parental (of the two parents) adversities, and the related external influences, during the intra-utero/perinatal life of each eutherian mammal organism, human included, can permanently set the structure and functionality of specific body systems (i.e., immune, endocrine, nervous and cardiovascular systems), predisposing them to early ageing and disease during adulthood. The pulmonary circulation system also appears to be one of its targets. Established evidence supports the strong association between developmental programming and pulmonary arterial remodeling and dysfunction. Here, a revised overview of this topic is reported, by stressing the efforts and advances in identifying the molecular and cellular mechanisms and pathways involved.

Keywords: Developmental programming, pulmonary circulation system, adult pulmonary arterial remodeling and dysfunction

INTRODUCTION

Lung represents an essential organ for human life, having an exclusive circulation system for functions and volume. Specifically, it has two circulations: the pulmonary and bronchial circulations^[1]. Moreover, pulmonary arteries (PAs) and veins have two different functions: the gas replacement and oxygenation of blood, respectively^[1]. In addition, pulmonary circulation has the distinctive feature of being able to accommodate the entire cardiac output, by preserving a high blood flow, but maintaining the intravascular arterial pressure at a reduced value. Another feature, which distinguishes the lung circulation system as unique, is the structure of arterial wall^[1]. Accordingly, PAs have walls, which appear thinner than those



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



of systemic arteries, because of a reduced vascular smooth muscle component and a relative absence of adventitial components^[1]. However, the lung circulation system has some factors able to control the pulmonary blood flow, like the systemic vascular system. They firstly include vascular structure, gravity, and mechanical effects of breathing^[1]. Furthermore, neural and humoral factors are also involved.

Another feature of PAs is the susceptibility of pulmonary vascular tone for hypoxia^[1]. Temporary hypoxia provokes pulmonary vasoconstriction, but, if persistent, it contributes to the onset of vascular remodeling and dysfunction, which results in the development of pulmonary hypertension (PH)^[2]. PH is defined by abnormally high pulmonary artery pressure, which can occur in numerous diseases and clinical situations. The hypoxemia represents one of the five major causes of PH. Accordingly, the 2015 ECS/ERS guidelines in the treatment and diagnosis of PH have assembled the different forms of PH related to diverse causes in five major groups^[3]. Consistent with the recent guidelines, genetic and environmental factors represent other crucial factors of PH susceptibility. For example, pathogen infections [i.e., human immunodeficiency virus (HIV) infection] and the related inflammatory responses constitute the common causes of their onset, by altering the normal pulmonary endothelium barrier and causing edema^[4,5]. Other inflammatory conditions not related to pathogens are also involved. For examples, autoimmune disorders, with a female predominance, are significantly associated with the onset of PH^[5]. Regarding genetic factors, several research groups, from diverse European and US countries, have recently summarized all the established and emerging molecular defects related or not to familial pulmonary arterial hypertension (PAH) forms, which include not only mutations in the bone morphogenetic protein receptor type II (*BMPR2*) gene, defects of the transforming growth factor beta pathway, but also defects in activin A receptor type II-like 1 (*ACVRL1*), endoglin (*ENG*), and members of the SMAD family^[6].

Recently, Gao *et al.*^[7] have emphasized that an impairment of the normal development of pulmonary vasculature is involved in the pathogenesis of different paediatric pulmonary pathologies, PH included. In addition, they have particularly suggested that the principal target of this process is the endothelium. Accordingly, they have speculated that the identification of mechanisms and pathways, preserving both endothelial maturation and function, might improve the development of the entire lung. Furthermore, they also hypothesized that these mechanisms and pathways have long-term favourable effects in diverse forms of neonatal PH related not only to preterm birth and onset of lung diseases, but also to congenital heart diseases^[7].

Consistent with these last findings, it appears reasonable that PH may also be the result of the developmental programming (DP) of adult diseases, as emphasized by fetal origin of adult diseases theory postulated by Barker^[8]. PH in neonates (PHN) has been demonstrated to be the result of several adverse events during intra-utero/perinatal life, including high-altitude living, maternal malnutrition, placental insufficiency related to environmental factors or diseases such as preeclampsia, infections (i.e., staphylococcus infection), drugs, alcohol, *etc.*^[3]. Currently, the knowledge of the related mechanisms and pathways involved is imperative, because of the increased morbidity and mortality related to this PH group and the rise of prevalence in the new-borns, linked to the increase in unfavourable environmental factors in our populations. Their identification might facilitate the development of effective therapies or measures. Here, established literature evidence will be described and discussed.

THE RELATIONSHIP BETWEEN DP AND PH: FOCUS ON EPIGENETIC FACTORS

There is, in literature, a well-established evidence of DP in eutherian mammals, humans included. DP is the result of evolution's machinery, which determines, on the late, the utero and placentation development for guaranteeing an optimal intrauterine life^[9].

Accordingly, protection and nutritional support are the utero and placenta's functions. On the other late,

this implies that foetal development happens by adapting to the micro-environment and maternal (or better paternal) endogenous and exogenous stressors. Consequently, both development and fate of tissues, organs, and systems of an offspring can result in an altered or greater susceptibility to onset of ageing and pathologies^[9]. The process of genomic imprinting is the principal driver of these alterations, and it consists of an uncommon epigenetic process^[9]. It provides effects on new-borns, principally through maternal DNA methylation imprints. Today, other epigenetic mechanisms (i.e., histone modifications, antisense noncoding RNA (ncRNA)-mediated silencing, long-range chromatin interactions, and expression of microRNA), have been suggested to mediate genomic imprinting^[9]. However, their actions remain unclear, even if it firstly supposes their capacity of modulating the expression of a wide range of imprinted genes restricted to specific foetal tissues, particularly the placenta, hypothalamus, and endothelium^[9]. Contemporarily, the developing placenta and hypothalamus influence the expression of the foetal genome, which results in the release of hormones able to impact the functions of both foetal and maternal hypothalamus. This control consents the foetal hypothalamus to regulate the development of organs and systems of the foetus, such as the pulmonary circulation^[9]. Maternal insults or stressors, including maternal nutritional changes, hormones (i.e., cortisol), other lifestyle stressors (e.g., obesity, smoking, drugs, alcohol), and the clinical status can alter maternal imprinting. This results in the onset of pathological conditions during pregnancy, including intrauterine growth restriction (IUGR) and preeclampsia^[9]. On the other hand, IUGR and preeclampsia have been demonstrated to be significantly associated with the development of several pathologies in neonates, such as PHN^[9]. IUGR neonates show prematurity and related immediate medical problems, and a notable susceptibility for hypertension, PH, cardiovascular diseases, diabetes, and neurodegenerative diseases in adult age, likely through the effects of foetal programming.

The principal target of foetal programming: the endothelium

Endothelial dysfunction is the pathological condition associated with the onset of many pathologies related not only to the cardiovascular system, but also to the cardio-pulmonary circuit, as well as to developmental of disease in other tissues, principally correlated to the ageing process. This close relationship derives from the function of endothelium itself, which is the essential element of stroma of all tissues. Our and other groups emphasise this concept as being of great importance in understanding the complex pathophysiology of diseases, such as those of the cardiovascular and pulmonary systems^[10-13].

Endothelium has been demonstrated to be the major target of fetal programming. Its alterations and dysfunction primordially seem to originate from adverse parental and foetal environment conditions. Accordingly, recent experimental data have demonstrated that the levels of vascular endothelial growth factor (VEGF), its receptors and transcription factors, useful for the correct maturation and differentiation of foetal endothelium, are modulated by several adverse conditions, such as chronic hypoxia, maternal food restriction, altered levels of glucocorticoids, and microRNA^[14]. Other findings report that chronic hypoxia and altered maternal clinical conditions can impact the maturation and differentiation of endothelium and the vasculature of all the tissue districts, ranging from foeto-placental arteries, carotid arteries, myocardium, to cerebrovascular systems, renal, liver, and PAs^[15-17]. Another study also demonstrates that the IUGR condition in rats affects the function of both endothelium cells and their progenitors. Specifically, IUGR appears to induce vasodilatation, by modulating the expression and function of some molecules and pathways, such as acetylcholine and nitric oxide (NO) pathways, respectively^[18]. Metabolic alterations related to IUGR condition have been also demonstrated to contribute to an altered development of endothelium and its dysfunction^[19]. Musa *et al.*^[20], have recently reviewed the results from about 230 studies, on the key role of maternal and intrauterine conditions on endothelium structural and function of offspring, by reporting all the related alterations mechanisms and pathways involved.

PHN AND FOETAL PROGRAMMING: FOCUS ON MECHANISMS AND PATHWAYS INVOLVED

One of five PH groups, established by 2015 ECS/ERS 2015 guidelines^[3], consists of PH diseases related to an

Table 1. Mechanisms and pathways identified using apposite PHN models

Mechanisms and pathways	Models
Functional reductions in soluble guanylyl cyclase (sGC) function and cyclic guanosine monophosphate (cGMP)-dependent vasorelaxation	Utero ligation of the ductus arteriosus and chronic perinatal hypoxia in sheep fetuses and newborns
Increased phosphodiesterase type 5 (PDE5) and enhanced Endothelin-1 (ET-1) contraction;	
Significant decrease of levels of endothelial nitric oxide synthase (eNOS)	
Significant reduction in calcium activated potassium channels (BKCa)	
Increase of these last molecules in chronic hypoxia	Antenatal and/or postnatal hypoxic exposure in mice
Important pulmonary arterial pressure (P_{PA})	
Altered relaxation and augmented contractility of pulmonary arteries (PAs)	
Hyperplasia of pulmonary arterial smooth muscle cells (PASMCs) and improved actin polymerization, and adventitial fibroblast proliferation.	
Rare group of the microvasculature and augmented smooth muscle actin expression in distal PAs, changes that are associated with down-regulation of the bone morphogenetic proteins (BMP) signaling pathway in affected lungs	Short-term hyperoxia in mice: a model of bronchopulmonary dysplasia (BPD)
Persistent alterations in lung structure.	
Vascular defects, predisposing the lung to PH later in life, while in neonates it induces an adaptive mechanism, which occurs in the right ventricular (RV) increasing the tolerance	Neonatal hyperoxia
Oxidative stress: high levels of reactive oxygen species (ROS) related to hyperoxia, mechanical ventilation, hypoxia, and inflammation	High altitude and assisted reproductive technologies (ART)
Epigenetic alterations	Maternal undernutrition
Systemic vascular dysfunction in the progeny from both animals and humans. This seems to be associated with an increase of ROS in placenta, which induce epigenetic alterations, such as lung DNA methylation epigenetic mechanisms, such as an altered DNA methylation and gene expressions of conserved pathways, such as Notch pathway	
End-products of endothelial-derived nitric oxide (NO) heme-oxidation, nitrate and nitrite produce exogenous NO, which mediates an increased vasoactive signaling activity during hypoxia and stress	Alteration in maternal microbiome

altered development of pulmonary microvasculature, including PHN, which affects newborns. Offspring with PHN have aberrantly reactive or overly muscular vessels and show acute and chronic states of PHN, characterized by difficulty to adapt to breathing during the birth transition and early postnatal period. PHN is associated with a high morbidity and mortality^[3]. The etiology's factors of PHN are diverse, ranging from high-altitude living, maternal malnutrition, placental insufficiency due to environmental factors or diseases, such as preeclampsia, to other pregnancy complications, such as infections (i.e., staphylococcus infection) or drugs^[3]. The number of newborns affected by PHN might increase, given the rise in adverse environmental factors or other causes in our Western society. Consequently, the investigations for identifying mechanisms and pathways are imperative. Of note are the experimental investigations on animal models, given the inadequate availability of patient tissues and inability to perform mechanistic studies in humans. Several animal PH's models have been developed for performing studies into the functional and structural changes, which occur during the development of pulmonary circulation and PH^[21-34]. Unfortunately, to date not a single preclinical model perfectly replicates human PH. Nonetheless, the models used provide the opportunity to characterize the development and progression of PH, to perform mechanistic studies, and to evaluate potential therapeutic treatments. In addition, the developed models could also permit to identify the mechanisms and pathways involved, which appear to be dependent on the type and grade of stress to which the fetus is subjected. They are illustrated in [Table 1](#).

The multitude of models reported in literature is described in detail in the next paragraph, as well as the mechanisms and pathways identified and reported in [Table 1](#).

The relevant models for PHN

Some relevant models for PHN have been developed and studied. Of note are the results obtained by the utero ligation of the ductus arteriosus and chronic perinatal hypoxia in sheep fetuses and newborns^[22]. They have demonstrated that the mechanisms associated with PH are dependent on the type and grade of stress to which the fetus is subjected^[22]. Specifically, similarities were observed between the ligation and

hypoxia models, in functional reductions in soluble guanylyl cyclase (sGC) function, cyclic guanosine monophosphate (cGMP)-dependent vasorelaxation, increased phosphodiesterase type 5 (PDE5) and enhanced Endothelin-1 (ET-1) contraction. In contrast, significant differences were found in the cellular processes between the two models: a significant decrease in the levels of endothelial nitric oxide synthase (eNOS) and calcium activated potassium channels (BKCa) in ligation models; an increase of these molecules in chronic hypoxia^[22]. Other models for PHN are the exposure-based models, including short-term neonatal hyperoxia, fetal and/or post-natal hypoxia and a two-hit model of prenatal hypoxia followed by postnatal hyperoxia. Among these, the antenatal and/or postnatal hypoxic exposure in mice are significantly associated with the onset of an important pulmonary arterial pressure (P_{PA}), altered relaxation and augmented contractility of PAs, hyperplasia of pulmonary arterial smooth muscle cells (PASMCs) and improved actin polymerization, and adventitial fibroblast proliferation^[23-25]. Short-term hyperoxia in mice, which is used as typical model of bronchopulmonary dysplasia (BPD), impacts the microvasculature and shows an augmented smooth muscle actin expression in distal PAs. These changes are also associated with the down-regulation of the bone morphogenetic proteins (BMP) signaling pathway in affected lungs^[26]. Relevant are the recent findings on short-term neonatal exposure to hyperoxia. This condition is characterized to induce not directly PH, but it may predispose adults to PH^[27], because of persistent alterations in lung structure. On the contrary, neonatal hyperoxia has been demonstrated to be advantageous for right ventricular (RV) hypertrophy. This has led to the hypothesis that this exposure shows two different effects: it can cause vascular defects, predisposing the lung to PH later in life, while in neonates it induces an adaptive mechanism, which occurs in the RV, increasing the tolerance^[27]. Other models are the high altitude^[28] and assisted reproductive technologies (ART)^[29], that have represented the models typically used by the Sartori group. They have consented to evidence the role of oxidative stress and/or epigenetic alterations during foetal programming and the altered onset of pulmonary circulation^[30,31]. Consistent with these efforts and advances, reactive oxygen species (ROS) seem to have an important role in the pathogenesis of neonatal pulmonary vascular diseases, such as PHN. High levels of ROS may be produced in conditions of hyperoxia, mechanical ventilation, hypoxia, and inflammation. These data may be of crucial relevance in individuals born premature, who show a high risk of the long-term complications of pulmonary vascular diseases, thereby contributing to the increase of incidence of adult cardiovascular disease^[32]. Maternal undernutrition^[31] during pregnancy has been used as another model for identifying the fundamental mechanisms, which occur in the altered development of pulmonary circulation and the onset of PHN^[31]. This condition provokes systemic vascular dysfunction in the progeny from both animals and humans. Precisely, in rats, restrictive diet during pregnancy (RDP) raises oxidative stress in the placenta. ROS induce epigenetic alterations and can cross the placental barrier. An altered lung DNA methylation has been detected and is correlated with pulmonary vascular dysfunction. This datum has been confirmed using the treatment with histone deacetylase inhibitors butyrate and trichostatin A in RDP newborns^[31]. These results suggest that the condition of undernutrition during gestation can contribute to an altered development of the cardiopulmonary system and the consequent onset of vascular dysfunction in the new generation through the actions of epigenetic mechanisms, such as an altered DNA methylation and gene expressions of conserved pathways, such as Notch pathway^[31]. Accordingly, another investigation has evidenced the key importance of Notch pathway in the developmental alterations of cardiopulmonary circuit and the onset of PHN^[33]. Recent evidence also underlines a key role of maternal microbiome in the onset of cardiac and pulmonary vascular diseases, such as PHN^[34]. Specifically, it has been reported that end-products of endothelial-derived NO heme-oxidation, nitrate and nitrite produce exogenous NO, which mediates an increased vasoactive signaling activity during hypoxia and stress. The levels of nitrate and nitrite depend on the enzymatic reduction of nitrate to nitrite by bacterial nitrate reductase enzymes, expressed by precise bacterial gut populations^[34]. Such as result, PH seems to be related to alterations in NO signaling, by suggesting a role of commensal oral bacteria in contributing to the onset of PH through the formation of nitrite, NO and other bioactive nitrogen oxides^[34]. This evidence is supported by oral supplementation with inorganic nitrate or nitrate-containing foods, which are shown to have pleiotropic, useful vascular effects in the setting of inflammation, endothelial dysfunction,

ischemia-reperfusion injury and in pre-clinical models of PH. Furthermore, the traditional high-nitrate diet is associated with beneficial outcomes in hypertension, obesity and cardiovascular diseases^[34].

POTENTIAL MEASURES OF INTERVENTIONS FOR IMPROVING FOETAL PROGRAMMING AND THE DEVELOPMENT OF TARGET BODY SYSTEMS?

It is well established that adverse events, taking place during the early periods of human development, mediate deleterious effects on health and disease molecular patterns of new progeny over his life course. This concept of health and disease origin^[8] is also leading to the study of the existence of relationships between the improving of foetal programming and the change of one determined lifestyle behaviour. For example, including the quantity of food intake and its quality, oral supplementation of prebiotics and/or probiotics, drug molecules, feeding preferences or willingness to engage in physical activities, *etc.*^[35]. Beneficial results from use of their administration could lead to propose them as very programs of health, like to those recently projected by our institutions and organizations for improving “health” in our growing aged society having a high susceptibility for age-related diseases, such as cardiovascular diseases, pulmonary diseases, type 2 diabetes, cancer and neurodegenerative diseases. Certainly, their results could also prove unsuccessful because of the free will that characterizes the human nature in assuming choices on the behaviours to adopt. This limits their application, and it makes more difficult the understanding that the behaviours of everyone may also be the result of genetic and neurologic determinants mixed with the environment, during the process of neurodevelopment. Another limitation might be the choice of tool for measuring the consequent beneficial or lack of beneficial effects. One tool or parameter might be represented by fetal growth. Poor fetal growth is significantly associated with the onset of many diseases mentioned above. Specifically, IUGR represents a typical model, and is caused by, but not restricted to, placental insufficiency, maternal malnutrition and smoking, congenital infections and anomalies, drugs, obesity and chromosomal abnormalities. In low and middle-income countries, IUGR achieves the proportion of 27%.

Some interventions are described in the subsequent paragraphs, stressing their advantageous effects.

Physical activity

Physical activity has been demonstrated to have many beneficial effects on diverse pathological conditions. In addition, it improves not only the physical health, but also the mental health. As stressed in my recent review, it represents a very useful intervention in old people^[35]. In addition, exercise increases brain connectivity between the frontal, posterior, and temporal cortices, influences hippocampal volume and serum levels of brain-derived neurotrophic factor (BDNF)^[36], a mediator of neurogenesis in the dentate gyrus of the hippocampus^[36]. From a neurobiological standpoint, exercise modulates the production and release of cortisol, endocannabinoids, BDNF, dopamine and serotonin^[36]. Specifically, the transient stress's response related to physical exercise evokes inhibitory effects from the secreted cortisol upon the hypothalamus and pituitary through medial prefrontal cortex receptors and reduces stress-induced over-excitability of the amygdala^[35,36]. Moreover, aerobic exercise decreases the quantity of competitive amino acids during muscle uptake, and enhances tryptophan's chances of crossing the blood-brain barrier, and consequently it mediates the augmentation of serotonin, an important neurotransmitter for emotional processing, satiety and memory functions^[35,36]. These might represent some of the potential targets of future research, particularly the study of the neurobiological effects of exercise in susceptible populations such as those born with IUGR^[35,36]. Consequently, further research might benefit from recruiting IUGR individuals as appropriate subjects for evaluating interventions on physical activity in well designed, large-scale longitudinal studies, for detecting potential beneficial effects. Furthermore, basic neurobiological examinations on the effects of exercise in this group might also be performed. They could facilitate the identification of the mechanisms that better adapt to such interventions^[35,36].

Microbiome interventions

Altered human microbiome, and particularly gut microbiome, is recognized to be significantly associated with the onset of various inflammatory diseases (i.e., age-related diseases)^[35]. In addition, today, it is also linked with spontaneous preterm birth and other adverse pregnancy outcomes [Table 1]. Accordingly, it is suggesting that the administration of probiotics and/or prebiotics can ameliorate several immune and inflammatory parameters. This is demonstrating that manipulation of gut microbiome may result in beneficial effects^[35]. In addition, a change in food intake and its quality are also proposed to be beneficial (see the next paragraph).

Mediterranean diet

The diet, and particularly the Mediterranean diet, represents a very advantageous intervention for the health of people, and some recent studies also confirm such relevance in both pregnancy and new-borns^[35,36]. Accordingly, a recent investigation has evaluated the effects of adherence to a Mediterranean diet in 997 mother-child pairs from Project Viva in Massachusetts, USA, and 569 pairs from the Rhea study in Crete, Greece. This large study has demonstrated that greater adherence to Mediterranean diet during pregnancy may protect against cardio/pulmonary damage in offspring^[37]. Similar data have been obtained in a study performed in 728 pregnant women who assumed a Mediterranean diet, and enrolled from TIMOUN Mother-Child Cohort Study conducted in Guadeloupe (French West Indies) between 2004 and 2007^[38]. In addition, some studies suggest that the women who prepare for pregnancy, and particularly adolescent pregnancies, should benefit of an appropriate nutrition and diet for decreasing adverse maternal and new-born outcomes^[39]. Although early life may be imperative for baby development, meticulous studies are essential to show the advantages to obtain using prenatal or postnatal diet's supplementation.

Melatonin and metformin

Useful results might derive by other approaches. Recently, it has been reported that the administration of antioxidants and anti-remodeling agents associated with the vasodilator therapies could have beneficial effects in new-borns^[40-46]. From this point of view, melatonin (N-acetyl-5-methoxytryptamine, an indoleamine molecule), appears to be an effective agent for PHN, markedly improving pulmonary vascular function. However, the data until now obtained derive only from animal studies. Consequently, future studies should be performed for: (1) optimizing doses and/or therapeutic windows to improve the functional and anti-remodeling effects in animal models, as preclinical studies ; and (2) extending these investigations to human pregnant women and new-borns^[40-46].

Another promising treatment might be the metformin, one drug normally used in diabetes therapy, even if its effects in pregnancy have been experimented only in the treatment of gestational diabetes^[47]. Consequently, more studies are needed to provide more evidence for the future use of metformin.

CONCLUSIONS AND PERSPECTIVES

Growing evidence from epidemiological, clinical and experimental studies has clearly revealed a close relationship between adverse in utero environment and the augmented risk of diverse diseases, such as PH in later life^[8]. Fetal stressors, such as hypoxia, high-altitude, malnutrition, and fetal exposure to nicotine, alcohol, cocaine and glucocorticoids can directly or indirectly act at cellular and molecular levels, by altering the cardio/pulmonary development and resulting in programming of heightened cardio/pulmonary vulnerability to diverse pathologies, such as PH^[8]. The underlying mechanisms and pathways are not completely identified. However, crucial is the role of epigenetic mechanisms in fetal origin^[9,48]. Predictably, pharmacological manipulations of epigenetic mechanisms present a promising interventional strategy. Indeed, several experimental studies in animals have offered exciting results, by using DNA methylation inhibitors and other agents, such as plant-derived isoflavone genistein, leptin, folate, fish oil, omega-3 and

vitamin D. Specifically, they can alter the corresponding abnormal epigenetic modification status and improve the adverse programming effects caused by prenatal stress^[49]. Advantageous effects have been obtained by modifying the diet and physical exercise, and performing interventions on the gut microbiome, as abovementioned. Furthermore, it has estimated, across gestation, that genetic and environmental influences vary during the various sensitive periods of pregnancy^[50]. Accordingly, environmental factors have stronger influence on fetal growth at early end of first trimester, but are overtaken by genetic influences in late gestation. In addition, the fetal growth can be used as an optimal tool for measuring and estimating the effects.

Meticulous studies are indispensable to improve our knowledge in this field and the development of appropriate treatments, such as prenatal or postnatal supplementations. This might facilitate the reduction of the incidence and prevalence of this disease in new generations. Currently, such preventive measures are limited. However, it is well established until now that some surgical treatments, including both balloon pulmonary angioplasty (BPA) and pulmonary endarterectomy (PEA), can improve long-term survival, clinical status and hemodynamics in patients affected by this pathology. BPA is preferentially used for inoperable chronic thromboembolic pulmonary hypertension (CTEPH) or residual PH after PEA. PEA is, indeed, performed for operable CTEPH. Recent systematic reviews suggest their efficacy and safety^[51,52].

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Suresh K, Shimoda LA. Lung circulation. *Compr Physiol* 2016;6:897-943.
2. Tudor RM. Pulmonary vascular remodeling in pulmonary hypertension. *Cell Tissue Res* 2017;367:643-9.
3. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: the joint task force for the diagnosis and treatment of pulmonary hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Respir J* 2015;46:903-75.

4. Guignabert C, Tu L, Girerd B, Ricard N, Huertas A, Montani D, Humbert M. New molecular targets of pulmonary vascular remodeling in pulmonary arterial hypertension: importance of endothelial communication. *Chest* 2015;147:529-37.
5. Batton KA, Austin CO, Bruno KA, Burger CD, Shapiro BP, Fairweather D. Sex differences in pulmonary arterial hypertension: role of infection and autoimmunity in the pathogenesis of disease. *Biol Sex Differ* 2018;9:15.
6. Machado RD, Southgate L, Eichstaedt CA, Aldred MA, Austin ED, Best DH, Chung WK, Benjamin N, Elliott CG, Eyries M, Fischer C, Gräf S, Hinderhofer K, Humbert M, Keiles SB, Loyd JE, Morrell NW, Newman JH, Soubrier F, Trembath RC, Viales RR, Grünig E. Pulmonary arterial hypertension: a current perspective on established and emerging molecular genetic defects. *Hum Mutat* 2015;36:1113-27.
7. Gao Y, Cornfield DN, Stenmark KR, Thébaud B, Abman SH, Raj JU. Unique aspects of the developing lung circulation: structural development and regulation of vasomotor tone. *Pulm Circ* 2016;6:407-25.
8. Barker DJ. The developmental origins of adult disease. *J Am Coll Nutr* 2004;23:588S-95S.
9. Keverne EB. Genomic imprinting, action, and interaction of maternal and fetal genomes. *Proc Natl Acad Sci U S A* 2015;112:6834-40.
10. Balistreri CR (Ed.). Endothelial progenitor cells (EPCs) in ageing and age-related diseases: from their physiological and pathological implications to translation in personalized medicine. *Mech Ageing Dev* 2016;159:1-80.
11. Balistreri CR. Endothelial progenitor cells. Basel: Springer International Publishing; 2017. p. 1-80.
12. Regina C, Panatta E, Candi E, Melino G, Amelio I, Balistreri CR, Annicchiarico-Petruzzelli M, Di Daniele N, Ruvolo G. Vascular ageing and endothelial cell senescence: molecular mechanisms of physiology and diseases. *Mech Ageing Dev* 2016;159:14-21.
13. Madonna R, Novo G, Balistreri CR. Cellular and molecular basis of the imbalance between vascular damage and repair in ageing and age-related diseases: as biomarkers and targets for new treatments. *Mech Ageing Dev* 2016;159:22-30.
14. Pearce WJ, Khorram O. Maturation and differentiation of the fetal vasculature. *Clin Obstet Gynecol* 2013;56:537-48.
15. Adeoye OO, Bouthors V, Hubbell MC, Williams JM, Pearce WJ. VEGF receptors mediate hypoxic remodeling of adult ovine carotid arteries. *J Appl Physiol* (1985) 2014;117:777-87.
16. Yzydorczyk C, Armengaud JB, Peyter AC, Chehade H, Cachat F, Juvet C, Siddeek B, Simoncini S, Sabatier F, Dignat-George F, Mitancher D, Simeoni U. Endothelial dysfunction in individuals born after fetal growth restriction: cardiovascular and renal consequences and preventive approaches. *J Dev Orig Health Dis* 2017;8:448-64.
17. Muñoz-Muñoz EC, Krause BJ, Uauy R, Casanello P. LGA-newborn from patients with pregestational obesity present reduced adiponectin-mediated vascular relaxation and endothelial dysfunction in fetoplacental arteries. *J Cell Physiol* 2018;233:6723-33.
18. Oliveira V, de Souza LV, Fernandes T, Junior SDS, de Carvalho MHC, Akamine EH, Michelini LC, de Oliveira EM, Franco MDC. Intrauterine growth restriction-induced deleterious adaptations in endothelial progenitor cells: possible mechanism to impair endothelial function. *J Dev Orig Health Dis* 2017;8:665-73.
19. Menendez-Castro C, Rascher W, Hartner A. Intrauterine growth restriction-impact on cardiovascular diseases later in life. *Mol Cell Pediatr* 2018;5:4.
20. Musa MG, Torrens C, Clough GF. The microvasculature: a target for nutritional programming and later risk of cardio-metabolic disease. *Acta Physiol (Oxf)* 2014;210:31-45.
21. Berger J, Bhandari V. Animal models of bronchopulmonary dysplasia. The term mouse models. *Am J Physiol Lung Cell Mol Physiol* 2014;307:L936-47.
22. Papamatheakis DG, Chundu M, Blood AB, Wilson SM. Prenatal programming of pulmonary hypertension induced by chronic hypoxia or ductal ligation in sheep. *Pulm Circ* 2013;3(4):757-80.
23. Blood AB, Terry MH, Merritt TA, Papamatheakis DG, Blood Q, Ross JM, Power GG, Longo LD, Wilson SM. Effect of chronic perinatal hypoxia on the role of rho-kinase in pulmonary artery contraction in newborn lambs. *Am J Physiol Regul Integr Comp Physiol* 2013;304:R136-46.
24. Fediuk J, Sikarwar AS, Nolette N, Dakshinamurti S. Thromboxane-induced actin polymerization in hypoxic neonatal pulmonary arterial myocytes involves Cdc42 signaling. *Am J Physiol Lung Cell Mol Physiol* 2014;307:L877-87.
25. Yang Q, Lu Z, Ramchandran R, Longo LD, Raj JU. Pulmonary artery smooth muscle cell proliferation and migration in fetal lambs acclimatized to high-altitude long-term hypoxia: role of histone acetylation. *Am J Physiol Lung Cell Mol Physiol* 2012;303:L1001-10.
26. Yee M, White RJ, Awad HA, Bates WA, McGrath-Morrow SA, O'Reilly MA. Neonatal hyperoxia causes pulmonary vascular disease and shortens life span in aging mice. *Am J Pathol* 2011;178:2601-10.
27. Goss KN, Cucci AR, Fisher AJ, Albrecht M, Frump A, Tursunova R, Gao Y, Brown MB, Petrache I, Tepper RS, Ahlfeld SK, Lahm T. Neonatal hyperoxic lung injury favorably alters adult right ventricular remodeling response to chronic hypoxia exposure. *Am J Physiol Lung Cell Mol Physiol* 2015;308:L797-806.
28. Scherrer U, Allemann Y, Rexhaj E, Rimoldi SF, Sartori C. Mechanisms and drug therapy of pulmonary hypertension at high altitude. *High Alt Med Biol* 2013;14:126-33.
29. Allemann Y, Stuber T, de Marchi SF, Rexhaj E, Sartori C, Scherrer U, Rimoldi SF. Pulmonary artery pressure and cardiac function in children and adolescents after rapid ascent to 3,450 m. *Am J Physiol Heart Circ Physiol* 2012;302:H2646-53.
30. Sartori C, Rimoldi SF, Rexhaj E, Allemann Y, Scherrer U. Epigenetics in cardiovascular regulation. *Adv Exp Med Biol* 2016;903:55-62.
31. Rexhaj E, Bloch J, Jayet PY, Rimoldi SF, Dessen P, Mathieu C, Tolsa JF, Nicod P, Scherrer U, Sartori C. Fetal programming of pulmonary vascular dysfunction in mice: role of epigenetic mechanisms. *Am J Physiol Heart Circ Physiol* 2011;301:H247-52.
32. de Wijs-Meijler DP, Duncker DJ, Tibboel D, Schermuly RT, Weissmann N, Merkus D, Reiss IKM. Oxidative injury of the pulmonary circulation in the perinatal period: short- and long-term consequences for the human cardiopulmonary system. *Pulm Circ* 2017;7:55-66.
33. Hussain M, Xu C, Ahmad M, Yang Y, Lu M, Wu X, Tang L, Wu X. Notch signaling: linking embryonic lung development and asthmatic

- airway remodeling. *Mol Pharmacol* 2017;92:676-93.
34. Koch CD, Gladwin MT, Freeman BA, Lundberg JO, Weitzberg E, Morris A. Enterosalivary nitrate metabolism and the microbiome: intersection of microbial metabolism, nitric oxide and diet in cardiac and pulmonary vascular health. *Free Radic Biol Med* 2017;105:48-67.
 35. Balistreri CR. Anti-inflamm-ageing and/or anti-age-related disease emerging treatments: a historical alchemy or revolutionary effective procedures? *Mediators Inflamm* 2018;2018:3705389.
 36. Nasello M, Schirò G, Crapanzano F, Balistreri CR. Stem cells and other emerging agents as innovative “drugs” in neurodegenerative diseases: benefits and limitations. *Rejuvenation Res* 2018;21:123-40.
 37. Chatzi L, Rifas-Shiman SL, Georgiou V, Joung KE, Koinaki S, Chalkiadaki G, Margioris A, Sarri K, Vassilaki M, Vafeiadi M, Kogevas M, Mantzoros C, Gillman MW, Oken E. Adherence to the Mediterranean diet during pregnancy and offspring adiposity and cardiometabolic traits in childhood. *Pediatr Obes* 2017;12:47-56.
 38. Saunders L, Guldner L, Costet N, Kadhel P, Rouget F, Monfort C, Thomé JP, Multigner L, Cordier S. Effect of a Mediterranean diet during pregnancy on fetal growth and preterm delivery: results from a French Caribbean Mother-Child Cohort Study (TIMOUN). *Paediatr Perinat Epidemiol* 2014;28:235-44.
 39. Christian P, Mullany LC, Hurley KM, Katz J, Black RE. Nutrition and maternal, neonatal, and child health. *Semin Perinatol* 2015;39:361-72.
 40. Astorga CR, González-Candia A, Candia AA, Figueroa EG, Cañas D, Ebensperger G, Reyes RV, Llanos AJ, Herrera EA. Melatonin decreases pulmonary vascular remodeling and oxygen sensitivity in pulmonary hypertensive newborn lambs. *Front Physiol* 2018;9:185.
 41. Torres F, González-Candia A, Montt C, Ebensperger G, Chubretovic M, Serón-Ferré M, Reyes RV, Llanos AJ, Herrera EA. Melatonin reduces oxidative stress and improves vascular function in pulmonary hypertensive newborn sheep. *J Pineal Res* 2015;58:362-73.
 42. Maarman GJ. Natural antioxidants as potential therapy, and a promising role for melatonin against pulmonary hypertension. *Adv Exp Med Biol* 2017;967:161-78.
 43. Hung MW, Yeung HM, Lau CF, Poon AMS, Tipoe GL, Fung ML. Melatonin attenuates pulmonary hypertension in chronically hypoxic rats. *Int J Mol Sci* 2017;18:pii: E1125.
 44. Sun H, Gusdon AM, Qu S. Effects of melatonin on cardiovascular diseases: progress in the past year. *Curr Opin Lipidol* 2016;27:408-13.
 45. Maarman G, Blackhurst D, Thienemann F, Blauwet L, Butrous G, Davies N, Sliwa K, Lecour S. Melatonin as a preventive and curative therapy against pulmonary hypertension. *J Pineal Res* 2015;59:343-53.
 46. Jin H, Wang Y, Zhou L, Liu L, Zhang P, Deng W, Yuan Y. Melatonin attenuates hypoxic pulmonary hypertension by inhibiting the inflammation and the proliferation of pulmonary arterial smooth muscle cells. *J Pineal Res* 2014;57:442-50.
 47. Zhu B, Zhang L, Fan YY, Wang L, Li XG, Liu T, Cao YS, Zhao ZG. Metformin versus insulin in gestational diabetes mellitus: a meta-analysis of randomized clinical trials. *Ir J Med Sci* 2016;185:371-81.
 48. Wang Y, Yan L, Zhang Z, Prado E, Fu L, Xu X, Du L. Epigenetic regulation and its therapeutic potential in pulmonary hypertension. *Front Pharmacol* 2018;9:241.
 49. Li Y, Gonzalez P, Zhang L. Fetal stress and programming of hypoxic/ischemic-sensitive phenotype in the neonatal brain: mechanisms and possible interventions. *Prog Neurobiol* 2012;98:145-65.
 50. Workalemahu T, Grantz KL, Grewal J, Zhang C, Louis GMB, Tekola-Ayele F. Genetic and environmental influences on fetal growth vary during sensitive periods in pregnancy. *Sci Rep* 2018;8:7274.
 51. Tanabe N, Kawakami T, Satoh T, Matsubara H, Nakanishi N, Ogino H, Tamura Y, Tsujino I, Ogawa A, Sakao S, Nishizaki M, Ishida K, Ichimura Y, Yoshida M, Tatsumi K. Balloon pulmonary angioplasty for chronic thromboembolic pulmonary hypertension: a systematic review. *Respir Investig* 2018;56:332-41.
 52. Tromeur C, Jais X, Mercier O, Couturaud F, Montani D, Savale L, Jevnikar M, Weatherald J, Sitbon O, Parent F, Fabre D, Mussot S, Dartevelle P, Humbert M, Simonneau G, Fadel E. Factors predicting outcome after pulmonary endarterectomy. *PLoS One* 2018;13:e0198198.

Review

Open Access



Complications of transcatheter aortic valve replacement and rescue attempts

Burak Can Depboylu¹, Serkan Yazman², Bugra Harmandar¹

¹Department of Cardiovascular Surgery, Mugla Sitki Kocman University, Faculty of Medicine, Mugla 48000, Turkey.

²Cardiovascular Surgery Clinic, Mugla Sitki Kocman University Education and Research Hospital, Mugla 48000, Turkey.

Correspondence to: Dr. Burak Can Depboylu, Department of Cardiovascular Surgery, Mugla Sitki Kocman University, Faculty of Medicine, Mugla 48000, Turkey. E-mail: burakdepboylu@mu.edu.tr

How to cite this article: Depboylu BC, Yazman S, Harmandar B. Complications of transcatheter aortic valve replacement and rescue attempts. *Vessel Plus* 2018;2:26. <http://dx.doi.org/10.20517/2574-1209.2018.39>

Received: 29 May 2018 **First Decision:** 6 Aug 2018 **Revised:** 17 Aug 2018 **Accepted:** 23 Aug 2018 **Published:** 21 Sep 2018

Science Editors: Mario F. L. Gaudino, Cristiano Spadaccio **Copy Editor:** Yuan-Li Wang **Production Editor:** Huan-Liang Wu

Abstract

As a novel treatment modality, transcatheter aortic valve replacement (TAVR) is widely used for patients with severe aortic valve stenosis who have high surgical risk worldwide. However, this promising alternative procedure has different types of complication risks including, cerebrovascular events, vascular complications, bleeding, coronary obstruction, myocardial infarction, valve regurgitation, valve malpositioning or migration, conduction disturbances and acute kidney injury which may occur during and/or after the procedure. These complications may be seen up to one third of the patients and some of them may need urgent surgical intervention and may have a higher risk of death. For preventing and overcoming these complications, pre-procedural evaluation of the patient by an effective “heart team” which consists of cardiologists, cardiac surgeons, radiologists and anesthesiologists in equal proportion is needed. Estimating the potential difficulties and complications, deciding the interventions to be performed in case of any complication may increase the success of the procedure and save the patients' lives. In this article, we reviewed the possible complications of the TAVR procedure and described rescue procedures in case of complications, in the context of the literature.

Keywords: Aortic valve stenosis, transcatheter aortic valve replacement, risk factors, complication, surgery, catheter

INTRODUCTION

Transcatheter aortic valve replacement (TAVR) was firstly described as catheter-based implantation of a crimped valve, to the stenotic native aortic valve via transapical access-antegrade approach in 2002, as an alternative treatment method to surgical aortic valve replacement (SAVR) for patients who have high or prohibitive surgical risk^[1,2]. Since 2002, TAVR is used increasingly all over the world. By the time, different access sites have been described and different TAVR valves have been developed [Figures 1 and 2].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Access sites and approaches for TAVR		
Access site	Approaches	
1- Trans-femoral access	Retrograde	
2- Trans-subclavian access	Retrograde	
3- Direct aortic access	Retrograde	
4- Trans-carotid access	Retrograde	
5- Trans-apical access	Antegrade	

Figure 1. Access sites and approaches for transcatheter aortic valve replacement

Although its efficacy has been proven in patients with aortic valve stenosis having high surgical risks, as a less invasive catheterization procedure, it has varying types of complications that may increase morbidity, require urgent surgical intervention and even cause death. These complications can occur anytime during and/or after the procedure, and include cerebrovascular events, vascular complications, bleeding, coronary obstruction, myocardial infarction, valve regurgitation, valve malpositioning or migration, conduction disturbances and acute kidney injury. With the advances in medical equipment and systems, improvements in procedural techniques together with increasing experience and advances in patients' imaging, these procedural complications decreased dramatically. However, if occur, complications still remain the major factors affecting the success of the procedure. To prevent and/or overcome these complications, all TAVR patients should be evaluated by the "heart team" which consists of cardiologists, cardiac surgeons, radiologists and anesthesiologists in equal proportion. The risks and/or difficulties of anesthesia and SAVR procedure should be put forth by the cardiac surgeons and anesthesiologists, and declared to the patient. Once the decision of performing TAVR procedure has been taken, structures and calcification loads of the aortic valve, aortic annulus, aorta and access vessels should be evaluated by cardiologists, cardiac surgeons and radiologists via CT images, angiogram and echocardiographic findings. The TAVR valve planned to be used, potential difficulties of the procedure and possible complications should be determined and in case of complications, rescue attempts should be planned before the procedure by the "heart team". The procedure should be performed in a hybrid operating room and surgical backup should be available whenever needed.

However, in all centers where TAVR is performed, it seems that a heart team with equal participation of specialists is not established and managed. Performing the procedure under this inappropriate condition may cause doctors to inform the procedure as a risk-free intervention to the patients, to get out of the TAVR indications such as performing the procedure according to the patient's wish only and to be caught unprepared against the complications.

Here, for highlighting pre-procedural evaluation of the patients and being prepared against the complications of TAVR, we reviewed the possible complications of the TAVR procedure and described rescue procedures and/or treatment options in case of complications, in the context of the literature.

VASCULAR COMPLICATIONS

The vascular complications of TAVR may be evaluated under two subheadings.

Minor/major vascular complications

Minor Vascular Complications

Vascular access injuries, those do not cause tissue malperfusion and do not require surgery^[3].

CE-marked TAVR valves			
Self balloon-expandable	Access-approach	Valve	
Self-expandable	Trans-femoral Trans-femoral	Porticovalve™ (Abbott, USA)	
Other design	Trans-femoral	LOTUS™ (Boston Scientific Corp., USA)	
Other design	Trans-femoral Direct-aortic	LOTUS Edge™ (Boston Scientific Corp., USA)	
Self-expandable	Trans-apical	ACURATE TA™ (Boston Scientific Corp., USA- Symetis SA, Ecublens, Switzerland)	
Self-expandable	Trans-femoral	ACURATE neo™ (Boston Scientific Corp., USA- Symetis SA, Ecublens, Switzerland)	
Other design	Trans-femoral	Direct Flow Medical Transcatheter Aortic Valve System (Direct Flow Medical Inc., USA)	
Balloon-expandable	Trans-femoral, trans-apical Trans-femoral, Trans-apical	SAPIEN® (Edwards Lifesciences, Irvine, CA, USA)	
Balloon-expandable	Trans-femoral, Trans-apical Direct-aortic	SAPIEN XT® (Edwards Lifesciences, Irvine, CA, USA)	
Balloon-expandable	Trans-femoral, Trans-apical Direct-aortic	SAPIEN 3® (Edwards Lifesciences, Irvine, CA, USA)	
Other design	Trans-apical	JenaValve™ (JenaValve, Munich, Germany-JenaValve Technology Inc., USA)	
Self-expandable	Trans-femoral	Allegra (NVT GmbH, Germany)	
Self-expandable	Trans-femoral	CoreValve Re Valving System™ (Medtronic Plc, Ireland)	
Self-expandable	Trans-femoral, Direct-aortic Trans-subclavian	CoreValve™ (Medtronic Plc, Ireland)	
Self-expandable	Trans-femoral, Direct-aortic Trans-subclavian	CoreValve Evolut™ (Medtronic Plc, Ireland)	
Self-expandable	Trans-femoral, Direct-aortic Trans-subclavian	CoreValve Evolut-R™ (Medtronic Plc, Ireland)	
Self-expandable	Trans-apical	Engager™ (Medtronic Plc, Ireland)	

Figure 2. CE-marked TAVR valves. TAVR: transcatheter aortic valve replacement

Major Vascular Complications

All other vascular injuries, those cause tissue malperfusion, require blood transfusion over 4 units or surgery.

Heart team has the key role in preventing and/or overcoming major vascular complications. Not only the status of aortic valve and device landing zone, a full evaluation including the status of the access-site, access artery diameter, its stenosis and/or calcification, sharp angulations and/or tortuosity of the conducting arteries, should be done by using computerized tomography and catheter angiography.

Vascular access-site/device landing zone complications

Vascular Access-Site Complications

Vascular access-site complications are mainly caused by the mismatch of access artery and sheaths of delivery system. Sex (female), calcification status of the access artery, ratio of the sheath to access artery diameter (> 1.05)^[4] and the experience of the operator were determined as major predictors of vascular access-site complications^[5,6]. With the improvements in the delivery systems (decreased diameters), improvements in the pre-procedural patient evaluation and increased surgical experience, the vascular access-site complications decreased nowadays^[7]. Despite all the improvements, if the conducting arteries have sharp angulations, tortuosity or untreated aneurysms, and the conducting artery lumen is narrower than 6 mm with calcifications, the trans-femoral, trans-subclavian and trans-carotid accesses are not recommended, instead, trans-apical or direct-aortic accesses should be used.

In case of any complication, angiographic evaluation of the artery may be the urgent diagnosis method and an acute hypotension without other causes may also support the diagnosis of major arterial injury. Urgent endovascular or surgical repair is recommended for treatment.

Device landing zone complications

Rupture of the device landing-zone is a rarely encountered complication (1%), but has a high mortality risk (48%-50%)^[8,9]. The presence of severe annular, sub-annular, left ventricular outflow tract calcifications and valve over sizing were determined as the predictors of this complication^[10]. From the perspective of tissue quality, patients older than 90 years, chronic steroid users and immunosuppressed hosts have a higher risk of annular injury. Device landing-zone complications such as injury, rupture or dissection of aorta, ventricular septal defect and aorto-ventricular fistula are mostly seen in implantation of balloon-expandable valves or in balloon dilatation of a self-expandable valve after implantation^[11]. Smaller annular area ($< 300 \text{ cm}^2$) may increase the annular rupture due to relative valve oversizing^[12]. Also, aggressive oversizing of the prosthesis, may decrease significant aortic regurgitation but induce conduction disorders requiring pacemaker implantations^[13].

In case of complication, trans-esophageal echocardiography may give critical information about new pericardial effusion or tamponade, aortic root injury and aortic dissection. The occurrence of an acute hypotension supports the diagnosis. If the problem is only aortic root hematoma with no rupture, hemodynamic support with inotropes, reversal of anticoagulation, then transfusion of fresh frozen plasma and close observation may be enough. Otherwise, if there is rupture, cardiac tamponade occurs frequently and reversal of anticoagulation, pericardial drainage or surgical repair are recommended^[14].

AORTIC VALVE REGURGITATION

Aortic valve regurgitation is frequently seen after TAVR and can be evaluated under two subheadings.

Paravalvular leak

The incidence of paravalvular leak is 50%-85%. Whilst most of them are mild, moderate and/or severe leaks are seen up to 24%^[15] that increase the mortality of the procedure up to 4 times in the first year^[16,17]. Occur-

rence of paravalvular leaks can be explained by 3 mechanisms: (1) prosthetic valve-annulus size mismatch; (2) inappropriate placement of the prosthetic valve; and (3) incomplete apposition of the stent due to deformed native structure.

Aortic root calcification, its degree and geometric distribution are the main factors affecting the native structure. Asymmetric and severe calcifications may deform the prosthesis resulting in paravalvular leaks. Assessing the aortic root calcification with echocardiographic examination and/or Agatston score, may decrease the risk of paravalvular leak^[18].

The use of self-expandable valves is a major determinant for significant paravalvular leak. The studies have shown that self-expandable valves were associated with moderate-severe paravalvular leak compared with balloon-expandable valves (19.8% vs. 12.2%)^[19].

Central leak

The incidence of moderate or severe central leak is 4.5%-11.7%^[20] and usually occurs due to structural dysfunction of the valve. Central leak can be the result of leaflet restriction or damage, during crimping or implantation as well as over dilatation of the valve^[21]. Post implantation dilatation of the prosthetic valve may also cause central leak^[22].

In case of any complication, aortic root angiography is performed for the quantification of central leak. Intra-procedural echocardiography may be performed for determining the severity of leak and the location of the prosthetic valve. Increase of left ventricular end-diastolic pressure and decrease of aortic diastolic blood pressure also support the diagnosis. If the leak is central, gentle probing of leaflets with a soft wire and/or catheter or delivery of a second prosthetic valve may solve the problem. The management of paravalvular leaks is controversial. Mild degrees may be clinically followed as they are thought to be not progressive. However, more severe degrees of leaks may deserve intervention. Usually, balloon post-dilatation is the first option, using a slightly oversized balloon.

Repositioning of the implanted prosthetic valve, delivery of a second prosthetic valve and percutaneous vascular occlusion devices may be the other choices for the treatment. However, in large and high volume leaks, for implanting the appropriate device, large sheaths may be needed. Particularly in self-expanding prostheses, valve struts and calcification of the annulus may complicate the advancement of delivery systems mainly when using large sheaths^[23].

Otherwise, SAVR should be performed for both types of leaks^[14].

PROSTHETIC VALVE MALPOSITIONING

Valve malpositioning usually occurs during or just after valve implantation. However, rare delayed migration cases together with acute heart failure and/or cardiogenic shock have been reported in literature^[24]. The incidence of the prosthetic valve malpositioning is about 1.3% (CoreValve® 2.3% vs. Edwards SAPIEN® valve 1.0%)^[20]. The predisposing factors for the prosthetic valve malpositioning can be listed as: (1) incorrect assessment of the aortic annulus; (2) incorrect implantation of the prosthetic valve; (3) insufficient or early termination of rapid ventricular pacing; (4) presence of prosthetic mitral valve; and (5) presence of severe mitral annular calcification extending to anterior leaflet and left ventricular outflow tract.

In case of any complication, aortography and trans-esophageal echocardiography are performed for evaluating the position and confirming the malposition or migration of prosthetic valve [Figure 3A and B].

Hemodynamic status of the patient, final position and the type of prosthetic valve determine the treatment. For self-expandable ones, if the prosthetic valve is still attached to the delivery system, it may be re-captured

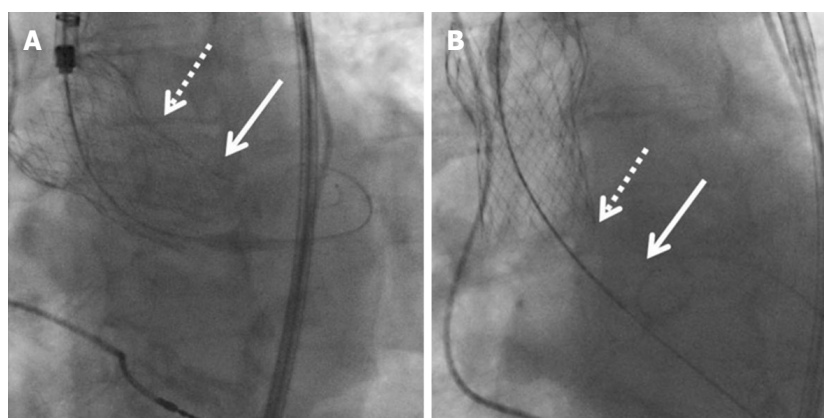


Figure 3. A: An angiographic view of CoreValve® prosthesis before final delivery; B: final position of prosthesis in ascending aorta before emergent surgery (solid arrows show the annulus of the native aortic valve and dotted arrows show the sino-tubular junction)

or deployed to descending aorta. If not, it may be snared in the aortic direction or a second prosthetic valve may be implanted as valve-in-valve. For migration of the balloon expandable ones, prosthetic valve may be pulled to descending aorta via an inflated balloon inside. In case of unsuccessful bailout maneuvers, urgent surgical removal of the prosthetic valve and SAVR should be performed^[14] [Figure 4A and B].

CORONARY OBSTRUCTION

The incidence of coronary obstruction is about 0.8% for the procedures which are performed to native aortic valve and 3.5% for the procedures which are performed to degenerative bioprosthetic aortic valve^[25,26]. The risk factors for coronary obstruction may be listed as: (1) low coronary ostium height (< 12 mm); (2) narrow sinus valsalva; (3) small sinotubular junction; (4) low sinus valsalva height (< 30 mm); (5) bulky calcification of the aortic valve leaflets; and (6) oversized prosthetic valve.

Closure of the coronary ostium by the calcific aortic valve leaflets is the most encountered cause of the coronary obstruction^[27] and also reported to be more frequent in women and in patients with prior surgical bioprosthesis. In the CHOICE trial, two patients belonging to the balloon-expandable valve group had coronary obstruction as opposed to none in the self-expandable valve group^[28].

In case of complication; coronary obstruction manifests itself with acute hypotension, segment (ST) elevation, ventricular arrhythmias and/or cardiac arrest. Because of the high hemodynamic collapse risk, an emergent aortography or selective angiography to the obstructed coronary artery with stent implantation should be performed. The patient may be placed on mechanical circulatory support for allowing the operators to gain time for intervention. Failure of percutaneous coronary intervention indicates the necessity of a coronary bypass grafting operation for the treatment of this complication.

MYOCARDIAL INFARCTION

The incidence of peri-procedural myocardial infarction is about 1.1% (in transapical approach 1.9% vs. in trans-arterial approach 0.8%)^[8,20]. The reasons of peri-procedural myocardial infarction can be listed as^[29]: (1) myocardial ischemia due to rapid ventricular pacing; (2) myocardial ischemia due to hypotension; (3) micro-embolisms to coronary arteries; (4) compression of the myocardium due to expansion of the prosthetic valve; 5. trauma to the ventricular apex in the trans-apical approach.

Presence of chest pain and/or shortness of breath, ST changes, pathological Q wave, hemodynamic instability, ventricular arrhythmia, new or worsened heart failure, elevated levels of cardiac biochemical markers

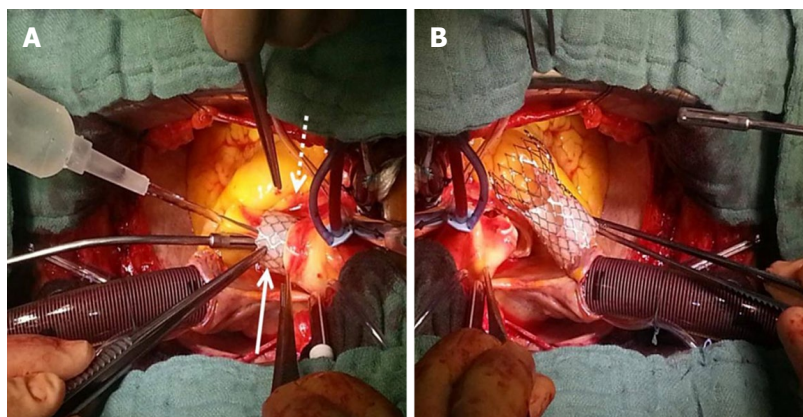


Figure 4. A: An intraoperative view of migrated CoreValve® prosthesis (solid arrow) through aortic incision at the level of sino-tubular junction (dotted arrow). The surgical field is flushed with cold saline solution to soften the rigid nitinol struts of the prosthetic aortic valve for smooth extraction through aortotomy; B: an intraoperative view of migrated Medtronic-CoreValve® prosthesis removed from ascending aorta

(particularly CK-MB) in the post-procedural 72 h, detection of the loss of viable myocardium on imaging and ventricular wall motion abnormality also indicate the peri-procedural myocardial infarction^[3].

In case of such complications, selective coronary angiography and percutaneous coronary interventions should be performed. According to the results, medical treatment and/or coronary artery bypass grafting operation may be the treatment options.

CEREBROVASCULAR COMPLICATIONS

The incidence of strokes and transient ischemic attacks in a month after TAVR procedure vary between 3%-7%^[30,31]. The majority of these cerebrovascular complications (50%-70%) are seen in the first 24 h after the procedure and neither the type of prosthetic valve, nor the access route has any effect over incidence of cerebrovascular complications^[20]. After the TAVR procedure, in one third of the patients, new onset atrial fibrillation may be encountered. The cerebrovascular complications that occur after the first 24 h are thought to be related with this new onset atrial fibrillation^[32]. Studies revealed that the origin of embolic material was usually native aortic valve leaflets or aortic wall^[33]. Thus, avoiding frequent aortic balloon dilatation and limiting the manipulations of large catheters in the aortic arch, were suggested to reduce the cerebrovascular complications^[34].

In case of complication, in large ischemic cerebrovascular events, mechanical retrieval of the embolic material via catheter may be performed. Otherwise, conservative treatment should be performed^[14]. Antiplatelet and anticoagulant agents should be used during and after the procedure. In the presence of newly onset atrial fibrillation, anti-arrhythmic drugs should also be added to the treatment.

BLEEDING

Life-threatening bleeding

Occurrence in critical areas, development of severe hypotension or shock, decrease of hemoglobin value more than 5 g/dL or requirement of red blood cells transfusion more than 4 units, indicate the life-threatening bleeding.

Major bleeding

Bleedings that do not meet the life-threatening bleeding criteria but cause the decrease of hemoglobin value

equal to or more than 3 g/dL and the ones those require 2-3 units of red blood cells transfusion can be defined as major bleeding.

Minor bleeding

All bleedings other than life-threatening and major bleedings can be described as minor bleeding.

Cardiac tamponade due to bleeding to the pericardium is seen in about 3%-4% of the patients who underwent TAVR and causes high rate of death (24%)^[35]. Of the access-site complications, 69% is bleeding and 23%-31% of them are life-threatening ones. Digestive tract, the retro-peritoneum, and the pleura may be listed as the other sources of bleedings.

In case of such complications, the anticoagulation should be reversed and if needed transfusion of fresh frozen plasma and/or red blood cells should be performed. Hemodynamic conditions and hemoglobin levels should be stabilized. If feasible, the source of the bleeding should be treated surgically.

CARDIAC CONDUCTION ABNORMALITIES

Conduction system damages are one of the major complications of TAVR and can be listed as: (1) prolonged atrio-ventricular (AV) conduction time; (2) AV block; (3) left bundle branch block; and (4) need for permanent pacemaker implantation.

The thickness of the ventricular septum, thickness of the non-coronary aortic cusp, implantation depth of the prosthetic valve in the left ventricular outflow tract, post implantation dilatation of the prosthetic valve, type of prosthetic valve and pre-existence of right bundle branch block can be listed as the risk factors for occurrence of conduction abnormalities^[36,37]. The incidence of conduction abnormalities after TAVR varies between 5.7%-42.5%^[38]. The incidence of AV block varies between 24.5%-25.8% for CoreValve® and 5.9%-6.5% for Edwards SAPIEN® valve^[39]. Besides the prosthetic valve, manipulation of the guide wires and catheter systems in the left ventricular outflow tract may also cause temporary or permanent conduction system injuries. Most of the conduction abnormalities occur during the procedure (after the isolated aortic balloon valvuloplasty and before the implantation of the prosthetic valve)^[40]. New left bundle branch block is the most seen conduction abnormality with the rate of 25%-85% for CoreValve® and 8%-30% for Edwards SAPIEN® valve^[41]. The risk of AV block is higher for CoreValve® due to its self-expandable design and the possible deeper implantation into the left ventricular outflow tract. For preventing the complications related to conduction pathways, patients should be carefully screened for risk factors.

In case of such complications, trans-venous pacemaker implantation with conversion to permanent pacemaker is the most common treatment option^[14].

ACUTE RENAL INJURY

The incidence of acute renal injuries after TAVR is about 22% and less than half of them are acute renal injuries in stage 2 or stage 3 (8.4%)^[42]. The predisposing factors for acute renal injuries can be listed as: (1) chronic renal disease; (2) peripheral vascular disease; (3) diabetes mellitus; (4) hypoperfusion during rapid ventricular pacing; and (5) aortic plaque embolism in the renal arteries.

In case of any renal complication, the cessation of nephrotoxic drugs and the start of hydration procedure should be performed. If needed hemodialysis may be the treatment option.

DEATH

The mortality incidence after TAVR varies between 5%-10%. No significant difference about mortality has been reported between the self-expandable and balloon expandable prosthetic valve implantation^[2,8]. How-

ever, significant difference is present between the trans-apical and trans-arterial implantation of the balloon expandable prosthetic valve^[20]. The cause of death is mostly originated from the heart (75%) and occurs in the first 48 h after the procedure. After the first 48 h, non-cardiac reasons are the most encountered ones with an incidence of 69%^[43]. Whilst heart failure, cardiac tamponade and arrhythmias are the most seen cardiac reasons; infection, sepsis and stroke are the most seen non-cardiac reasons of death.

CONCLUSION

TAVR procedure is increasingly used all over the world each day. Despite all procedural improvements and technical advances, TAVR procedure still has severe complication risks. It seems that the most important point of preventing and/or overcoming these complications is having an effective heart team. A good patient evaluation by each member of the team, appropriate patient selection, determining the procedural difficulties before the procedure may reduce the complications. Being prepared against the complications, may allow the most needed time to perform the rescue attempts and save the patients' lives.

DECLARATIONS

Authors' contributions

Design: Depboylu B

Literature research, data analysis, manuscript writing: Depboylu B, Yazman S

Manuscript editing, manuscript revision: Harmandar B

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Cribier A, Eltchaninoff H, Bash A, Borenstein N, Tron C, Bauer F, Derumeaux G, Anselme F, Laborde F, Leon MB. Percutaneous transcatheter implantation of an aortic valve prosthesis for calcific aortic stenosis: first human case description. *Circulation* 2002;106:3006-8.
2. Smith CR, Leon MB, Mack MJ, Miller DC, Moses JW, Svensson LG, Tuzcu EM, Webb JG, Fontana GP, Makkar RR, Williams M, Dewey T, Kapadia S, Babaliaros V, Thourani VH, Corso P, Pichard AD, Bavaria JE, Herrmann HC, Akin JJ, Anderson WN, Wang D, Pocock SJ; PARTNER Trial Investigators. Transcatheter versus surgical aortic-valve replacement in high-risk patients. *N Engl J Med* 2011;364:2187-98.
3. Leon MB, Piazza N, Nikolsky E, Blackstone EH, Cutlip DE, Kappetein AP, Krucoff MW, Mack M, Mehran R, Miller C, Morel MA, Petersen J, Popma JJ, Takkenberg JJ, Vahanian A, van Es GA, Vranckx P, Webb JG, Windecker S, Serruys PW. Standardized endpoint definitions for transcatheter aortic valve implantation clinical trials: a consensus report from the Valve Academic Research Consortium.

- Eur Heart J 2011;32:205-17.
4. Chaudhry MA, Sardar MR. Vascular complications of transcatheter aortic valve replacement: a concise literature review. *World J Cardiol* 2017;9:574-82.
 5. Généreux P, Webb JG, Svensson LG, Kodali SK, Satler LF, Fearon WF, Davidson CJ, Eisenhauer AC, Makkar RR, Bergman GW, Babaliaros V, Bavaria JE, Velazquez OC, Williams MR, Hueter I, Xu K, Leon MB; PARTNER Trial Investigators. Vascular complications after transcatheter aortic valve replacement: insights from the PARTNER (Placement of AoRTic TraNscathetER Valve) trial. *J Am Coll Cardiol* 2012;60:1043-52.
 6. Van Mieghem NM, Chieffo A, Dumonteil N, Tchetché D, van der Boon RM, Buchanan GL, Marcheix B, Vahdat O, Serruys PW, Fajadet J, Carrié D, Colombo A, de Jaegere PP. Trends in outcome after transfemoral transcatheter aortic valve implantation. *Am Heart J* 2013;165:183-92.
 7. Hayashida K, Lefèvre T, Chevalier B, Hovasse T, Romano M, Garot P, Mylotte D, Uribe J, Farge A, Donzeau-Gouge P, Bouvier E, Cormier B, Morice MC. True percutaneous approach for transfemoral aortic valve implantation using the Prostar XL device: impact of learning curve on vascular complications. *JACC Cardiovasc Interv* 2012;5:207-14.
 8. Généreux P, Head SJ, Van Mieghem NM, Kodali S, Kirtane AJ, Xu K, Smith C, Serruys PW, Kappetein AP, Leon MB. Clinical outcomes after transcatheter aortic valve replacement using valve academic research consortium definitions: a weighted meta-analysis of 3,519 patients from 16 studies. *J Am Coll Cardiol* 2012;59:2317-26.
 9. Barbanti M, Yang TH, Rodés Cabau J, Tamburino C, Wood DA, Jilaihawi H, Blanke P, Makkar RR, Latib A, Colombo A, Tarantini G, Raju R, Binder RK, Nguyen G, Freeman M, Ribeiro HB, Kapadia S, Min J, Feuchtner G, Gurtvich R, Alqoofi F, Pelletier M, Ussia GP, Napodano M, de Brito FS Jr, Kodali S, Norgaard BL, Hansson NC, Pache G, Canovas SJ, Zhang H, Leon MB, Webb JG, Leipsic J. Anatomical and procedural features associated with aortic root rupture during balloon-expandable transcatheter aortic valve replacement. *Circulation* 2013;128:244-53.
 10. Blanke P, Reinöhl J, Schlensak C, Siepe M, Pache G, Euringer W, Geibel-Zehender A, Bode C, Langer M, Beyersdorf F, Zehender M. Prosthesis oversizing in balloon-expandable transcatheter aortic valve implantation is associated with contained rupture of the aortic root. *Circ Cardiovasc Interv* 2012;5:540-8.
 11. Revilla Martínez MI, Gutiérrez García H, San Román Calvar JA. Interventricular septum rupture after transcatheter aortic valve implantation. *Eur Heart J* 2012;33:190.
 12. Nakashima M, Watanabe Y. Transcatheter aortic valve implantation in small anatomy: patient selection and technical challenges. *Interv Cardiol* 2018;13:66-8.
 13. Debry N, Sudre A, Elquodeimat I, Delhayé C, Schurtz G, Bical A, Koussa M, Fattouch K, Modine T. Prognostic value of the ratio between prosthesis area and indexed annulus area measured by MultiSlice-CT for transcatheter aortic valve implantation procedures. *J Geriatr Cardiol* 2016;13:483-8.
 14. Otto CM, Kumbhani DJ, Alexander KP, Calhoon JH, Desai MY, Kaul S, Lee JC, Ruiz CE, Vassileva CM. 2017 ACC expert consensus decision pathway for transcatheter aortic valve replacement in the management of adults with aortic stenosis: a report of the American college of cardiology task force on clinical expert consensus documents. *J Am Coll Cardiol* 2017;69:1313-46.
 15. Lerakis S, Hayek SS, Douglas PS. Paravalvular aortic leak after transcatheter aortic valve replacement: current knowledge. *Circulation* 2013;127:397-407.
 16. Athappan G, Patvardhan E, Tuzcu EM, Svensson LG, Lemos PA, Fraccaro C, Tarantini G, Sinning JM, Nickenig G, Capodanno D, Tamburino C, Latib A, Colombo A, Kapadia SR. Incidence, predictors, and outcomes of aortic regurgitation after transcatheter aortic valve replacement: meta-analysis and systematic review of literature. *J Am Coll Cardiol* 2013;61:1585-95.
 17. Sinning JM, Vasa-Nicotera M, Chin D, Hammerstingl C, Ghanem A, Bence J, Kovac J, Grube E, Nickenig G, Werner N. Evaluation and management of paravalvular aortic regurgitation after transcatheter aortic valve replacement. *J Am Coll Cardiol* 2013;62:11-20.
 18. Ryś M, Hryniewiecki T, Michałowska I, Stokłosa P, Różewicz-Juraszek M, Chmielak Z, Dąbrowski M, Mirotka K, Szymański P. Quantitative estimation of aortic valve calcification in multislice computed tomography in predicting the development of paravalvular leaks following transcatheter aortic valve replacement. *Postępy Kardiologii Interwencyjnej* 2018;14:85-9.
 19. Généreux P, Head SJ, Hahn R, Daneault B, Kodali S, Williams MR, van Mieghem NM, Alu MC, Serruys PW, Kappetein AP, Leon MB. Paravalvular leak after transcatheter aortic valve replacement: the new Achilles' heel? A comprehensive review of the literature. *J Am Coll Cardiol* 2013;61:1125-36.
 20. Khatri PJ, Webb JG, Rodés-Cabau J, Fremes SE, Ruel M, Lau K, Guo H, Wijeyesundera HC, Ko DT. Adverse effects associated with transcatheter aortic valve implantation: a meta-analysis of contemporary studies. *Ann Intern Med* 2013;158:35-46.
 21. Holmes DR Jr, Mack MJ, Kaul S, Agnihotri A, Alexander KP, Bailey SR, Calhoon JH, Carabello BA, Desai MY, Edwards FH, Francis GS, Gardner TJ, Kappetein AP, Linderbaum JA, Mukherjee C, Mukherjee D, Otto CM, Ruiz CE, Sacco RL, Smith D, Thomas JD. 2012 ACCF/AATS/SCAI/STS expert consensus document on transcatheter aortic valve replacement. *J Am Coll Cardiol* 2012;59:1200-54.
 22. Al-Attar N, Himbert D, Vahanian A, Nataf P. Severe intraprosthesis regurgitation by immobile leaflet after trans-catheter aortic valve implantation. *Eur J Cardiothorac Surg* 2011;39:591-2.
 23. Estévez-Loureiro R, Benito-González T, Gualis J, Pérez de Prado A, Cuellas C, Fernandez-Vazquez F. Percutaneous paravalvular leak closure after CoreValve transcatheter aortic valve implantation using an arterio-arterial loop. *J Thorac Dis* 2017;9:E103-8.
 24. Pang PY, Chiam PT, Chua YL, Sin YK. A survivor of late prosthesis migration and rotation following percutaneous transcatheter aortic valve implantation. *Eur J Cardiothorac Surg* 2012;41:1195-6.
 25. Dvir D, Webb J, Brecker S, Bleiziffer S, Hildick-Smith D, Colombo A, Descoutures F, Hengstenberg C, Moat NE, Bekeredjian R, Napodano M, Testa L, Lefevre T, Guetta V, Nissen H, Hernández JM, Roy D, Teles RC, Segev A, Dumonteil N, Fiorina C, Gotzmann M,

- Tchetche D, Abdel-Wahab M, De Marco F, Baumbach A, Laborde JC, Kornowski R. Transcatheter aortic valve replacement for degenerative bioprosthetic surgical valves: results from the global valve-in valve registry. *Circulation* 2012;126:2335-44.
26. Paradis JM, Del Trigo M, Puri R, Rodés-Cabau J. Transcatheter valve-in-valve and valve-in-ring for treating aortic and mitral surgical prosthetic dysfunction. *J Am Coll Cardiol* 2015;66:2019-37.
27. Ribeiro HB, Nombela-Franco L, Urena M, Mok M, Pasian S, Doyle D, DeLarochellière R, Côté M, Laflamme L, DeLarochellière H, Allende R, Dumont E, Rodés-Cabau J. Coronary obstruction following transcatheter aortic valve implantation: a systematic review. *JACC Cardiovasc Interv* 2013;6:452-61.
28. Abdel-Wahab M, Mehili J, Frerker C, Neumann FJ, Kurz T, Tölg R, Zachow D, Guerra E, Massberg S, Schäfer U, El-Mawardy M, Richardt G; CHOICE investigators. Comparison of balloon-expandable versus self-expandable valves in patients undergoing transcatheter aortic valve replacement: the CHOICE randomized clinical trial. *JAMA* 2014;311:1503-14.
29. Rodés-Cabau J, Gutiérrez M, Bagur R, De Larochellière R, Doyle D, Côté M, Villeneuve J, Bertrand OF, Larose E, Manazzoni J, Pibarot P, Dumont E. Incidence, predictive factors, and prognostic value of myocardial injury following uncomplicated transcatheter aortic valve implantation. *J Am Coll Cardiol* 2011;57:1988-99.
30. Miller DC, Blackstone EH, Mack MJ, Svensson LG, Kodali SK, Kapadia S, Rajeswaran J, Anderson WN, Moses JW, Tuzcu EM, Webb JG, Leon MB, Smith CR; PARTNER Trial Investigators and Patients; PARTNER Stroke Substudy Writing Group and Executive Committee. Transcatheter (TAVR) versus surgical (AVR) aortic valve replacement: occurrence, hazard, risk factors, and consequences of neurologic events in the PARTNER trial. *J Thorac Cardiovasc Surg* 2012;143:832-43.
31. Stortecky S, Windecker S, Pilgrim T, Heg D, Buellesfeld L, Khattab AA, Huber C, Gloekler S, Nietlispach F, Mattle H, Jüni P, Wenaweser P. Cerebrovascular accidents complicating transcatheter aortic valve implantation: frequency, timing and impact on outcomes. *EuroIntervention* 2012;8:62-70.
32. Nombela-Franco L, Webb JG, de Jaegere PP, Toggweiler S, Nuis RJ, Dager AE, Amat-Santos IJ, Cheung A, Ye J, Binder RK, van der Boon RM, Van Mieghem N, Benitez LM, Pérez S, Lopez J, San Roman JA, Doyle D, Delarochellière R, Urena M, Leipsic J, Dumont E, Rodés-Cabau J. Timing, predictive factors, and prognostic value of cerebrovascular events in a large cohort of patients undergoing transcatheter aortic valve implantation. *Circulation* 2012;126:3041-53.
33. Van Mieghem NM, Schipper ME, Ladich E, Faqiri E, van der Boon R, Randjari A, Schultz C, Moelker A, van Geuns RJ, Otsuka F, Serruys PW, Virmani R, de Jaegere PP. Histopathology of embolic debris captured during transcatheter aortic valve replacement. *Circulation* 2013;128:e478-9.
34. Grube E, Naber C, Abizaid A, Sousa E, Mendiz O, Lemos P, Kalil Filho R, Mangione J, Buellesfeld L. Feasibility of transcatheter aortic valve implantation without balloon pre-dilation: a pilot study. *JACC Cardiovasc Interv* 2011;4:751-7.
35. Rezq A, Basavarajaiah S, Latib A, Takagi K, Hasegawa T, Figini F, Cioni M, Franco A, Montorfano M, Chieffo A, Maisano F, Corvaja N, Alfieri O, Colombo A. Incidence, management, and outcomes of cardiac tamponade during transcatheter aortic valve implantation: a single-center study. *JACC Cardiovasc Interv* 2012;5:1264-72.
36. Piazza N, Nuis RJ, Tzikas A, Otten A, Onuma Y, García-García H, Schultz C, van Domburg R, van Es GA, van Geuns R, de Jaegere P, Serruys PW. Persistent conduction abnormalities and requirements for pacemaking six months after transcatheter aortic valve implantation. *EuroIntervention* 2010;6:475-84.
37. Roten L, Wenaweser P, Delacrétaz E, Hellige G, Stortecky S, Tanner H, Pilgrim T, Kadner A, Eberle B, Zwahlen M, Carrel T, Meier B, Windecker S. Incidence and predictors of atrioventricular conduction impairment after transcatheter aortic valve implantation. *Am J Cardiol* 2010;106:1473-80.
38. Bates MG, Matthews IG, Fazal IA, Turley AJ. Postoperative permanent pacemaker implantation in patients undergoing trans-catheter aortic valve implantation: what is the incidence and are there any predicting factors? *Interact Cardiovasc Thorac Surg* 2011;12:243-53.
39. Nazif TM, Dizon JM, Hahn RT, Xu K, Babaliaros V, Douglas PS, El-Chami MF, Herrmann HC, Mack M, Makkar RR, Miller DC, Pichard A, Tuzcu EM, Szeto WY, Webb JG, Moses JW, Smith CR, Williams MR, Leon MB, Kodali SK; PARTNER Publications Office. Predictors and clinical outcomes of permanent pacemaker implantation after transcatheter aortic valve replacement: the PARTNER (Placement of AoRticTraNscathetER Valves) trial and registry. *JACC Cardiovasc Interv* 2015; 8:60-9.
40. Nuis RJ, Van Mieghem NM, Schultz CJ, Tzikas A, Van der Boon RM, Maugenes AM, Cheng J, Piazza N, van Domburg RT, Serruys PW, de Jaegere PP. Timing and potential mechanisms of new conduction abnormalities during the implantation of the Medtronic CoreValve System in patients with aortic stenosis. *Eur Heart J* 2011;32:2067-74.
41. Colombo A, Latib A. Left bundle branch block after transcatheter aortic valve implantation: inconsequential or a clinically important endpoint? *J Am Coll Cardiol* 2012;60:1753-5.
42. Takagi H, Niwa M, Mizuno Y, Goto SN, Umemoto T; All-Literature Investigation of Cardiovascular Evidence Group. Incidence, predictors, and prognosis of acute kidney injury after transcatheter aortic valve implantation: a summary of contemporary studies using Valve Academic Research Consortium definitions. *Int J Cardiol* 2013;168:1631-5.
43. Van Mieghem NM, van der Boon RM, Nuis RJ, Schultz C, van Geuns RJ, Serruys PW, Kappetein AP, van Domburg RT, de Jaegere PP. Cause of death after transcatheter aortic valve implantation. *Catheter Cardiovasc Interv* 2014;83:E277-82.

Original Article

Open Access



Age-associated features of oxidative stress as marker of vascular aging in comorbid course of hypertension and type 2 diabetes mellitus

Valeriya Nemtsova¹, Olexander Bilovol¹, Irina Ilchenko¹, Anna Shalimova²

¹The Clinical Pharmacology and Internal Diseases Department, Kharkiv National Medical University, Kharkiv 61039, Ukraine.

²Department of Chronic Non-communicable Disease Prevention, Government Institution "National Institute of Therapy named by L.T. Malaya of National Ukrainian Academy of Medical Science", Kharkiv 61039, Ukraine.

Correspondence to: Dr. Anna Shalimova, Department of Chronic Non-communicable Disease Prevention, Government Institution "National Institute of Therapy named by L.T. Malaya of National Ukrainian Academy of Medical Science", Kharkiv, 61039, Ukraine. E-mail: anna.shalimova83@gmail.com

How to cite this article: Nemtsova V, Bilovol O, Ilchenko I, Shalimova A. Age-associated features of oxidative stress as marker of vascular aging in comorbid course of hypertension and type 2 diabetes mellitus. *Vessel Plus* 2018;2:27. <http://dx.doi.org/10.20517/2574-1209.2018.48>

Received: 25 Jun 2018 **First Decision:** 27 Jul 2018 **Revised:** 8 Aug 2018 **Accepted:** 31 Aug 2018 **Published:** 28 Sep 2018

Science Editor: Igor A. Sobenin **Copy Editor:** Cui Yu **Production Editor:** Zhong-Yu Guo

Abstract

Aim: To evaluate activity of oxidative stress (OS) as marker of vascular aging in different age groups of patients with combined course of arterial hypertension (HT) and type 2 diabetes mellitus (T2DM).

Methods: 126 patients (average age 57.8 ± 6.2 years) with stage II HT and compensated T2DM were divided into 2 subgroups: 2a ($n = 59$) - aged 45-60 years; 2b ($n = 97$) - aged 61-75 years; 30 patients with isolated stage II HT (comparison group), 20 practically healthy individuals (control group). The activity of antioxidative [glutathione peroxidase, sulfhydryl groups (SH-groups)] and oxidative [malonic dialdehyde (MDA)], 8-hydroxy-2-deoxyguanosine (8-OH-dG) systems in blood serum, were studied.

Results: A significant increase in MDA levels ($P < 0.05$) and SH-groups ($P < 0.05$) compared with healthy volunteers was observed. Patients in 2b group had lower MDA values than in 2a ($6.25 \pm 0.33 \mu\text{mol/L}$, $7.07 \pm 0.44 \mu\text{mol/L}$, respectively, $P > 0.05$). In the 2b group, in comparison with 2a patients, a decrease in thiol status was observed ($P > 0.05$). The level of 8-OH-dG was increased in patients with HT and T2DM, but there was also an age-associated increase in the average 8-OH-dG in the 2b group.

Conclusion: The age-associated changes in the OS in comorbid course of HT and T2DM did not have significant differences. Nevertheless, the presence of correlations between various indexes that are included in the concept of "vascular



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



aging" and indicators of oxidant-antioxidant systems in different age groups allows us to make an assumption about the significant influence of the oxidative status on the status of vascular age, especially in the older age group persons.

Keywords: Hypertension, type 2 diabetes mellitus, oxidative stress, vascular aging

INTRODUCTION

A significant increase in the proportion of older people in the population of developed countries is accompanied by an increase in mortality from the main diseases of old age-diseases of the cardiovascular system, malignant neoplasms, neurodegenerative processes, reduced resistance to infection and diabetes mellitus.

According to the United Nations Organization prognosis, by 2025 the number of people over 60 will reach 1.2 billion (15% of the world's total population). Therefore, the concept of healthy aging, developed in 2001 by the United Nations Organization, is classified as one of the highest priority areas of medicine.

Aging is considered to be a natural physiological process. At the same time, there are data which demonstrate that physiological aging is observed in only 3%-6% of the human population while in other cases accelerated aging is observed. Aging is a biological process that develops with age and manifests as a gradual decrease in the adaptive capabilities of the organism. These changes can play a significant role in the development of different pathologies.

Medico-biological studies involving elderly, old people and centenarians are recognized as necessary to identify age-associated risk factors and specific markers that would optimize therapy for age-associated diseases, especially taking into consideration that cardio-vascular mortality remains high, despite therapeutic and prophylactic efforts. There is also a need to develop new pathophysiological models for a better understanding of cardiovascular risks (CVRs) based on new age-associated concepts.

Since age is a marker of the cumulative effect of risk factors and the overall integral index of the development of many chronic diseases [cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM), malignant diseases], Nilsson *et al.*^[1] proposed the concept of "early vascular aging", which is a new concept for studying patients with high CVR or patients with early family manifestations of cardiovascular events. "Vascular age" (VA) generally includes many determinants, the main ones of which are: endothelial dysfunction, pulse wave velocity (PWV), central arterial pressure and carotid intima-media complex thickness (CIMCT). These parameters can be considered as "tissue biomarkers" of vascular lesions, which may be more sensitive than "circulating biomarkers" (e.g., C-reactive protein, hyperglycemia, dyslipidemia) and in combination with classical risk factors show better additional results predicting cardiovascular complications^[2]. At present, the Framingham scale and the European scale for assessing the 10-year risk of cardiovascular mortality (SCORE) are widely used, in which age is one of the most important CVR determinants and is associated with a number of morphological and functional changes in vessels^[3].

As knowledge accumulates, it is becoming increasingly evident that aging and major chronic age-related diseases have the same basic molecular and cellular mechanisms^[4]. It is believed that in the first place they are associated with mild chronic systemic inflammation. To denote this phenomenon a group of scientists led by Franceschi *et al.*^[5] proposed the term "inflammaging". Today inflammaging is a widely accepted theory of aging. Global age-related systemic inflammation in many organs is involved in the pathogenesis of the most associated diseases, but until now it has not been fully determined whether these conditions are the cause or consequence of age-related systemic inflammation. Inflammation is one of the central pathogenetic mechanisms at all stages of development of atherosclerosis and its complications. What

mechanisms are involved in the development of age-associated chronic inflammation is not definitively established.

The age-associated increase in oxidative stress can contribute to the development of chronic inflammation and the progression of various diseases. Today the theory of oxidative stress is considered one of the most popular theories explaining not only aging, but also the initiation, as well as the progression of many modern diseases, in particular cardiovascular and diabetes mellitus. Recently, oxidative stress has been actively studied in order to better understand the mechanisms of protection and the relationship between oxidative damage and the aging process^[6].

Population aging became the leading demographic feature of Ukraine as well^[7]. Hypertension (HT), T2DM, ischemic heart disease (IHD), cerebral vascular disease with different severity of clinical symptoms and in different combinations are the most common in elderly people^[4,7].

The purpose of this study was to evaluate plasma parameters of oxidant-antioxidant systems as markers of vascular aging in patients of different age groups with a combined course of arterial HT and T2DM.

METHODS

126 patients (55 males and 71 females) from 45-75 years old (mean age: 57.8 ± 6.2 years) with stage II HT (mean duration of disease 10.2 ± 3.7 years) and well controlled T2DM (mean duration of disease: 4.1 ± 2.4 years) were examined and according to the current World Health Organization (WHO) age classification^[8] were divided into 2 subgroups: group 2a ($n = 59$) - patients with HT in combination with T2DM aged 45-60 years; group 2b ($n = 97$) - patients with a combined course of HT and T2DM aged 61-75 years. As a comparison group, patients with isolated stage II H ($n = 30$), identical in age and sex to the main group (average age 53.8 ± 4.6 years) were studied. The control group consisted of 20 healthy individuals, matched for age and sex.

The study did not include patients with symptomatic HT, uncontrolled HT, type 1 diabetes, decompensated T2DM and other endocrine disorders, clinical signs of IHDs or severe concomitant chronic diseases. The excluding criteria were also: taking iodine medications, glucocorticoids, amiodarone, lithium medications, and medications containing estrogens and pregnancy. For patient selection, the diagnostic criteria of HT approved by the European recommendations on diagnosis and treatment of HT^[9] were used. The diagnosis of T2DM was established according to the approved by order of the Ministry of Health of Ukraine dated on 21.12.2012 № 1118 "On Approval and implementation of medical-technological documents for the standardization of medical aid in type 2 diabetes"^[10] and in accordance with the recommendations of the American Diabetes Association and the European Association for the study of diabetes^[11].

On a background of dietary recommendations, all patients received basic therapy in accordance with international and national recommendations for the management of patients with appropriate pathology^[9-11]. Before being included in the study, all patients had been receiving antihypertensive therapy for at least 6 months in individually selected doses with the use of angiotensin - converting enzyme (ACE) inhibitors or angiotensin II receptors blockers (ACE inhibitors/ARBs), diuretics (indapamide or torasemide). Some of the patients received calcium antagonists (amlodipine or lercanidipine). As an antidiabetic therapy, patients with T2DM received metformin in individually selected doses from 1000-2000 mg per day, 49 patients (29.51%) additionally were using sulfonylurea derivatives.

Blood pressure (BP) levels were assessed in all patients by means of blood pressure obtained from three measurements at 2-min intervals in a sitting position.

Determination of total cholesterol (TC), triglycerides (TG) and cholesterol of high density lipoproteins (HDL cholesterol) were performed in serum enzymatically by photolorimetric method with sets produced by Human (Germany). The content of cholesterol in the low density lipoprotein (LDL cholesterol) was calculated by the formula of Friedewald W. T. with consideration of measurement in mmol/L: $LDL\ cholesterol = cholesterol - (HDL\ cholesterol + TG/2.22)$.

Determination of the concentration of fasting glucose was performed by the glucose oxidase method using analyzer Humolizer (made in Germany). The level of glycated hemoglobin (HbA_{1c}) was measured by enzyme immunoassay (ELISA) using a set of reagents Hummer (USA). To determine the insulin resistance (IR) index HOMA-IR was used, which was calculated with the formula: $[(Glucose\ fasting) \times (fasting\ insulin)]\ mmol/mL/22.5$.

Besides the indicators of carbohydrate and lipid metabolism all patients underwent measurement of the concentration of insulin in blood serum by the method of ELISA using a kits DRG Instrument GmbH (Germany) on a semi-automatic ELISA analyzer “ImmunoChem-2100”, HighTechnology, Inc. (USA).

To study the antioxidative system, the activity of glutathione peroxidase (GPO) and the level of sulphydryl groups (SH-groups) were assessed. GPO plays an important role in protecting biological cell membranes against oxidative damage by increasing the concentration of reduced glutathione (oxidised glutathione ratio - GSSG-R) in the process of aerobic glycolysis. SH-groups are the organic compounds that contain a sulphhydryl group. Among all the antioxidants that are available in the body, they constitute the major portion of the total body antioxidants and they play a significant role in defense against reactive oxygen species (ROS). The level of malonic dialdehyde (MDA) was used as a marker of the lipid peroxidation and oxidative system activity. The activity of GPO (KF 1.11.1.9) in Ethylenediaminetetraacetic acid (EDTA)-hemolysate was determined by the decrease in the content of reduced glutathione during a 5-min incubation of a test sample of hemolysate in the presence of oxidizing substrate - cumene hydroperoxide by the photometric method^[12]. The SH-groups and MDA were determined in serum using a photometric method^[12]. The following reagents were used: thiobarbituric acid (Organika, Germany), dithiobisnitrobenzoic acid (Merck, Germany), restored glutathione (Sigma-Aldrich, Germany), cumene hydroperoxide (Merck, Germany). The determination of 8-hydroxy-2-deoxyguanosine (8-OH-dG) in blood serum, as one of the biomarkers of oxidative damage, was carried out by ELISA with kits “Bio-Vendor” (Czech Republic).

Ultrasound examination of the common CIMCT was performed according to the standard procedure on the device “LOGIQ5”.

The results obtained are presented as the average value of parameters (M) and standard error (m). Processing of statistical data was performed using the software package “Statistics for Windows 8.0”. The student criterion and Pearson’s chi-squared test were used to estimate the differences between groups with normal distribution. The differences were considered statistically significant at $P < 0.05$.

The study was performed in compliance with the basic provisions of the Helsinki declaration of the world medical association on ethical principles of scientific and medical research involving humans (1964-2000) and the order of the ministry of health of Ukraine dated 23.09.2009 № 690. The article is a fragment of the research work of the department of clinical pharmacology and internal medicine of Kharkiv National Medical University “Optimization of diagnosis and treatment of comorbid pathology (HT and diabetes mellitus type 2) on the basis of evaluation of cardio-hemodynamics, metabolism and pharmacogenetic

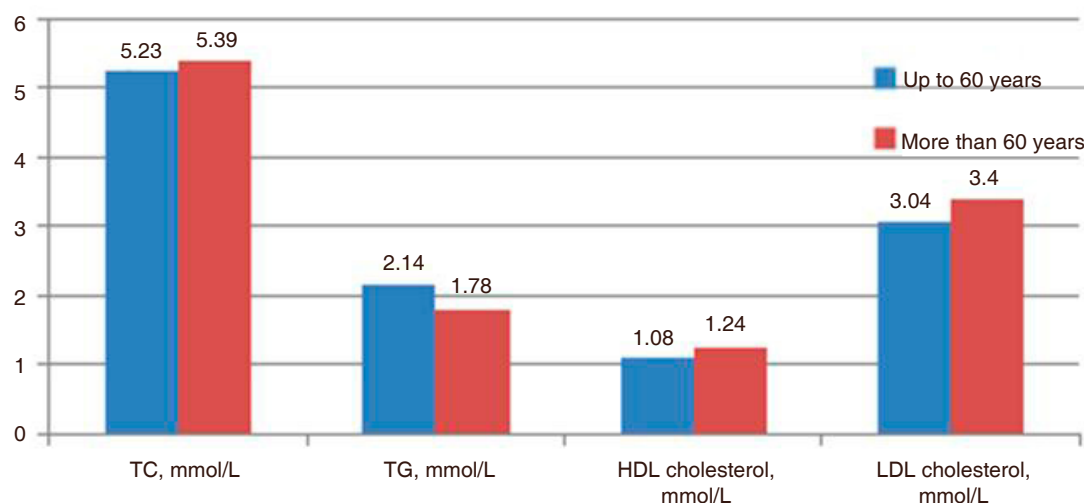


Figure 1. The state of lipid metabolism in patients with AH and DM2T, depending on the age group. TC: total cholesterol; TG: triglycerides; HDL cholesterol: cholesterol of high density lipoproteins; LDL cholesterol: cholesterol of low density lipoproteins

analysis". The study was approved by the commission on bioethics at the Kharkiv National Medical University, consistent with the principles outlined in the Helsinki declaration.

RESULTS

Comparative analysis of lipid and carbohydrate metabolism parameters, as was expected, showed the presence of dyslipidemia both among patients with isolated HT and in the combined course of HT and T2DM in comparison with controls. Inclusion of compensated T2DM to HT was not accompanied by a significant aggravation of lipid metabolism disorders, but increased the index of IR almost 2-fold compared with patients with isolated HT and 4-fold compared with the control group (group 1: 4.40 ± 0.51 , group 2: 8.26 ± 0.68 , control: 2.23 ± 0.36 respectively, $P < 0.05$).

The distribution of patients with HT and T2DM in the 2 age categories did not reveal significant differences in the disorders of both lipids, except HDL cholesterol, and carbohydrate metabolism [Figure 1]. In the 2b subgroup patients, despite a higher level of fasting glucose (2a subgroup: 8.59 ± 0.72 mmol/L and 2b: 9.15 ± 0.67 mmol/L, respectively, $P > 0.05$), there was a decrease in the signs of insulin resistance (HOMA-IR: 2a: 8.88 ± 1.15 and 2b: 7.92 ± 0.86 , respectively, $P > 0.05$). It is believed that the increase in IR leads to the depletion of antioxidant protection. In our work in patients with comorbid pathology with an increase in the age group, there was a unidirectional decrease in the signs of both IR and indexes of both oxidative and antioxidant systems [Table 1]. In patients of the older age group, the correlation analysis revealed the presence of an average positive relationship between GPO and HOMA-IR ($P = 0.046$, $r = 0.374$).

When evaluating the carotid intima-media thickness (CIMT) we were guided by the data obtained in the Atherosclerosis Risk in Communities (ARIC) study, in which it was demonstrated that the calculation of VA, taking into account gender, age and race, can be made on the basis of the CIMT measurement^[2]. Stein et al.^[13] have shown that the assessment of VA using a non-invasive CIMT measurement, can more accurately determine the age as one of the main indicators in the evaluation individual risk of cardiovascular disease.

As is known, the presence of HT and T2DM themselves are characterized by a tendency to increase the CIMT, which we observed in our patients, although it is worth noting that this increase was in comparison

Table 1. Age-associated comparative assessment of oxidative-antioxidant systems in patients with H and DM2T

Index	2a subgroup (n = 59)	2b subgroup (n = 97)
8-OH-dG, ng/L	14.07 ± 1.03	17.23 ± 0.97*
GPO, $\mu\text{mol}/\text{min}/\text{gHb}$	5.73 ± 0.36	5.23 ± 0.23
MDA, $\mu\text{mol}/\text{L}$	7.07 ± 0.44	6.25 ± 0.33
SH-groups, $\mu\text{mol}/\text{L}$	584.38 ± 14.56	567.36 ± 15.03
CIMT RCCA, mm	0.08 ± 0.00	0.08 ± 0.00
CIMT LCCA, mm	0.08 ± 0.00	0.08 ± 0.00
Insulin, $\mu\text{IU}/\text{mL}$	24.55 ± 4.24	19.71 ± 2.17
HOMA-IR	8.88 ± 1.15	7.92 ± 0.86
SBP, mmHg	152.92 ± 5.85	143.64 ± 2.82
DBP, mmHg	92.50 ± 3.29	88.88 ± 1.75
PBP, mmHg	60.42 ± 3.72	54.76 ± 2.15

* $P < 0.05$: compared to the 2a group; HOMA-IR: insulin resistance index; MDA: malonic dialdehyde; GPO: glutathione peroxidase; SH-groups: sulfhydryl groups; 8-OH-dG: 8-hydroxy-2-deoxyguanosine; CIMT RCCA: carotid intima-media thickness of right common carotid artery; CIMT LCCA: carotid intima-media thickness of left common carotid artery; SBP: systolic blood pressure; DBP: diastolic blood pressure; PBP: pulse blood pressure

with control group, and in 67% not reach the threshold for the European recommendations (CIMT thickness ≤ 0.9 mm)^[14]. The subdivision of patients with HT and T2DM into age groups was accompanied by a slight increase in this parameter, which did not reach significance (2a subgroup - CIMT of right common carotid artery (RCCA) - 0.086 ± 0.004 mm, CIMT of left common carotid artery (LCCA) - 0.086 ± 0.003 mm, 2b subgroup - CIMT RCCA - 0.088 ± 0.003 mm and CIMT LCCA - 0.087 ± 0.002 mm, respectively, $P > 0.05$). Ultrasound examination of carotid arteries revealed the presence of atherosclerotic plaques in 39 patients (30.95%), 5 of which (12.82%) belong to the 2a subgroup, and 34 patients (87.18%) - 2b subgroup.

Oxidative stress is defined as an imbalance between the concentration of oxidation products and the activity of antioxidant processes in the body. Oxidative stress promotes the oxidation of a number of molecules, such as DNA, lipids, and proteins, which are associated with various processes, including aging.

Despite the available data on the beneficial effect of modern antihypertensive^[15] and antidiabetic^[16] therapy on oxidative stress, the results of our study demonstrate the presence of intensive OS in patients with the combined course of HT and T2DM. This manifests in a significant increase in MDA levels ($P < 0.05$), and a decrease in levels of SH-groups ($P < 0.05$) compared with healthy volunteers.

MDA is the main end product in the process of lipid peroxidation. Data from recent years suggest using the MDA level as a marker of the risk of complications in patients with T2DM, especially inadequately compensated^[17].

In the studies of Carracedo *et al.*^[18] it was shown that people with age showed a significant increase in MDA. In our work, patients in the older age group had lower MDA values than those under 60 (6.25 ± 0.33 $\mu\text{mol}/\text{L}$ and 7.07 ± 0.44 $\mu\text{mol}/\text{L}$, respectively, $P > 0.05$).

The main role in protecting against the influence of OS is played by an antioxidant system (AOS), one of the main components of which is the thiol disulfide system. An important biomarker reflecting the state of this system is the organism thiol status. Thiol status indicates the total level of SH-groups of proteins and free SH-groups. In patients with the combined course of HT and T2DM [Table 2], there was a significant decrease in thiol status in comparison with the control group (573.52 ± 10.91 $\mu\text{mol}/\text{L}$ and 712.26 ± 11.08 mmol/L , respectively, $P < 0.05$).

Table 2. Indexes of lipid, carbohydrate metabolism, oxidant and antioxidant systems in patients with isolated hypertension, combination of H and DM2T compared with controls

Index	Control (n = 20)	Isolated H (group1, n = 30)	H with DM2T (group2, n = 126)
TC, mmol/L	4.77 ± 0.52	5.95 ± 0.23	5.34 ± 0.21
TG, mmol/L	1.03 ± 0.30	1.63 ± 0.13	1.91 ± 0.14
VLDL cholesterol, mmol/L	0.54 ± 0.22	0.72 ± 0.06	0.85 ± 0.08
HDL cholesterol, mmol/L	1.45 ± 0.30	1.41 ± 0.06	1.18 ± 0.04*
LDL cholesterol, mmol/L	2.6 ± 0.33	3.7 ± 0.24	3.28 ± 0.21
Glucose, mmol/L	4.62 ± 1.08	5.45 ± 0.12	8.25 ± 0.30*
HbA1c, %	4.62 ± 1.08	6.22 ± 0.15	7.32 ± 0.20*
Insulin, µIU/mL	9.84 ± 2.20	19.77 ± 2.06	22.89 ± 2.20
HOMA-IR	2.23 ± 0.36	4.40 ± 0.51	8.26 ± 0.68*
MDA, µmol/L	4.07 ± 0.22	6.11 ± 0.31	6.55 ± 0.27
GPO, µmol/min/gHb	406.20 ± 31.2	346.80 ± 15.01	324.60 ± 12.01
SH-groups, µmol/L	712.26 ± 11.08	570.54 ± 12.64	573.52 ± 10.91
8-OH-dG, ng/L	6.66 ± 0.97	16.26 ± 0.83	15.89 ± 0.76

* $P < 0.05$: compared to the 1st group; TC: total cholesterol; TG: triglycerides; VLDL cholesterol, HDL cholesterol: cholesterol of high density lipoproteins; LDL cholesterol: cholesterol of low density lipoproteins; HbA1c: glycated hemoglobin; HOMA-IR: insulin resistance index; MDA: malonic dialdehyde; GPO: glutathione peroxidase; SH-groups: sulfhydryl groups; 8-OH-dG: 8-hydroxy-2-deoxyguanosine

This trend persisted even when patients were divided into age groups: in patients of the older age group in comparison with younger patients a decrease in thiol status was observed ($P > 0.05$). These changes were observed against the background of an insignificant age-dependent decrease of GPO, which indicates a decrease of the antioxidant protective force [Table 1].

Previously, it was shown that guanine is the most oxidizable of the four bases included in the DNA structure^[19]. Its oxidation product, as a result of ROS exposure, is 8-OH-dG, which can be determined in various biological tissues and liquids. In our study the correlation analysis revealed 8-OH-dG bonds practically only in the group of individuals under 60 years: the average positive correlation between 8-OH-dG and CIMT ($P = 0.038$, $r = -0.569$), a high positive relationship between 8-OH-dG and SBP ($P = 0.027$, $r = 0.765$) 8-OH-dG and PBP ($P = 0.046$, $r = 0.715$).

The correlation analysis also revealed the age-dependence of various correlations between the studied parameters. Thus, in the group under 60 years a weak negative relationship was found between the SH-groups and the TG levels ($P = 0.002$, $r = -0.04$) and HDL cholesterol levels ($P = 0.042$, $r = -0.206$). In the combined course of HT and T2DM in patients of the older age group the correlation analysis revealed the presence of an average positive association between 8-OH-dG levels and HDL cholesterol ($P = 0.045$, $r = 0.543$), GPO and TC ($P = 0.026$, $r = 0.405$), GPO and LDL cholesterol ($P = 0.027$, $r = 0.410$), GPO and HOMA-IR ($P = 0.046$, $r = 0.374$).

DISCUSSION

It is known that oxidative stress increases with age and its progressive development can be considered as one of the aging markers. It is generally accepted that an increase in OS during aging is a consequence of a decrease in the effectiveness of antioxidant protection. The quantitative determination of 8-OH-dG is suggested as one of the markers of free-radical processes occurring in the body under normal circumstances and with the development of various pathological processes. It is believed that an increased level of 8-OH-dG is associated with the aging process, as well as with many pathological conditions, including diabetes mellitus and HT. In the studies of Wua et al.^[19] the correlations between the level of oxidative DNA damage and the severity of diabetic nephropathy and retinopathy were demonstrated.

In our study, we observed not only an increase in this index in patients with HT and T2DM compared to the control group, but there was also an age-associated increase in the average 8-OH-dG in the 2b age group, which coincides with the results of Wang *et al.*^[20], who demonstrated a significant relationship between the plasma level of 8-OHdG and the age of the studied subjects.

Our results indicate that the combined course of HT and T2DM is characterized by the presence of pronounced oxidative stress, which manifests in a significant increase in the intensity of lipid peroxidation, the marker of DNA damage which increased against the background of a decreased activity of antioxidant protection.

Despite the fact that the age-associated changes in oxidative stress in comorbid course of HT and T2DM did not have significant differences, the presence of correlations between various indexes that are included in the concept of “vascular aging” and indicators of oxidant-antioxidant systems in different age groups allows us to make an assumption about the significant influence of the oxidative status on the status of VA, especially in the older age group persons.

Given that elderly age is generally characterized by polymorbidity and with age the risk of age-associated diseases development, including HT and T2DM and their complications, increases, one active area of research is to study the peculiarities of oxidative stress development in older age groups.

Thus, to date the concept of VA allows us to take a new view at the assessment of CVR: on the one hand, as a biological model of aging, on the other, it makes it possible to analyze the state of many risk factors in different age groups.

DECLARATIONS

Authors' contributions

Study design, manuscript review: Nemtsova V, Bilovol O

Development of methodology: Nemtsova V

Collection of data, analysis and/or interpretation of data, writing (not revising) all or sections of the manuscript: Nemtsova V, Bilovol O, Ilchenko I, Shalimova A

Supervision: Bilovol O

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there were no conflicts of interest.

Ethical approval and consent to participate

The study protocol was supported by the Ethics Committee of the Kharkiv National Medical University, Ukraine.

Consent for publication

Each patient was informed the study and gave their consent.

Copyright

© The Author(s) 2018.

REFERENCES

1. Nilsson PM, Boutouyrie P, Laurent S. Vascular aging: a tale of EVA and ADAM in cardiovascular risk assessment and prevention. *Hypertension* 2009;54:3-10.
2. Sinkevich DA, Protasov KV, Dzyzinsky AA. The concept of vascular age as a new approach to evaluation of cardiovascular risk. *Siberian Med J* 2011;6:9-13. (in Russian)
3. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a “set up” for vascular disease. *Circulation* 2003;107:139-46.
4. Luchikhina LV, Mendel OI, Mendel V, Golukhov GN. Osteoarthritis and age. Role of aging in the etiology and pathogenesis of the disease. *Mod rheumatology* 2017;11:4-11. (in Russian)
5. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, Salvioli S. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev* 2007;128:92-105.
6. Hammad EV, Belousova ON, Khmelnsky AV, Poltoratsky AN, Shchekaturov AA. Modern biomaterials of aging for stratification of risks associated with development of age-associated diseases (review) // Scientific Bulletins of BelSU. Series: medicine. Available from: <https://cyberleninka.ru/article/n/sovremennyye-biomaterialy-stareniya-dlya-stratifikatsii-riskov-razvitiya-vozrast-assotsirovannyh-zabolevaniy-obzor-literatury>. [Last accessed on 19 Sep 2018]
7. Kovalenko VM, Kornatsky VM. Diseases of the circulatory system as a medical and social and socio-political problem (analytical and statistical manual). Kiev; 2014. p. 279. (in Ukrainian)
8. Age classification of the World Health Organization. Available from: <https://citifox.ru/2016/05/05/vozrastnaya-klassifikatsiya-vsemirnaya/>. [Last accessed on 19 Sep 2018]
9. Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F; Task Force Members. 2013 ESH/ESC guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2013;31:1281-357.
10. Ministry of the Health of Ukraine. Order of the Ministry of Health of Ukraine dated 21.12.2012 № 1118 “on approval and implementation of medical-technological documents for the standardization of medical aid in type 2 diabetes”. Available from: <https://medprosvita.com.ua/nakaz-moz-ukrayini-vid-21-12-2012-n-1118-pro-zatver/>. [Last accessed on 19 Sep 2018]
11. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the study of diabetes. *Diabetes Care* 2015;38:140-9.
12. Arutyunov AV, Dubinina EE, Zybina N.N. Methods for assessing free radical oxidation and the body's antioxidant system. Guidelines. St. Petersburg: IKF “Foliant”; 2000. p. 104 (in Russian)
13. Stein JH, Fraizer MC, Aeschlimann SE, Nelson-Worel J, McBride PE, Douglas PS. Vascular age: integrating carotid intima-media thickness measurements with global coronary risk assessment. *Clin Cardiol* 2004;27:388-92.
14. Ostroumova OD, Zhukova OV, Erofeeva AG, Tolkalenov AV. Thickness of carotid arteries intima-media complex in patients with H - the possibility of a fixed combination Logimax. *Rus Med J* 2009;8:548 (in Russian)
15. E.A.Prochorovich. Hypotensive therapy: a new combination and new possibilities. *Consilium Medicum* 2013;10:121-5.
16. Alireza Esteghamati, Delaram Eskandari, Hossein Mirmiranpour, Sina Noshad, Mostafa Mousavizadeh, Mehdi Hedayati, Manouchehr Nakhjavan. Randomized clinical trial: the effect of metformin on markers of oxidative stress and antioxidant reserves in patients with newly diagnosed type 2 diabetes. *Obes Metabolism* 2012;3:41-2.
17. Lodovici M, Bigagli E, Luceri C, Mannucci E, Rotella CM, Raimondi L. Gender-related drug effect on several markers of oxidation stress in diabetes patients with and without complications. *Eur J Pharmacol* 2015;766:86-90.
18. Carracedo J, Ramírez-Carracedo R, Martínez de Toda I, Vida C, Alique M, De la Fuente M, Ramírez-Chamond R. Protein carbamylation: a marker reflecting increased age-related cell oxidation. *Int J Mol Sci* 2018; doi: 10.3390/ijms19051495.
19. Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OH-dG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetes. *Clin Chim Acta* 2004;339:1-9.
20. Wang CC, Chen WL, Liou SH. P081 The difference between plasma and urinary 8-hydroxy-2-deoxyguanosine biomarkers measured by liquid chromatography tandem mass spectrometry. *Occup Environ Med* 2016;73:A147.

Review

Open Access



Transcatheter aortic valve replacement: is anesthesiologic management linked to surgical outcomes?

Chiara Candela, Annalaura Di Pumpo, Alessandro Centonze, Fabrizio Cucciniello, Domenico Sarubbi, Felice Eugenio Agrò

Department of Anesthesiology, Università Campus Bio-Medico di Roma, Rome 00128, Italy.

Correspondence to: Dr. Chiara Candela, Department of Anesthesiology, Università Campus Bio-Medico di Roma, Rome 00128, Italy. E-mail: c.candela@unicampus.it

How to cite this article: Candela C, Di Pumpo A, Centonze A, Cucciniello F, Sarubbi D, Agrò FE. Transcatheter aortic valve replacement: is anesthesiologic management linked to surgical outcomes? *Vessel Plus* 2018;2:28.
<http://dx.doi.org/10.20517/2574-1209.2018.31>

Received: 14 May 2018 **First Decision:** 25 Jul 2018 **Revised:** 7 Sep 2018 **Accepted:** 7 Sep 2018 **Published:** 29 Sep 2018

Science Editors: Mario F. L. Gaudino, Cristiano Spadaccio **Copy Editor:** Yuan-Li Wang **Production Editor:** Zhong-Yu Guo

Abstract

Aortic valve replacement (AVR) is the current standard treatment for severe aortic stenosis, nonetheless, many patients are not suitable to AVR because of high risk related to advanced age, impaired cardiac function, or comorbidities. Given these considerations, transcatheter aortic valve replacement or implantation (TAVR or TAVI) has emerged in the last decade as an alternative to surgery and has become the treatment of choice for severe aortic stenosis in patients with prohibitive surgical risk. In the context of this kind of hybrid procedure, the anesthesiologist plays a central role because the choice of anesthetic technique is strongly related to clinical features of the patients and technical considerations, which must be discussed collegially with the surgeons. The choice of anesthesiologic management is different among hospitals, but it is generally based on preoperative comorbidities, procedural approach used for TAVR and even hospital logistic. Some centers used to perform TAVR under general anesthesia (GA), some else under local anesthesia plus sedation (LAS), some of them start their TAVR program under GA, but convert in LAS when the team get enough experience. Also, anesthesiologists involved in TAVR procedures must be part of a "heart team", and should be confident with anesthesia for cardiovascular surgery, mechanical circulatory support, and with transesophageal echocardiography. The aim of this article is to provide a general overview about anesthetic techniques in TAVR and to evaluate pathways for future researches.

Keywords: Transcatheter aortic valve implantation, anesthesia, aortic valve disease



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



INTRODUCTION

Aortic stenosis is the most common and dangerous cardiac valvular disease, which a reported incidence of 2%-4% of patients over 65 years^[1,2]. Aortic valve replacement (AVR) is the current standard treatment for severe aortic stenosis^[3], nonetheless, many patients are not suitable to AVR because of high risk related to advanced age, impaired cardiac function, relevant comorbidities such as chronic kidney disease or chronic obstructive pulmonary disease. In addition, heavily calcified aortas, previous mediastinal radiation, and redo valvular surgery expose patients to a prohibitive risk for standard AVR. Given these considerations, transcatheter aortic valve replacement or implantation (TAVR or TAVI) has emerged in the last decade as an alternative to surgery and has become the treatment of choice for severe aortic stenosis in patients with prohibitive surgical risk^[4-6]. The goal of this procedure is minimizing surgical trauma by avoiding sternotomy, aortotomy, cardiopulmonary bypass (CPB) and by implanting the prosthetic valve on beating heart, thereby avoiding cardiac arrest, in order to decrease perioperative risks and improve patient outcomes^[7]. Depending on patient characteristics, TAVR can be performed using different access sites including transfemoral (TF), trans-subclavian/trans-axillary, transaortic, or transapical approaches^[8-10]. The most commonly used approach to performing TAVR is the TF approach by retrograde deployment of the valve passing through the ascending aorta. Most of the centers prefer TF approach because it is less invasive and is associated to a reduced percentage of cardiac related complications, therefore it continues to be the main approach used on patients without severe vascular disease^[11]. Alternative access sites are used on patients with severe peripheral vascular disease and short vessel segments with iliac-femoral arteries diameters < 7 mm^[12,13].

In the context of this kind of hybrid procedure, the anesthesiologist plays a central role because the choice of anesthetic technique is strongly related to clinical features of the patients and technical considerations, which must be discussed collegially with the operators. The most important consideration for the anesthesiologist member of the care team is the type of anesthesia most suited for the patient. The choice of anesthesiologic management is different among hospitals, but it is generally based on preoperative comorbidities, procedural approach used for TAVR and even hospital logistics^[14-18]. Some centers used to perform TAVR under general anesthesia (GA), some else under local anesthesia with a mild sedation (LAS), some of them start their TAVR program under GA, but convert in LAS when the team get enough experience. The aim of the anesthesiologist should be to provide less-invasive anesthesia/analgesia without compromising the safety or comfort of the patient. The aim of this article is to provide a general overview about anesthetic techniques in TAVR and to evaluate pathways for future researches.

FROM AVR TO TAVR... AND FROM GA TO LAS

Although GA is considered mandatory in case of transapical or transaortic approach, there has recently been a significant increase in literature showing the safety and efficacy of local anesthesia with LAS compared with GA when TF approach is performed^[19,20]. At the beginning of a TAVR program, most centers initially chose to provide GA for this procedure, but as the team developed with enough experience and confidence with the procedure, many hospitals started to convert GA in LAS. Actually, the preferred anesthetic management is equally split among centers providing GA vs. sedation for TAVR^[21].

The induction of GA can be performed with a variety of agents, often with a reduced dosage and a very slow administration because of advanced age and decreased cardiac function. Surgical stimulation is not much painful, so the procedure does not need elevated dosage of opioids. Inhalational agents may have some advantages on myocardial protection^[22-26] thanks to a pharmacological preconditioning and postconditioning action. Studies performed on patients undergoing coronary artery bypass graft (CABG) surgery, showed lower postoperative values of cardiac troponin because of the cardioprotective effect of inhalational agents. Short-acting drugs, such as Remifentanyl, that are rapidly cleared are preferred to

Table 1. Comparison of general anesthesia (GA) and local anesthesia with a mild sedation (LAS) in TAVR

GA		LAS	
Advantage	Disadvantages	Advantages	Disadvantages
Safety in starting TAVR program	Myocardial depression due to anesthetic drugs	Hemodynamic stability	Patient discomfort
Safety in predicted difficult airway	Weaning from mechanical ventilation	Reduced in-hospital stay	Not safe in predicted difficult airway
Safety in expected technical complications	Increased in-hospital stay and ICU stay	Reduced ICU stay	Not safe in starting TAVR program
Patient immobility	More invasive (catheterization, CVC, mechanical ventilation)	Reduced delirium	Not safe in expected technical complications
Easy use of TEE		Less invasive (catheterization, CVC, mechanical ventilation)	

TAVR: transcatheter aortic valve replacement; TEE: transesophageal echocardiography; ICU: intensive care unit; CVC central venous catheter

ease extubation at the end of the procedure. Airway control is performed by endotracheal intubation. Any supraglottic device is not advised because of the use of transesophageal echocardiography.

The reason why most centers initially perform TAVR under GA is the safety of anesthesiologic management and the easier management of procedural complications. TAVR has already been demonstrated to be an effective and safe procedure, but it may have important and very dangerous complications leading to true catastrophic events that are often life threatening such as coronary artery occlusion, annular rupture, prosthesis embolization, major vascular injuries, cardiac tamponade, aortic dissection, and/or ventricular perforation^[27,28].

Although conversion to GA because of emergency complications is not common, ranging from 2%-5%^[29-32], most of these events cause serious hemodynamic instability and any delay of ventilation can worsen significantly patient outcome.

Similar considerations are needed for major vascular injuries such as vascular dissection, vascular perforation, and hematoma often requiring blood transfusions. Even in these circumstances conversion to GA may be necessary.

Further advantages of performing TAVR under GA include patient immobility during valve positioning, reduction of breathing artifacts, patient tolerability for the length of the procedure, but above all, facilitating the use of transesophageal echocardiography (TEE). TEE is an useful instrument during the procedure to assist optimal valve placement and prompt recognition of complication such as tamponade or interference with mitral valve. TEE guides the advancement of guidewires and the delivery system and allows to evaluate the effects of the balloon aortic valvuloplasty and the position of the prosthesis at deployment, also it allows to perform a post-implant valve assessment to identify residual regurgitation or paravalvular leaks. Also, 3D TEE may give additional information about structures, catheters and device^[33,34].

Whereas many institutions still perform TAVR under GA, many clinicians in recent years have proposed local anesthesia with or without mild sedation. Several drugs and compounds have been used as monotherapy or in combination for sedating patients during TAVR, including dexmedetomidine, remifentanyl, midazolam, ketamine and propofol^[15,31,35-39].

Local anesthesia combined with conscious sedation provides multiple advantages compared with GA [Table 1]. These benefits are especially noticeable in an old age and a high level of frailty patient

population. Indeed, anesthetic drugs may have depressant effects on myocardial tissue and vasodilatory activity resulting in hemodynamic instability, hypotension and bradycardia which may reduce vital organ perfusion pressure, leading to several postoperative complications such as neurological deficits, myocardial ischemia or renal dysfunction^[40,41]. The use of LAS as an anesthetic choice for TAVR permits to avoid collateral effects of general anesthetics, minimizing hemodynamic instability.

It is furthermore not surprising that using LAS decreases pulmonary complications such as respiratory failure and pneumonia by avoiding mechanical ventilation^[28].

Also, has been showed a significant reduction in postprocedural delirium, which has been showed to prolong in-hospital stay and impair long-term survival. Delirium after TAVR occurs early in the post-operative period, with a percentage around 13%. Patients who developed postprocedural delirium more frequently underwent non-TF procedures under GA^[42].

REGISTRY DATA ANALYSIS

Since LAS has emerged as an alternative to GA, many groups have conducted systematic reviews and meta-analysis in order to determine if the change in anesthetic management has modified the outcome.

In the French Aortic National CoreValve and Edwards 2 (FRANCE 2) registry, data from 2326 patients who underwent TF-TAVR were analyzed, comparing patients receiving GA *vs.* LAS^[43]. This analysis highlighted similar clinical outcomes for GA and LAS about procedure success, 30-day and 1-year survival rates, incidence of complications such as myocardial infarction, stroke, and vascular and bleeding complications. The only significant difference in outcomes was that there is a higher incidence of postprocedural aortic regurgitation in the LAS group. This result is probably due to the less frequent use of TEE support during TAVR under LAS.

However, the new model of Edwards valve minimizes postoperative aortic regurgitation, indeed further studies demonstrated that residual postprocedural aortic regurgitation is completely absent or insignificant in patients implanted with this third-generation valve under LAS^[44,45].

Data from the Italian CoreValve registry also analyzed a cohort of 1316 patients to assess the safety and non-inferiority of LAS *vs.* GA. The authors demonstrated that, in experienced centers which have gone over their initial learning period with TAVR, LAS can be performed safely with good clinical outcomes, with no significant difference in myocardial infarction, stroke or mortality than GA group^[27].

In a recent review the authors screened publications (randomized controlled trials and observational studies) published between 1 January 2006 and 26 June 2016 that compared LAS to GA in an adult study population undergoing TAVR, to identify the potential favorable effects of LAS compared with GA. They analyzed differences between LAS and GA in terms of 30-day mortality, in-hospital mortality and other endpoints that address safety and complications rates^[46]. The authors showed no significant difference in the 30-day mortality rate among the two groups. Similarly, the in-hospital mortality rate did not demonstrate any significant difference between the study groups. Instead, the authors revealed a significant decrease in both intraprocedural and postprocedural catecholamine need in the LAS group. During TAVR, 31% of the LAS group received catecholamines, in contrast, the rate was 65.0% in the GA group.

As explained previously, this result in LAS group is probably due to the absence of hemodynamic effects of general anesthetics, such as vasodilation and myocardial depression. Regarding catecholamine administration, inotropes are more used than vasopressors, since patients suitable for TAVR often have a

depressed cardiac function which is the main factor of hemodynamic instability.

Conversion from TAVR to open heart surgery was infrequently occurring in 2.5% of the LAS group and 2.9% of the GA group, without any significant difference between the groups.

The main reasons for conversion from LAS to GA were vascular and procedural complications, hypotension, respiratory complications and insufficient patient compliance or patient discomfort.

The meta-analysis did not reveal a significant difference between the groups in the rate complications such as major and minor vascular complications, major and life-threatening bleeding, acute kidney injury, myocardial infarctions and stroke.

Only three studies reported a slightly lower frequency of pneumonia in LAS group, but the difference between the two groups did not reach statistical significance. It is possible to speculate that this tendency reflects minor risk of ventilator associated pneumonia (VAP), however is not possible to better analyze this aspect because too small number of articles reported this outcome. In this review an important difference between the two groups was highlighted: the length of hospital stay was significantly shorter in the LAS group (MD - 1.49 days) and the length of intensive care unit (ICU) stay was found to be shorter for patients in the LAS group (MD - 0.47 days). Since the rate of periprocedural complications is similar in both groups, such result is probably due to the time needed for the transfer of ventilated patients to the ICU, where extubation can occur with some delay after the procedure^[28].

As reported by Gauthier *et al.*^[47] ICU admission may be related with a nosocomial infection, with a lower risk of infectious complications for patients who received LAS for TAVR, by avoiding bladder catheterization, central venous catheter insertion and mechanical ventilation.

Another difference between the two groups was a higher rate of pacemaker (PMK) implantations in the patients who underwent LAS for TAVR occurring in 17.5% of patients compared with 12.8% of the GA group.

A third-degree atrioventricular block is a frequent periprocedural complication requiring a PMK. This result may be due to increased patient movement during valve positioning because of discomfort or poor patient compliance. In some case has been also reported an anxiogenic effect due to decreased cerebral blood flow during rapid ventricular pacing^[37]. Furthermore, the use of GA eases precise valve positioning thanks to a short interruption of mechanical ventilation and patient immobility.

DISCUSSION

Analyzing international literature, it appears clear that both anesthesiologic techniques GA and LAS are safe and none of them influence negatively the patient outcome.

From many studies a new trend towards minimally invasive anesthesia for TAVR has emerged, especially regarding the TF approach which is the most used technique for TAVR. The choice of anesthetic management generally depends on the patient's clinical profile and the procedural technical characteristics, but a center's experience and internal organization also play an important role in the decision-making. When a TAVR program starts, many operators might choose to perform the procedure with GA because of the uncertainty of a new procedure, initial low volume, operator's learning curve and the possible complications more frequent in centers with low volume of TAVR^[48]. As the learning curve of the operators reaches a new plateau and the techniques of TAVR evolve, the procedure time becomes shorter and the complications decrease; this needs around two years of continuous activity or 50 consecutive cases^[48]. Others revealed a learning curve with an improvement in complications rate, after the initial 86 cases using

the Edwards valve and 40 cases using the CoreValve^[49]. Due to this improvement in the procedure time and complications rate, the care team should be able to perform safely LAS for patients undergoing TAVR. Although minimal invasive anesthesiologic management is always more widespread, in some clinical situations could be advantageous provide TAVR under GA. This is particularly true in patients unable to maintain adequate immobility throughout the procedure. This may include patients with neurological impairment and patients with advanced heart failure with pulmonary edema that impede to keep supine position for prolonged periods of time. Even if TEE can be used also under sedation, the planned use of TEE may necessitate GA, because of long time esophageal stimulation. The general opinion is that TEE can help during valve deployment and in rapid identification of complications; nevertheless, its routine use may not be justified.

A further clinical situation in which providing GA may be safer than LAS is when patient is expected to be at high risk for intraprocedural complications because of anatomic conformation.

Prevention of these complications should be based on patient screening and selection by a dedicated “heart team”. In such cases, the use of multimodality imaging may play an even more important role, with the aim to evaluate patient suitability for the proposed access site and to select prosthesis size based on aortic measurement. Preprocedural program is also useful to ensure if proposed device can be safely deployed, based on device characteristics and the anatomic relationships between the aortic valve and root, left ventricle and coronary ostia^[50]. As experience suggests, if pre-procedural evaluation estimates that there might be a mechanical complication, GA is recommended rather than sedation. The same management is recommended for patient with difficult airway management because, in case of emergency conversion from LAS to GA, any delay in endotracheal intubation may be unsafe.

Another important consideration in management of patients undergoing TAVR should be considered: over the last decade, TAVR has emerged to become the preferred alternative for high-risk patients with severe aortic symptomatic stenosis. Nevertheless, new perspective seems destined to expand indications for TAVI towards lower risk, younger and asymptomatic populations^[51,52]. In such a case, a less invasive strategy, using LAS instead of GA, seems to be even more appropriate in order to make TAVR procedure even safer, faster, with fewer risks and to achieve an easier post-operative management.

CONCLUSION

Preoperative anesthesiologic management should be based on the experience of the team, preferring GA in the initial phases of the program (about 50 cases). Selection of anesthetic technique should be individualized on the patient's clinical status, preferring GA in case of difficult airway and in case of predicted technical difficulty. Instead, patients with advanced respiratory disease or renal impairment or patients with high risk in developing delirium after GA, should be treated by LAS.

Whether the team provide GA or LAS, the hybrid operating room must be equipped with devices for managing difficult airways and emergency scenarios. Also, there is an agreement that anesthesiologists involved in performing TAVR must be part of a “heart team”, who must be confident with anesthesia for cardiovascular surgery, with mechanical circulatory support, and with TEE.

DECLARATIONS

Authors' contributions

Concept, drafting, data collection, final approval: Candela C, Di Pumpo A
Critical revision, final approval: Centonze A, Cucciniello F

Supervision, critical revision, final approval: Sarubbi D, Agrò FE

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Iung B, Baron G, Butchart EG, Delahaye F, Gohlke-Bärwolf C, Levang OW, Tornos P, Vanoverschelde JL, Vermeer F, Boersma E, Ravaut P, Vahanian A. A prospective survey of patients with valvular heart disease in Europe: the euro heart survey on valvular heart disease. *Eur Heart J* 2003;24:1231-43.
2. Oppizzi M. Echocardiography in the perioperative decision making of patients with aortic stenosis. *HSR Proc Intensive Care Cardiovasc Anesth* 2009;1:7-15.
3. Carabello BA, Paulus WJ. Aortic stenosis. *Lancet* 2009;373:956-66.
4. Cribier A, Eltchaninoff H, Bash A, Borenstein N, Tron C, Bauer F, Derumeaux G, Anselme F, Laborde F, Leon MB. Percutaneous transcatheter implantation of an aortic valve prosthesis for calcific aortic stenosis: first human case description. *Circulation* 2002;106:3006-8.
5. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP 3rd, Guyton RA, O’Gara PT, Ruiz CE, Skubas NJ, Sorajja P, Sundt TM 3rd, Thomas JD; ACC/AHA Task Force Members. 2014 AHA/ACC guideline for the management of patients with valvular heart disease: executive summary: a report of the American College of Cardiology/American heart association task force on practice guidelines. *Circulation* 2014;129:2440-92.
6. Webb JG, Wood DA. Current status of transcatheter aortic valve replacement. *J Am Coll Cardiol* 2012;60:483-92.
7. Leon MB, Kodali S, Williams M, Oz M, Smith C, Stewart A, Schwartz A, Collins M, Moses JW. Transcatheter aortic valve replacement in patients with critical aortic stenosis: rationale, device descriptions, early clinical experiences, and perspectives. *Semin Thorac Cardiovasc Surg* 2006;18:165-74.
8. Latsios G, Gerckens U, Grube E. Transaortic transcatheter aortic valve implantation: a novel approach for the truly “no-access option” patients. *Catheter Cardiovasc Interv* 2010;75:1129-36.
9. Johansson M, Nozohoor S, Kimblad PO, Harnek J, Olivecrona GK, Sjögren J. Transapical versus transfemoral aortic valve implantation: a comparison of survival and safety. *Ann Thorac Surg* 2011;91:57-63.
10. Moynagh AM, Scott DJ, Baumbach A, Khavandi A, Brecker SJ, Laborde JC, Brown S, Chowdhary S, Saravanan D, Crean PA, Teehan S, Hildick-Smith D, Trivedi U, Khogali SS, Bhabra MS, Roberts DH, Morgan KP, Blackman DJ; UK CoreValve Collaborative. CoreValve transcatheter aortic valve implantation via the subclavian artery: comparison with the transfemoral approach. *J Am Coll Cardiol* 2011;57:634-5.
11. Mack MJ, Brennan JM, Brindis R, Carroll J, Edwards F, Grover F, Shahian D, Tuzcu EM, Peterson ED, Rumsfeld JS, Hewitt K, Shewan C, Michaels J, Christensen B, Christian A, O’Brien S, Holmes D; STS/ACC TVT Registry. Outcomes following transcatheter aortic valve replacement in the United States. *JAMA* 2013;310:2069-77.
12. Greenbaum AB, O’Neill WW, Paone G, Guerrero ME, Wyman JF, Cooper RL, Lederman RJ. Caval-aortic access to allow transcatheter aortic valve replacement in otherwise ineligible patients: initial human experience. *J Am Coll Cardiol* 2014;63:2795-804.
13. Mylotte D, Sudre A, Teiger E, Obadia JF, Lee M, Spence M, Khamis H, Al Nooryani A, Delhay C, Amr G, Koussa M, Debry N, Piazza N, Modine T. Transcarotid transcatheter aortic valve replacement: feasibility and safety. *JACC Cardiovasc Interv* 2016;9:472-80.
14. Ree RM, Bowering JB, Schwarz SK. Case series: anesthesia for retrograde percutaneous aortic valve replacement—experience with the first 40 patients. *Can J Anaesth* 2008;55:761-8.

15. Behan M, Haworth P, Hutchinson N, Trivedi U, Laborde JC, Hildick-Smith D. Percutaneous aortic valve implants under sedation: our initial experience. *Catheter Cardiovasc Interv* 2008;72:1012-5.
16. Billings FT 4th, Kodali SK, Shanewise JS. Transcatheter aortic valve implantation: anesthetic considerations. *Anesth Analg* 2009;108:1453-62.
17. Basciani RM, Eberle B. Percutaneous aortic valve implants under sedation: our initial experience. *Catheter Cardiovasc Interv* 2009;74:148-9.
18. Sellevold OF, Guarracino F. Transcatheter aortic valve implantation: recent advances and future. *Curr Opin Anaesthesiol* 2010;23:67-73.
19. Guarracino F, Landoni G. Con: transcatheter aortic valve implantation should not be performed under general anesthesia. *J Cardiothorac Vasc Anesth* 2012;26:736-9.
20. Motloch LJ, Rottlaender D, Reda S, Larbig R, Bruns M, Müller-Ehmsen J, Strauch J, Madershahian N, Erdmann E, Wahlers T, Hoppe UC. Local versus general anesthesia for transfemoral aortic valve implantation. *Clin Res Cardiol* 2012;101:45-53.
21. Brecker SJ, Bleiziffer S, Bosmans J, Gerckens U, Tamburino C, Wenaweser P, Linke A; ADVANCE Study Investigators. Impact of anesthesia type on outcomes of transcatheter aortic valve implantation (from the multicenter ADVANCE study). *Am J Cardiol* 2016;117:1332-8.
22. Tritapepe L, Landoni G, Guarracino F, Pompei F, Crivellari M, Maselli D, De Luca M, Fochi O, D'Avolio S, Bignami E, Calabrò MG, Zangrillo A. Cardiac protection by volatile anaesthetics: a multicentre randomized controlled study in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass. *Eur J Anaesthesiol* 2007;24:323-31.
23. Bignami E, Biondi-Zoccai G, Landoni G, Fochi O, Testa V, Sheiban I, Giunta F, Zangrillo A. Volatile anesthetics reduce mortality in cardiac surgery. *J Cardiothorac Vasc Anesth* 2009;23:594-9.
24. Landoni G, Fochi O, Tritapepe L, Guarracino F, Belloni I, Bignami E, Zangrillo A. Cardiac protection by volatile anesthetics. A review. *Minerva Anesthesiol* 2009;75:269-73.
25. De Hert SG, Turani F, Mathur S, Stowe DF. Cardioprotection with volatile anesthetics: mechanisms and clinical implications. *Anesth Analg* 2005;100:1584-93.
26. Chiari P, Bouvet F, Piriou V. Anaesthetic-induced myocardial preconditioning: fundamental basis and clinical implications. *Ann Fr Anesth Reanim* 2005;24:383-96.
27. Petronio AS, Giannini C, De Carlo M, Bedogni F, Colombo A, Tamburino C, Klugmann S, Poli A, Guarracino F, Barbanti M, Latib A, Brambilla N, Fiorina C, Bruschi G, Martina P, Ettori F. Anaesthetic management of transcatheter aortic valve implantation: results from the Italian CoreValve registry. *EuroIntervention* 2016;12:381-8.
28. Goren O, Finkelstein A, Gluch A, Sheinberg N, Dery E, Matot I. Sedation or general anesthesia for patients undergoing transcatheter aortic valve implantation--does it affect outcome? An observational single-center study. *J Clin Anesth* 2015;27:385-90.
29. Wiegerinck EM, Boerlage-van Dijk K, Koch KT, Yong ZY, Vis MM, Planken RN, Eberl S, de Mol BA, Piek JJ, Tijssen JG, Baan J Jr. Towards minimally invasiveness: transcatheter aortic valve implantation under local analgesia exclusively. *Int J Cardiol* 2014;176:1050-2.
30. Piayda KD, Gafoor S, Bertog S, Doss M, Vaskelyte L, Matic P, Franke J, Hofmann I, Staiger N, Reinartz M, Sievert H. True first-line local-anesthesia only protocol for transfemoral TAVI. *J Invasive Cardiol* 2015;27:501-8.
31. Durand E, Borz B, Godin M, Tron C, Litzler PY, Bessou JP, Bejar K, Fraccaro C, Sanchez-Giron C, Dacher JN, Bauer F, Cribier A, Eltchaninoff H. Transfemoral aortic valve replacement with the Edwards SAPIEN and Edwards SAPIEN XT prosthesis using exclusively local anesthesia and fluoroscopic guidance: feasibility and 30-day outcomes. *JACC Cardiovasc Interv* 2012;5:461-7.
32. Yamamoto M, Meguro K, Mouillet G, Bergeend E, Monin JL, Lim P, Dubois-Rande JL, Teiger E. Effect of local anesthetic management with conscious sedation in patients undergoing transcatheter aortic valve implantation. *Am J Cardiol* 2013;111:94-9.
33. Chin D. Echocardiography for transcatheter aortic valve implantation. *Eur J Echocardiogr* 2009;10:i21-9.
34. Gonçalves A, Marcos-Alberca P, Zamorano JL. Echocardiography: guidance during valve implantation. *EuroIntervention* 2010;6:G14-9.
35. Bufton KA, Augoustides JG, Cobey FC. Anesthesia for transfemoral aortic valve replacement in North America and Europe. *J Cardiothorac Vasc Anesth* 2013;27:46-9.
36. Park HS, Kim KM, Joung KW, Choi IC, Sim JY. Monitored anesthesia care with dexmedetomidine in transfemoral percutaneous transcatheter aortic valve implantation: two cases report. *Korean J Anesthesiol* 2014;66:317-21.
37. Dehédin B, Guinot PG, Ibrahim H, Allou N, Provenchère S, Dilly MP, Vahanian A, Himbert D, Brochet E, Radu C, Nataf P, Montravers P, Longrois D, Depoix JP. Anesthesia and perioperative management of patients who undergo transfemoral transcatheter aortic valve implantation: an observational study of general versus local/regional anesthesia in 125 consecutive patients. *J Cardiothorac Vasc Anesth* 2011;25:1036-43.
38. Ben-Dor I, Looser PM, Maluenda G, Weddington TC, Kambouris NG, Barbash IM, Hauville C, Okubagzi P, Corso PJ, Satler LF, Pichard AD, Waksman R. Transcatheter aortic valve replacement under monitored anesthesia care versus general anesthesia with intubation. *Cardiovasc Revasc Med* 2012;13:207-10.
39. Babaliaros V, Devireddy C, Lerakis S, Leonardi R, Iturra SA, Mavromatis K, Leshnower BG, Guyton RA, Kanitkar M, Keegan P, Simone A, Stewart JP, Ghasemzadeh N, Block P, Thourani VH. Comparison of transfemoral transcatheter aortic valve replacement performed in the catheterization laboratory (minimalist approach) versus hybrid operating room (standard approach): outcomes and cost analysis. *JACC Cardiovasc Interv* 2014;7:898-904.
40. Watterson LM, Morris RW, Westhorpe RN, Williamson JA. Crisis management during anaesthesia: bradycardia. *Qual Saf Health Care*

- 2005;14:e9.
41. Morris RW, Watterson LM, Westhorpe RN, Webb RK. Crisis management during anaesthesia: hypotension. *Qual Saf Health Care* 2005;14:e11.
 42. Abawi M, Nijhoff F, Agostoni P, Emmelot-Vonk MH, de Vries R, Doevendans PA, Stella PR. Incidence, predictive factors, and effect of delirium after transcatheter aortic valve replacement. *JACC Cardiovasc Interv* 2016;9:160-8.
 43. Oguri A, Yamamoto M, Mouillet G, Gilard M, Laskar M, Eltchaninoff H, Fajadet J, Iung B, Donzeau-Gouge P, Leprince P, Leguerrier A, Prat A, Lievre M, Chevreul K, Dubois-Rande JL, Chopard R, Van Belle E, Otsuka T, Teiger E; FRANCE 2 Registry Investigators. Clinical outcomes and safety of transfemoral aortic valve implantation under general versus local anesthesia: subanalysis of the French Aortic National CoreValve and Edwards 2 registry. *Circ Cardiovasc Interv* 2014;7:602-10.
 44. Wöhrle J, Gonska B, Rodewald C, Seeger J, Scharnbeck D, Rottbauer W. Transfemoral aortic valve implantation with the new Edwards Sapien 3 valve for treatment of severe aortic stenosis-impact of valve size in a single center experience. *PLoS One* 2016;11:e0151247.
 45. Kodali S, Thourani VH, White J, Malaisrie SC, Lim S, Greason KL, Williams M, Guerrero M, Eisenhauer AC, Kapadia S, Kereiakes DJ, Herrmann HC, Babaliaros V, Szeto WY, Hahn RT, Pibarot P, Weissman NJ, Leipsic J, Blanke P, Whisenant BK, Suri RM, Makkar RR, Ayele GM, Svensson LG, Webb JG, Mack MJ, Smith CR, Leon MB. Early clinical and echocardiographic outcomes after SAPIEN 3 transcatheter aortic valve replacement in inoperable, high-risk and intermediate-risk patients with aortic stenosis. *Eur Heart J* 2016;37:2252-62.
 46. Ehret C, Rossaint R, Foldenauer AC, Stoppe C, Stevanovic A, Dohms K, Hein M, Schälte G. Is local anaesthesia a favourable approach for transcatheter aortic valve implantation? A systematic review and meta-analysis comparing local and general anaesthesia. *BMJ Open* 2017;7:e016321.
 47. Gauthier C, Astarci P, Baele P, Matta A, Kahn D, Kefer J, Momeni M. Mid-term survival after transcatheter aortic valve implantation: results with respect to the anesthetic management and to the access route (transfemoral versus transapical). *Ann Card Anaesth* 2015;18:343-51.
 48. Lunardi M, Pesarini G, Zivelonghi C, Piccoli A, Geremia G, Ariotti S, Rossi A, Gambaro A, Götting L, Faggian G, Vassanelli C, Ribichini F. Clinical outcomes of transcatheter aortic valve implantation: from learning curve to proficiency. *Open Heart* 2016;3:e000420.
 49. Arai T, Lefèvre T, Hovasse T, Hayashida K, Watanabe Y, O'Connor SA, Benamer H, Garot P, Cormier B, Bouvier E, Morice MC, Chevalier B. Evaluation of the learning curve for transcatheter aortic valve implantation via the transfemoral approach. *Int J Cardiol* 2016;203:491-7.
 50. Bloomfield GS, Gillam LD, Hahn RT, Kapadia S, Leipsic J, Lerakis S, Tuzcu M, Douglas PS. A practical guide to multimodality imaging of transcatheter aortic valve replacement. *JACC Cardiovasc Imaging* 2012;5:441-55.
 51. Arora S, Vavalle JP. Transcatheter aortic valve replacement in intermediate and low risk patients-clinical evidence. *Ann Cardiothorac Surg* 2017;6:493-7.
 52. Hamm CW, Arsalan M, Mack MJ. The future of transcatheter aortic valve implantation. *Eur Heart J* 2016;37:803-10.

Review

Open Access



A current view of G protein-coupled receptor - mediated signaling in pulmonary hypertension: finding opportunities for therapeutic intervention

Derek Strassheim¹, Vijaya Karoor^{1,2}, Kurt Stenmark^{2,3}, Alexander Verin⁴, Evgenia Gerasimovskaya^{2,3}

¹Departments of Medicine, University of Colorado Denver, Aurora, CO 80045, USA.

²Cardiovascular and Pulmonary Research laboratories, University of Colorado Denver, Aurora, CO 80045, USA.

³Department of Pediatrics, Pulmonary and Critical Care Medicine, University of Colorado Denver, Aurora, CO 80045, USA.

⁴Vascular Biology Center, Augusta University, Augusta, GA 30912, USA.

Correspondence to: Dr. Derek Strassheim, Departments of Medicine, University of Colorado Denver, 12700 E. 19th Avenue, Box B131, Research 2, Room 6470D, Aurora, CO 80045, USA. E-mail: derek.strassheim@ucdenver.edu

How to cite this article: Strassheim D, Karoor V, Stenmark K, Verin A, Gerasimovskaya E. A current view of G protein-coupled receptor - mediated signaling in pulmonary hypertension: finding opportunities for therapeutic intervention. *Vessel Plus* 2018;2:29. <http://dx.doi.org/10.20517/2574-1209.2018.44>

Received: 8 Jun 2018 **First Decision:** 23 Jul 2018 **Revised:** 29 Aug 2018 **Accepted:** 30 Aug 2018 **Published:** 30 Sep 2018

Science Editor: Alexander D. Verin **Copy Editor:** Cai-Hong Wang **Production Editor:** Zhong-Yu Guo

Abstract

Pathological vascular remodeling is observed in various cardiovascular diseases including pulmonary hypertension (PH), a disease of unknown etiology that has been characterized by pulmonary artery vasoconstriction, right ventricular hypertrophy, vascular inflammation, and abnormal angiogenesis in pulmonary circulation. G protein-coupled receptors (GPCRs) are the largest family in the genome and widely expressed in cardiovascular system. They regulate all aspects of PH pathophysiology and represent therapeutic targets. We overview GPCRs function in vasoconstriction, vasodilation, vascular inflammation-driven remodeling and describe signaling cross talk between GPCR, inflammatory cytokines, and growth factors. Overall, the goal of this review is to emphasize the importance of GPCRs as critical signal transducers and targets for drug development in PH.

Keywords: Pulmonary hypertension, vascular remodeling, vasoconstriction, vascular inflammation, GPCR, intracellular signaling

INTRODUCTION

Pulmonary hypertension (PH) is a complex disease of unknown etiology. The pulmonary circulation responds to hypoxia by vasoconstriction, thereby diverting blood to oxygen rich regions. However, prolonged hypoxic vasoconstriction leads to remodeling of pulmonary arteries (PAs) and increased PA pressure.



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Increased pressure initially results in compensatory cardiac hypertrophy, but eventually causes de-compensatory cardiac remodeling and death by heart failure. Recent research indicates that PH in all its forms, especially associated with left heart disease, is more common than previously thought^[1]. Current PH therapies that include endothelin-1 (ET1) receptor antagonists, prostacyclin analogs, cGMP- phosphodiesterase (PDE) inhibitors, and Ca^{2+} channel blockers impede, but do not stop the disease process, emphasizing the need for a finding of alternate treatments^[2]. Over the years, preclinical research in PH has identified many protein targets, but very few have translated to the bench side. By contrast, G-protein-coupled receptors (GPCRs), the largest superfamily in the genome, play an important role in the development of PH and can be easily targeted by drugs^[3]. The heart, a prime target for development of new PH therapies, expresses 200 GPCRs^[4]. GPCR signaling cascades are critical for cardiovascular function and are targeted for the treatment of hypertension and heart failure by agonist and antagonist strategies. Here we reviewed the current knowledge on GPCR signaling in cardiac, vascular, and blood cells and highlighted some critical outcomes in PH, such as vasoconstriction/vasodilation responses, vascular inflammation, vascular and cardiac remodeling, and endothelial dysfunction (ED).

GPCR-MEDIATED SIGNALING

GPCRs are a family of 7-transmembrane domain proteins, forming a deep binding pocket for the extracellular ligand, agonist, which activates the receptor. Intracellular loops make contact to heterotrimeric G-proteins of 4 different classes ($\text{G}\alpha_s$, $\text{G}\alpha_i$, $\text{G}\alpha_q$, $\text{G}\alpha_{12}$) [Table 1]^[5]. Agonist binding to GPCRs stimulates GDP/GTP exchange on $\text{G}\alpha$ subunits, converting them into the active state and promote dissociation of $\text{G}\beta\gamma$ subunits. G proteins interact with multiple effectors, leading to generation of second messengers, including cAMP, 1,2-diacylglycerol, phosphatidylinositol-3, 4, 5-trisphosphate (PIP₃), and Ca^{2+} . These signaling events are translated into complex hierarchy of kinase network [PKA, PKC, Akt, Ca^{2+} /calmodulin-dependent protein kinase (CAMK)] leading to the regulation of gene expression and cellular functions. There are four families of $\text{G}\alpha$ subunits with multiple members. α_s exists as multiple transcripts 42 short and 44kD long forms. α_i subfamily has α_{i1} , α_{i2} , α_{i3} , α_z , α_{O1} , α_{O2} ; the α_q subfamily has α_{11} , α_{14} , and α_{16} , and the $\alpha_{12/13}$ family. The β subunits are β_{1-5} ; and the γ subunits are $\gamma_{1-5,7,8,10,11,13}$. The $\beta\gamma$ subunits, like $\text{G}\alpha$ subunits, activate intracellular effector pathways including MAPK cascades, Rac1, phospholipase C- β (PLC- β), phosphoinositide 3 kinase γ (PI3K- γ , and ion channels and show variation as to the GPCR- $\text{G}\alpha$ -complexes they interact with. Termination of G protein activation cycle occurs by the transition of $\text{G}\alpha$ subunits to GDP-bound state, that is catalyzed by GTPase activating proteins (GAPs), known as regulators of G-protein signaling (RGS proteins). There are 31 proteins, containing the RGS domain that function as GTPase enzymes, terminating G-protein signaling^[6,7].

GPCR SIGNALING IN VASOCONSTRICTION AND VASCULAR REMODELING

Vasoconstriction is driven by Ca^{2+} -dependent phosphorylation of myosin light chain (MLC) on Ser¹⁹-MLC, whereas vasodilators oppose this event^[8-10] [Figure 1, Tables 1 and 2]. In vascular smooth muscle cells (VSMC), the vasoconstrictor response is mediated by G_i , G_q , or $\text{G}_{12/13}$ -coupled GPCRs for ET1, angiotensin II (Ang II), serotonin, and thrombin^[11-16]. G_i and G_q activate PLC pathways, increasing Ca^{2+} and receptor operated calcium entry (ROCE) via transient receptor potential cation channel subfamily C member 6 (TRPC₆) channels. TRPC₆-activation occurs by several mechanisms, including direct ERK1/2-mediated phosphorylation of TRPC₆. Secondly, phosphoinositide-4, 5-bisphosphate (PIP₂), the substrate for PLC, is an inhibitor of TRPC₆^[17,18]. Activation of $\text{G}_{12/13}$ by vasoconstrictor GPCRs stimulates $\text{G}_{12/13}$ -dependent RhoA GEFs to increase the activity of, RhoA. In turn, RhoA activates Rho associated kinase (ROCK), which leads to increased Ser¹⁹-MLC and thereby, vasoconstriction^[19,20]. Vasodilators, such as prostaglandin I₂ (PGI₂), acting via G_s -coupled (IP) receptor on VSMC, activate PKA and decrease intracellular Ca^{2+} , leading to reduced MLC phosphorylation on Ser¹⁹ [Figure 1, Table 1].

Vasodilators decrease intracellular Ca^{2+} by inhibiting PLC β and TRPC₆. The mechanism involves PKA/PKG-mediated phosphorylation of PLC β and TRPC₆ (on Ser²⁸) and by phosphorylation of RGS4, which

Table 1. G protein-coupled receptor physiology and pathology in pulmonary hypertension

Physiology	Ligand-receptor-reference	Cell	G-protein	Important pathways	PH pathology
Vasodilation	Adenosine-A _{2A} -AR; PGI ₂ -IP ^[110-112]	VSMC	G _s	PKA	+
EC-eNOS-NO dependent vasodilation	Adenosine-A _{2A} -AR; Apelin-APJ; Relaxin-RXFP; Opioid-KOR ^[50,51,66,110-112,178,179,182,245,246]	EC	G _i	PKG	+
Vasoconstriction	ET ₁ /ET _A ; Ang II-AT ₁ ; TXA ₂ -TP ₁ ; PAF/PAFR; Shingosine-1-P/S1P ₁₋₅ ; Ca ²⁺ -CaSR ^[12,21,42,47,54-56,58,69,249,250]	VSMC	G _q /G _i	Ca ²⁺	-
Anti-inflammatory	Adenosine-A _{2A} -AR; PGI ₂ -IP ^[110]	VSMC	G _s	PKA	+
	PGI ₂ -IP; adenosine-A _{2A} AR ^[232,239]	Macrophage	G _s	PKA	+
	PGI ₂ -IP; adenosine-A _{2A} AR ^[110]	Fibroblast	G _s	PKA	+
	PGI ₂ -IP; Adenosine-A _{2A} -AR ^[110]	EC	G _s	PKA	+
Pro-inflammatory	ET ₁ -ET _A ; MCP1-CCR2; RANTES-CCR5; TXA ₂ -TP ^[69,163]	VSMC	G _q /G _i	Ca ²⁺	-
	LTB ₄ -LTB ₄ R; MCP1-CCR2 ^[163,164]	Macrophage	G _q /G _i	Ca ²⁺	-
	PAF-PAFR; TXA ₂ -TP ^[16,167,169]	EC	G _q /G _i	Ca ²⁺	-
	AngII-AT ₁ ; succinate-GPR91; thrombin-PAR ^[205,206]	Cardiac myocyte	G _q /G _i	Ca ²⁺	-
Cardiac fibrosis	Thrombin-PAR ₁₋₄ ^[223,225]	Cardiac fibroblast	G _q /G _i /G _{12/13}	Ca ²⁺ /RhoA	-

+: PH-protective; -: PH-pathogenic; VSMC: vascular smooth muscle cells; EC: endothelial cell

inhibits G_q-dependent activation of PLCβ^[21-23]. Vasodilator GPCRs that increase cAMP may also activate cAMP-binding domain in exchange factor EPAC1, a GEF for the small molecular weight G-protein Rap1, a member of Ras superfamily. Rap1 activates ARAP3, a Rho GAP, which in turn, inhibits RhoA, leading to reduced MLC phosphorylation and vasodilation^[24,25]. Vasodilation also occurs via endothelial cell (EC)-dependent production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS), which is activated by Akt or ERK1/2 by phosphorylation on Ser¹¹⁷⁷ residue^[26]. Highly permeable NO readily enters VSMC, stimulates soluble guanylate cyclase (sGC) and activates cGMP-PKG, antagonizing Ca²⁺ action on phospho-Ser¹⁹-MLC and promoting vasodilation. More specifically, NO-sGC-cGMP-PKG-axis inhibits Ca²⁺ increase by stimulating TRPC6 phosphorylation at Thr⁶⁹, decreasing ROCE and increasing vasodilation^[27]. PKG phosphorylates and activates RGS2, and RGS4, that leads to the inhibition of G_i/G_q-regulated PLC activity and termination of the vasoconstrictor Ca²⁺ signal^[23]. Both PKG and PKA phosphorylate and inhibit RhoA and increase the activity of myosin light chain phosphatase (MLCP), thereby decreasing MLC contraction^[28,29]. MLCP is also activated by vasodilators by PKG-mediated phosphorylation of a MLCP inhibitory subunit^[20]. In addition, PKG and PKA reduce the ability of RhoA to inhibit the delayed rectifier potassium channel (KDR), which attenuates extracellular Ca²⁺ entry^[30]. The enzyme PDE5A, a target of sildenafil therapy in PH, hydrolyzes cGMP to counter the effects of NO-cGMP-PKG signaling. However, other PDEs, including cAMP PDEs, play important roles^[31]. Vasoconstrictors activate PDE5A to reduce cGMP in VSMC by RhoA/PKC-mediated inhibition of protein phosphatase 1 (PP1), thereby increasing phosphorylation of PDE5A and activating it^[32]. GPCRs, including those for adenosine, ATP, adiponectin, apelin, prostaglandin E₂ (PGE₂), PGI₂ generally increase NO from EC, which diffuses to VSMC, or directly increase cAMP in VSMCs^[33-39].

As a final summation statement, all current PH therapies intersect GPCR actions by modulating critical signaling effects. Firstly they, ultimately inhibit intracellular Ca²⁺ signaling and vasoconstriction. This includes the cGMP-PDE inhibitors, soluble guanylate cyclase (sGC) activators, PGI₂ analogs, Ca²⁺-channel blockers, and ET-1 receptor antagonists. Secondly, they exert anti-inflammatory effects on vascular cells, as all of these therapeutics are known to do^[2,40,41].

GPCR ligand-dependent vasoconstrictor response

Vasoconstrictor ligands, including ET-1, TxA₂, and serotonin are increased in serum of PH patients; for serotonin a 4-5 fold increase has been reported, (8.8 ± 0.6 nmol/L) vs. (38.8 ± 7.3 nmol/L)^[42-47]. Serotonin,

Table 2. Current G protein-coupled receptor clinical trials in pulmonary hypertension

Clinical trials name	Sponsor	Drug	Target
Tomorrow	Acetilon	Macitentan	ET _A /ET _B antagonist
ADAPT	United therapeutics	Orenitram	IP agonist
	Lung biotechnology	BPS-314d oral treprostanil	IP agonist
	Arena pharmaceuticals	APD-811	IP agonist
INSPIRE	Liquidia technologies	Inhaled treprostanil	IP agonist

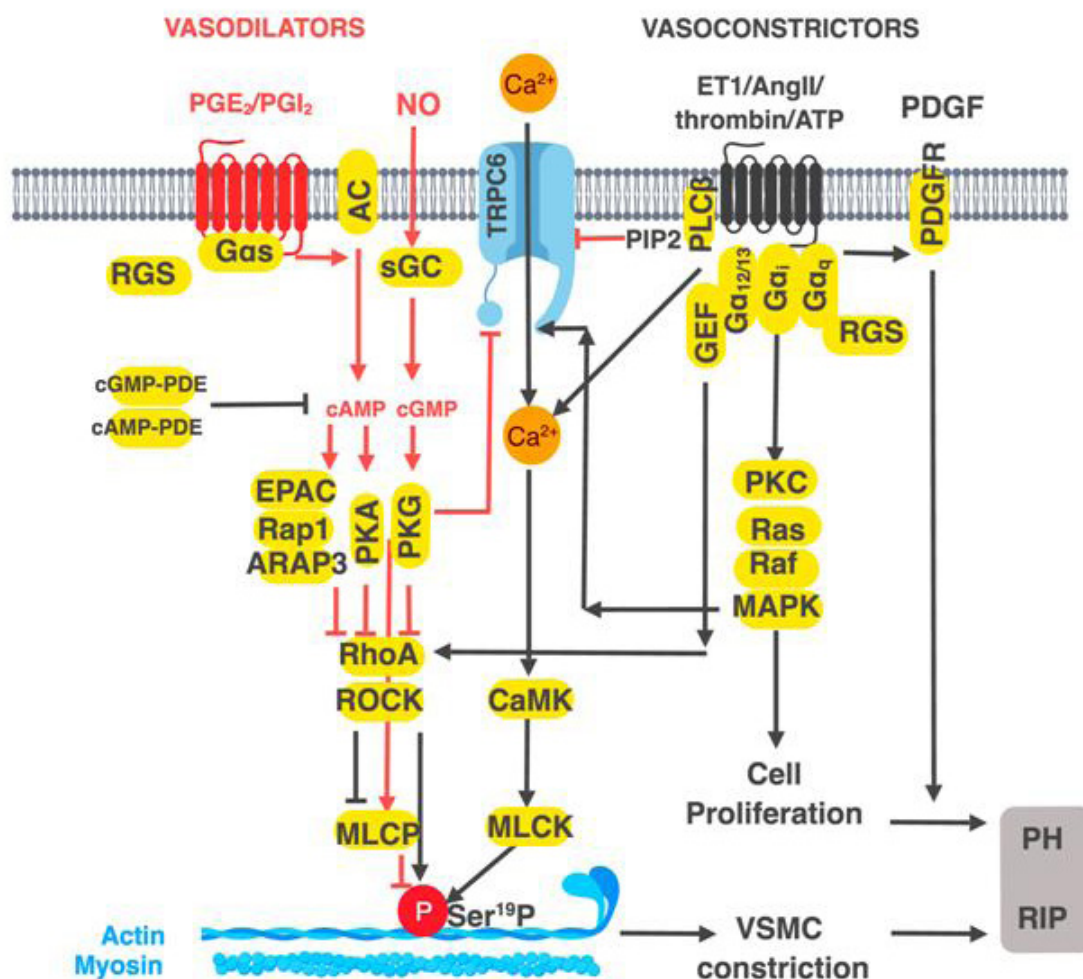


Figure 1. Schematic presentation of the mechanisms by which G protein-coupled receptors (GPCRs) regulate vascular tone and vascular smooth muscle cells (VSMC) proliferation. Vasoconstrictors like Ang II, ET1, thrombin, activate G_{α_i}, G_{α_q}, or G_{12/13}-coupled GPCRs, increase Ca²⁺ via PLCβ activity, and receptor operated calcium channels such as TRPC6. Increase in PLCβ activity decreases PIP2 relieving tonic inhibition of TRPC6. Increase in Erk1/2 activity by G_{12/13}-coupled GPCRs activates TRPC6 by phosphorylation leading to increased Ca²⁺ entry and calmodulin-dependent protein kinase (CAMK) activation. CAMK increases MLCK activity by phosphorylation, which in turn phosphorylates MLC phosphorylation causing vasoconstriction. GPCRs coupled to G_{12/13} increase RhoA activity and the downstream kinase ROCK. ROCK increases MLC phosphorylation by inhibiting MLCP, or by direct phosphorylation. Vasoconstrictors, such as PGI₂ acting via G_s-coupled receptors activate PKA thereby inhibit Ca²⁺ increase by PKA-mediated phosphorylation of PLCβ and TRPC6. In ECs, G_i, or G_q-coupled GPCRs, increase, PI3K-Akt signaling and activate eNOS by phosphorylation at Ser¹¹⁷⁷. NO diffuses to nearby VSMC, activating soluble guanylate cyclase, increasing cGMP, activating PKG, and inhibiting TRPC6 by phosphorylation. PKG also activates the GAPs for G_q, RGS2 and RGS4 to inhibit PLCβ activity thereby attenuating Ca²⁺ entry. Both PKG- and PKA inhibit RhoA by direct phosphorylation and promote vasodilation

acting via 5-HT_{1B}-G_i coupled and 5-HT_{2A/2B}-G_q coupled GPCRs, stimulates VSMC proliferation via the activation of the transcription factor GATA-4 and increase of cytokine generation from leukocytes, such as dendritic cells^[48]. TxA₂ level in PH is elevated due to up-regulation of thromboxane-A synthase^[46]. Increased presence of inflammatory cytokines, such as TNF α and IFN γ , stimulates ET1 release from VSMC, believed to be an important source of the vasoconstrictor ET-1 in PH. This effect of cytokines and ET1 is antagonized by the PGI₂-IP axis^[49].

GPCR ligand-dependent vasodilator response

In contrast to vasoconstrictors, several vasodilators are decreased in PH, promoting vasoconstriction in pulmonary vascular system. Apelin, the ligand for CVD protective GPCR (APJ), modestly falls in PH patients (1.25 ng/mL vs. 0.89 ng/mL, $P = 0.037$)^[50-52]. Decreased PGI₂ synthase (PGIS) in ECs also plays a role in vasodilation and inflammation^[45,46].

Increased activity of vasoconstrictor GPCRs

GPCR activity is frequently altered in diseases via internalization, phosphorylation, and expression levels. In lung, increased activity of TxA₂ and its G_q-coupled GPCR (TP) occurs via palmitoylation of TP and increasing the proportion of the active receptor at the plasma membrane, consistent with pathophysiological action of TP in PH^[53-56]. Similarly, increased expression of other GPCRs involved in PH pathogenesis has been noted for ET1 (ET_A) and serotonin receptors, 5-HT_{1B}R and 5-HT_{2B}R in COPD-PH patients^[54,55,57,58].

Decreased activity of vasodilator GPCRs

In PH, decreased serum concentrations of PGI₂ is accompanied by decrease in levels of the receptor IP, reducing the effectiveness of PGI₂ therapy^[59]. Similarly, chronic stimulation of PGI₂-IP axis, occurring with prostacyclin therapy in PH patients, is likely to even further down regulate the PGI₂-IP axis via heterologous desensitization, compounding a pathogenic situation^[60-62]. GPCRs such as IP, which increase cAMP-PKA, frequently exert anti-inflammatory effects, inhibiting key pro-inflammatory/pro-proliferative transcription factors, including NF- κ B^[63,64], Hippo pathway transcription factors Yap-Taz (co-factors for the pro-proliferative transcription factor TEAD1) and, no doubt, many others^[65]. Induction of anti-inflammatory/anti-proliferative PPAR γ is also another mechanism, by which PGI₂ acts^[66]. PPAR γ , along with sibling, transcription factors PPAR β / δ all are protective in PH and other cardiovascular diseases^[34,66-71]. The induction of PPAR γ activity by PGI₂ was once thought to be a direct binding event to the PPAR γ , but it now appears to occur by indirect mechanism. Activation of PKA or p38MAPK by PGI₂-IP stimulates the cAMP response element-binding protein (CREB) by phosphorylation. Activated CREB turns on the transcriptional co-activator, peroxisome proliferator-activated receptor gamma co-activator 1 α (PGC1 α) gene, increases PGC1 α activity and stimulates PPAR γ , leading to protective anti-inflammatory effects^[71]. Molecular targets of PPAR γ include inhibition of NF- κ B and hypoxic activation of HIF-1 α ^[72]. HIF-1 α is clearly important in VSMC proliferation occurring in PH, as it helps the cell switch to a glycolytic/Warburg metabolic phenotype and has been connected to the increased expression of Ca²⁺ entry channel, TRPC6, both aiding VSMC proliferation^[73-76]. Targeted KO of HIF-1 α inhibitor protein, prolyl-hydroxylase domain containing protein 2 (PHD2), reduced O₂-driven proteolysis of HIF-1 α , thereby increasing HIF-1 α -dependent proliferation of VSMC^[76]. There are 3 PHD (PHD1-3) enzymes, which in presence of O₂ hydroxylate proline residues, 402 and 564, ultimately resulting in the proteolysis of HIF-1 α . A small molecule drug, R59949, a PDH inhibitor, has shown potential to combat PH in the hypoxic mouse model^[76].

Post-receptor mechanisms leading to increased vasoconstrictor GPCR response

In VSMC, Angiotensin II (Ang II) up regulates G_i expression, thereby increasing the activation of PLC β and mobilization of Ca²⁺, further enhancing vasoconstriction and proliferation by a post-receptor mechanism^[77]. Of the PH pre-clinical therapeutics, RhoA-ROCK inhibitor, fasudil and statins both act at post GPCR level^[78,79]. Statins, such as simvastatin, can work in combination with sildenafil, the cGMP-PDE inhibitor, likely an important feature of any new therapy. Although some studies reported no drug combina-

tion yet tested, the combination could be more effective for patients' survival than any monotherapy^[2,80,81]. Statins may work in PH models by inhibition of isoprenoid intermediates, farnesyl pyrophosphate and geranyl-geranyl pyrophosphate, essential for the post-translational isoprenylation, membrane localization, and activation of Ras and Rho small GTP-binding protein families, respectively, thus inhibiting RhoA-ROCK^[82].

Post-receptor mechanisms leading to decreased vasodilator GPCR responses

Post-receptor mechanisms also operate to limit vasodilator response in PH, such as the several hits to the critical NO-cGMP-PKG vasodilation system. Firstly, inflammatory cytokines down regulate eNOS and up-regulate reactive oxygen species (ROS), including superoxide^[83-85]. Secondly, due to peroxynitrite formation, NO level is depleted^[86]. Thirdly, vasodilator response can be limited due to increased PDE5_A expression^[87,88]. Up regulation of both cAMP-PDEs, and cGMP-PDE is an important pathological event, which decreases effectiveness of vasodilator GPCRs and needs further investigation^[89]. The PDEs are a complex family of enzymes with 21 genes, and 11 subfamilies, and some share little sequence identity^[31]. Due to a combination of post-receptor mechanisms, increased expression of cAMP- and cGMP-PDEs, inhibition of eNOS activity, and decreased NO availability (as a result of ROS production), the effects of vasodilators in PH are diminished.

HOW GPCRS FUNCTION IN VASCULAR INFLAMMATION-DRIVEN REMODELING

GPCRs induce cytokine/chemokine production from leukocytes, VSMC, ECs, fibroblasts, and cardiac myocytes and are pathogenic in PH. Up regulation of SDF-1 in activated T cell results to the expression and secretion of RANTES and Monocyte Chemo-attractant protein 1 (MCP-1). These chemokines promote proliferation of VSMC, matrix remodeling, and ROS production^[90-92]. Additionally, GPCRs like serotonin receptor and purinergic P₂Y₁₄R, promote migration of bone marrow derived blood cells, essential to the development of PH^[93,94].

DAMAGE MOLECULAR PATTERNS AS A POTENTIAL CONTRIBUTOR TO VASCULAR INFLAMMATION IN PH

The driving forces behind vascular inflammation in PH are unclear, but it is likely that sterile inflammation-damage molecular pattern (DAMP) systems play a role. Purinergic receptors are also critical in DAMP responses. ATP, ADP, or adenosine are released from extracellular stimuli-activated, hypoxic, or damaged cells and play prominent roles in inflammatory and secretory responses associated with tissue repair. Of the 19 purinergic receptors, 12 are GPCRs nucleotide P2YR_{1, 2, 4, 6, 11-14} and adenosine A₁, A_{2A}, A_{2B}, A₃, and the remaining 7 purinergic receptors P2X₁₋₇, are ligand gated cation channels^[95-100]. Macrophage activation in PH is potentiated by the P₂Y₆^[101-103]. Some data suggest antagonizing the ATP-activated P₂X₁ purinergic receptor could be beneficial in PH^[104]. Both P₂Y₁ and P₂Y₁₂ purinergic receptors have been shown to be partially responsible for PA pressure increase due to hypoxia^[105]. Hypoxia-induced ATP release from PA adventitial fibroblasts and vasa vasorum endothelial cells (VVEC) induces mitogenic and angiogenic responses in VVEC in autocrine/paracrine manner^[95,96,106] [Figure 2]. Released ATP and ADP are degraded rapidly to adenosine. Activation of the A_{2A} adenosine receptor has been reported to be protective against PH, but the activation of A_{2B}-AR results in pathogenic effects^[107-112]. The involvement of DAMPS-GPCRs in PH is understudied, and therapeutic possibilities remain to be explored.

PATHOGENIC CHEMOKINE GPCRS

Small G-proteins in chemokine receptor-stimulated VSMC proliferation

In VSMC, MCP-1 acting via G_i-coupled CCR2, stimulates G_i-dependent proliferation, that also involves activation of the small G proteins^[113]. One of the mechanisms includes p115RhoGEF-dependent activation of

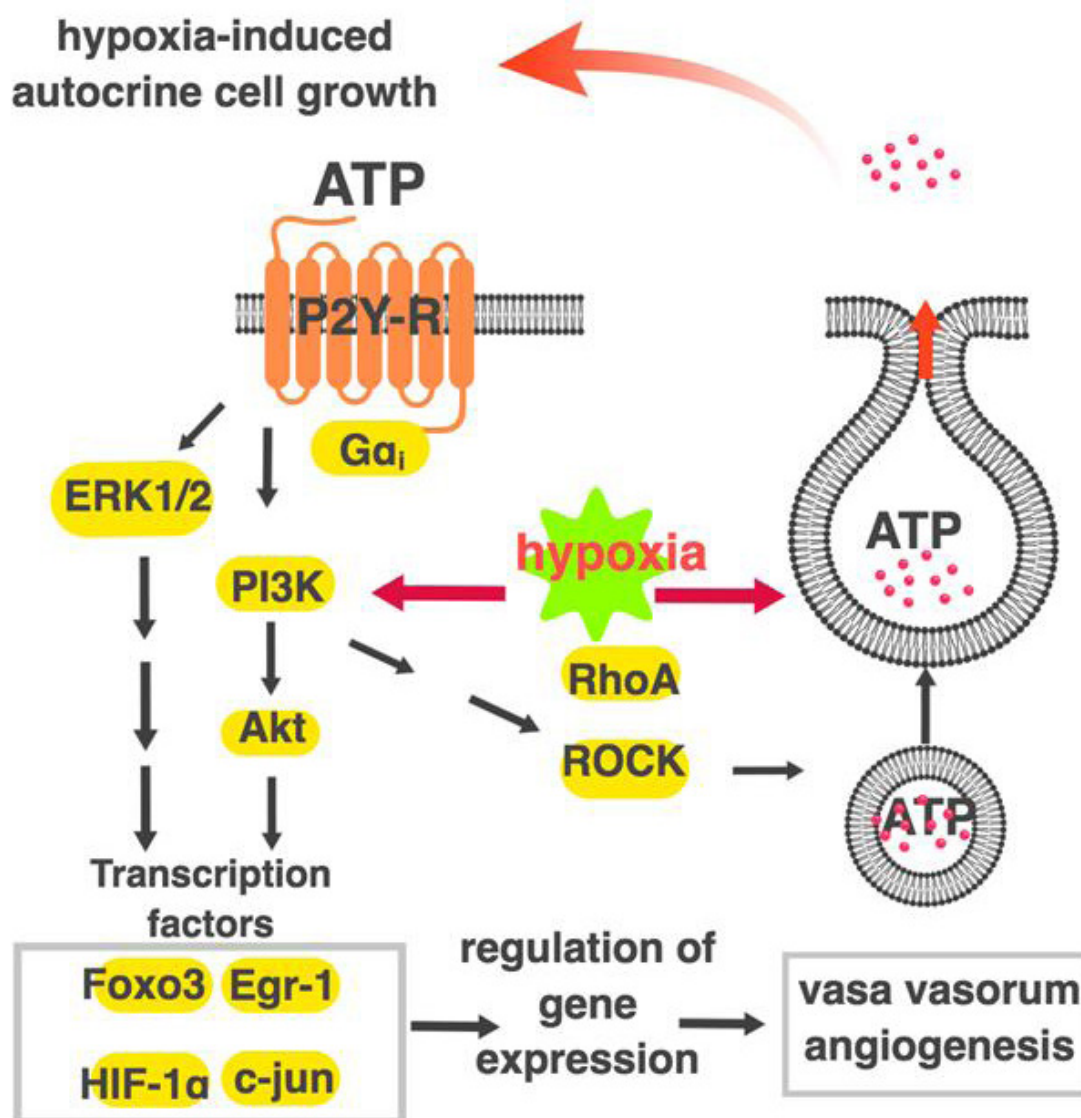


Figure 2. Schematic diagram illustrating a role of PI3K, Rho and ROCK pathways in hypoxia-induced ATP release and ATP-mediated angiogenic effects in vasa vasorum endothelial cells. Activation of PI3K/Rho/ROCK pathway in response to hypoxia results in regulated ATP release from VVEC. In turn, extracellular ATP triggers/initiates P2YR-dependent activation of PI3K/Rho/ROCK pathway leading to angiogenic responses in vasa vasorum endothelial cells. VVEC: vasa vasorum endothelial cells

the Rac and Nuclear factor of activated T-cells (NFAT1)-dependent up-regulation of cyclin D1 expression in VSMC^[113].

Involvement of ROS in chemokine receptor-stimulated responses

ROS is a pathogenic factor in PH by mechanisms, which include reducing NO; promoting VSMC proliferation; initiating sterile inflammation-DAMP response; and promoting vasoconstriction via increased membrane depolarization^[74,114]. G_i-coupled GPCRs, such as MCP-1, SDF-1, thrombin, PAF, and purinergic receptors, stimulate ROS production^[115-117]. ROS are produced as bactericidal compounds in large amounts in phagocytes (neutrophils, monocytes, macrophages) and, in a lesser amounts, in vascular cells. In phagocytes, chemokines, such as N-Formylmethionyl-leucyl-phenylalanine, PAF, complement C5a (C5a), LTB₄, and MCP-1 are G_i-coupled-GPCRs and activate Rac1-NAD(P)H oxidase-superoxide system. NOX2 is a neutrophil NADPH oxidase responsible for producing increased amounts of superoxide. There are 7 NOX

like oxidases, NOX1-5 DUOX1, 2 of which are expressed in vascular cells, and their activation involves Rac1 stimulation by the GEFs, such as engulfment and cell motility protein 1 (ELMO1)^[115,117,118]. The superoxide generated by NOX enzymes in the extracellular space, is converted to H₂O₂, some of which enters the cell to stimulate proliferation. H₂O₂ induces proliferation by changing the balance in protein kinase-protein phosphatase networks by inhibiting key protein phosphatases via the oxidation of labile sensitive cysteine in the active site^[119].

The involvement of HIF-1 α in chemokine/GPCR action with respect to PH

HIF-1 α and HIF-2 α may play a pathophysiological role in PH, and the action of GPCRs overlaps with that of HIFs^[76,120,121]. Firstly, some GPCRs, such as those for estrogen G-protein coupled estrogen receptor-1 (GPER), ET₁ (ET_A), PGE₁ (EP₁), and PGI₂ (IP), can activate HIF-1 α even under normoxic conditions^[122-131]. Secondly, ROS increased by GPCRs signaling, inhibit PHD proteins by oxidative inactivation, which in turn promotes HIF1 α activation and its pathological action in PH^[132-135]. Thirdly, hypoxic activation of HIF-1 α up regulates G_i-coupled receptor for SDF-1, CXCR4, implicated in PH by promoting VSMC proliferation^[136-139]. Moreover, hypoxia can stimulate ATP release from vasa vasorum endothelial cells (VVEC) by PI3K-dependent mechanism to promote angiogenesis in an autocrine manner [Figure 2]. This mechanism implicates purinergic GPCR-dependent activation of HIF-1 α and HIF-2 α that may amplify hypoxia-induced vasa vasorum expansion [Figure 3].

INTERACTION OF INFLAMMATORY CYTOKINES AND GROWTH FACTORS WITH GPCRS SIGNALING IN PH

PDGF-induced proliferation of VSMC is believed to be a major factor in PH. It is known to be dependent on Akt activation that can occur in co-operation with some GPCRs, termed trans-activation^[140]. Ang II receptor works in concert with PDGF-receptor tyrosine kinase, promoting Akt-dependent VSMC proliferation^[77,141-143]. Thrombin-PAR trans-activates the TGF- β receptor to promote VSMC proteoglycan synthesis^[144]. It is of some interest that PGI₂ has been described as unable to significantly inhibit PDGF-induced VSMC proliferation, suggesting that other PDGF-neutralizing strategies are needed in PH^[145]. MCP-1 and IL-6 also work together to induce VSMC proliferation^[146]. Activation of inflammatory TXA₂-TP inhibits FGF-2- or VEGF-stimulated angiogenesis, which could relate to vascular pruning in cardiac and pulmonary vessels, and is an example of GPCR-cytokine interaction^[41,147-149]. Protective interactions of GPCRs with cytokines and growth factors could include the ability of PGI₂-IP to inhibit the IFN γ -induced inflammation, dependent upon induction of suppressor of cytokine signaling 3 (SOCS3)^[150]. The GPCR GPR4 expressed on ECs, promotes angiogenesis in a Notch-dependent manner^[151]. Vessel architecture is maintained by the ligand-receptor pair jagged expression on EC and Notch expression on VSMC, keeping VSMC in a differentiated non-proliferating state^[152-156]. Both HIF-1 α -induced VEGF for reparative angiogenesis and hypoxia-induced epithelial to mesenchymal transition require Ras family member, RhoE, which activation involves SDF-1 GPCR, CXCR4 signaling^[157]. RhoE aids in HIF-1 α maintenance and is induced by cAMP via G_s-coupled GPCRs^[158]. Cardiac angiogenesis is believed to be critically protective in heart disease and potentially links SDF-1, cAMP, RhoE, HIF-1 α , and VEGF into signaling networks^[159].

INTERSECTIONS OF EICOSANOIDS AND GPCRS IN VASCULAR INFLAMMATION

Many eicosanoids induced by vascular inflammation, have short half-lives and must therefore be produced at the site of action either by monocyte/macrophages, ECs, fibroblasts, cardiac myocytes, or fibroblasts^[160,161]. Injection of the GPCR-G_q/G_i-coupled ligand, PAF into rat lung causes rapid increase in PA pressure, linked to LTB₄ production. LTB₄-LTB₄R, and PAF-PAFR coupled G_q/G_i are macrophage activators and plays a pathological role in PH^[162-169]. PGE₂, an important eicosanoid, which activates several GPCRs, such as G_q-coupled EP₁, G_s-coupled EP_{2/4} and G α_i /G α_{13} -coupled EP₃, EP₃ promotes PH by increasing Rho/TGF- β 1 signaling^[170]. Protective eicosanoids, like PGI₂, exert anti-inflammatory effects following LPS-induced lung injury and PH-induced cardiac inflammation and is active against T cells and macro-

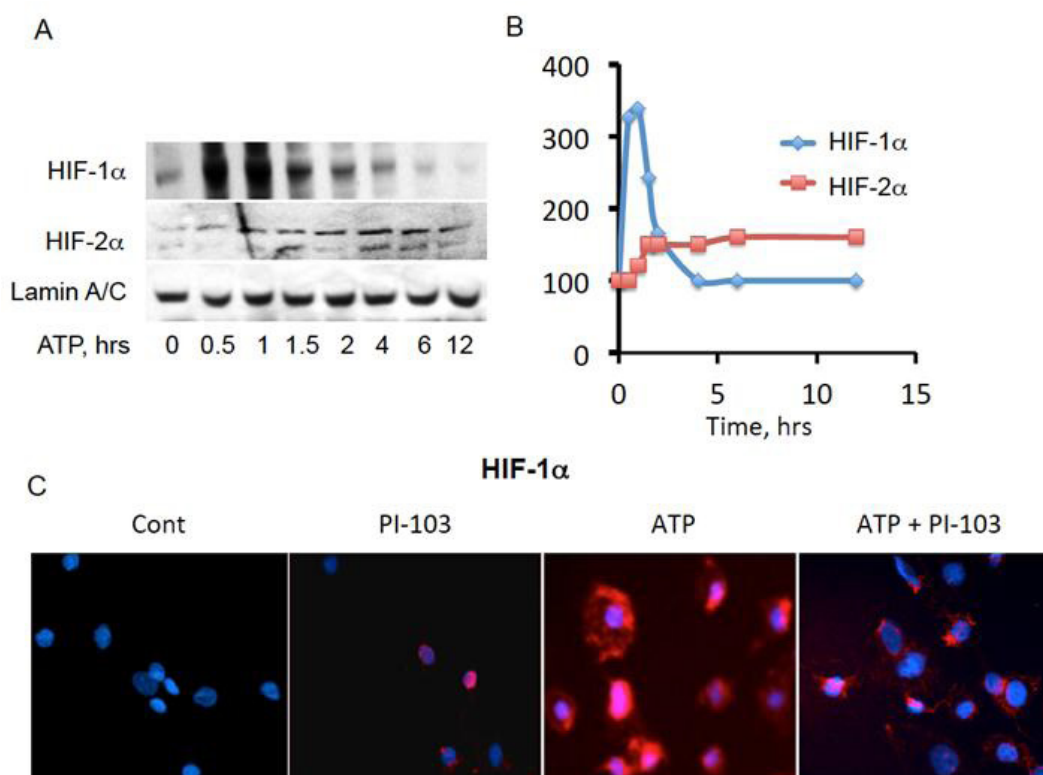


Figure 3. Extracellular ATP up regulates HIF-1 α and HIF-2 α transcription factors in pulmonary artery vasa vasorum endothelial cells. A, B: ATP (10 μ mol/L), applied to VVEC, results in activation of both HIF-1 α and HIF-2 α with distinct time courses. VVEC were serum starved for 18 h and stimulated for indicated times. Nuclear fractions were subjected for Western blot analysis for HIF-1 α , HIF-2 α , and lamin A/C expression; C: cells were stained for HIF-1 α at 1 h post stimulation with ATP (10 μ mol/L), with or without PI3K inhibitor, PI-103 pretreatment (0.5 μ mol/L, 15 min). VVEC: vasa vasorum endothelial cells

phages^[41,171-174].

INFLAMMATION-DRIVEN ENDOTHELIAL DYSFUNCTION (ED) AS A MECHANISM OF VASCULAR REMODELING: INVOLVEMENT OF GPCRS

Inflammatory stimuli, IL-1 or TNF α down-regulate eNOS, attenuate reparative angiogenesis, promote EC apoptosis, and increase endothelial to mesenchymal transition (EMT) - all of which contribute to ED^[46,83,175,176]. TxA₂, acting on both ECs and VSMCs, is pathological in PH and inhibits VEGF- or FGF-2-promoted angiogenesis^[46,147-149,165]. By contrast, many PH protective GPCR agonists (apelin, PGI₂) increase eNOS activity by phosphorylation of Ser¹¹⁷⁷ or by increasing eNOS expression^[50-52,177-179]. Some PH therapeutics, apelin and sildenafil, increase recruitment of endothelial cell progenitors, thereby counteracting ED^[180-184].

THROMBOSIS AND PLATELET ACTIVITY CROSS TALK WITH VASCULAR INFLAMMATION AND GPCR ACTION

Platelets from patients with the sub-form of PAH, due to thromboembolic PAH, exhibit increased reactivity to thrombin, which stimulates the G_q/G_i-coupled protease activated receptor 1 (PAR1), promoting VSMC proliferation^[185,186]. Thrombin receptors exist on EC and have been reported to inhibit angiogenesis.

RV REMODELING AND FAILURE

Cardiac myocytes (CMs) are terminally differentiated cells. The compensatory cardiac hypertrophy is en-

tirely due to increased CM cell size, rather than proliferation. The adult heart is 56% CM, 27% fibroblasts, 10% VSMC, and 7% ECs, and these ratios change little between the four chambers^[187]. During PH, the ratios of fibroblasts increases, and the ratio of ECs/CMs decreases^[188]. The transition to heart failure has been linked to endothelial dysfunction due to insufficient reparative angiogenesis - a loss of capillaries supplying cardiac myocytes with O₂, leading to capillary pruning, inflammation, and ROS production^[147-149,188-193].

Pathological role of GPCRs in cardiac myocyte with respect to RV failure

The hypertrophy response is engaged when increased Ca²⁺ - and cAMP-dependent contractile signals lead to activation of NFAT, MEF2, and GATA₄. These signals are driven by GPCR agonists, such as Ang II, thrombin, ET1, PGF2 α , β -AR^[194-197]. Typical gene expression changes include decreased expression of sarcoplasmic reticulum Ca²⁺ re-uptake channel (SERCA2), increased expression of slow twitch contractile protein myosin heavy chain β 9 (β -MHC, *a.k.a.* MyH7), and decreased expression of the fast twitch α -MHC/MyH6, amongst others^[198,199]. The transcription factor, Egr-1 has been linked to the down regulation of cardiac SERCA2 in hypertrophy and was found to be overexpressed in PAs of PH patients^[200-202]. GPCR-induced increase in intracellular Ca²⁺ stimulates PKD activity, promoting nuclear export of histone deacetylase 5 (HDAC5), thereby activating MEF2 to initiate hypertrophic gene program^[203,204]. GPR91, a receptor for succinate expressed in CMs, promotes cardiac hypertrophy by coupling to G_i/G_q-PI3K-Akt signaling^[205,206]. Succinate may be accumulated during cardiac remodeling due to changes in metabolism, and when released from the cells, promotes positive feedback loop by activating GPR91 leading to hypertrophy, or as also reported, to CM apoptosis via caspase3^[188].

Protective role of GPCRs in cardiac myocyte with respect to RV failure

The estrogen-activated GPER, found in CM, has been considered cardio-protective in a PI3K-Akt-dependent mechanism^[207,208]. RGS proteins 2, 4, 10, 14 modulate cardiac hypertrophy by inhibiting the G_i/G_q-PLC β -Ca²⁺ signaling axis. PKG activates RGS2 by phosphorylation, inhibiting G_s, G_q, and G_i signaling, which in turn, attenuates β -AR-induced hypertrophy and that of other GPCRs^[209-212]. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) exert CV protective actions by the activation of cGMP-dependent PKG, which phosphorylates and activates RGS4, aiding its inhibition of GPCR-G_q-PLC β -Ca²⁺ axis^[213]. RGS6 promotes cardiac myocyte apoptosis associated with decompensation due to its capacity to increase ROS^[214]. RGS10 inhibits the cardiac hypertrophy induced by Ang II^[215]. RGS14 protects against aortic banding-induced cardiac hypertrophy and fibrosis, decreasing ERK1/2 hypertrophy signals^[216].

ACTION OF GPCRS ON ENDOTHELIAL CELLS WITH RESPECT TO RV FAILURE

ED, occurring in failing RV, interconnects with fibrosis, as this appears to be a factor in the decreased capillary density-ED observed in hypertrophy and with the altered metabolism of CM, critical towards HF^[217,218]. ED can result in potentially uncontrolled inflammation of local RV tissue and in turn can lead to EC apoptosis, down regulation of eNOS and PGIS. TGF- β , which is pathologic in PH, is induced by inflammation, promotes lung and heart fibrosis, but also promotes ED by inhibiting differentiation of endothelial progenitor cells (EPCs) into ECs to repopulate damaged endothelium, counteracting the effects of endothelium protective GPCR ligand, apelin^[219,220]. Cardiovascular protective GPER is found in ECs, promotes angiogenesis, and could be significant in defending against endothelial dysfunction^[207,221,222].

VASCULAR FIBROBLASTS AND CARDIAC FIBROSIS

Cardiac fibrosis, seen in animal models of PH, involves expansion of fibroblast populations, their differentiation to myofibroblast, and the stiffening of the extracellular matrix by synthesis of collagens^[198]. Fibroblasts also can derive from EMT via conversion of EC to fibroblasts^[175]. GPCRs promoting cardiac fibrosis include G_q-PLC-Ca²⁺ - coupled 5-HT_{2B}, Ang II, and endothelin CPCR. The thrombin receptor, PAR1 is the most highly expressed GPCR in cardiac fibroblasts, therefore is a potentially important pro-fibrotic GPCR^[223-225]. P₂Y₆-purinergic receptors are reported to enhance pressure overload-induced fibrosis

by increasing TGF- β 1 and CTGF release^[226]. The p38 α MAPK, activated by Ang-II or non-GPCR stimuli, such as TGF- β 1, or cyclic stretch, has been identified as a master switch, common to many different receptors stimulating fibrosis^[198]. The ligand relaxin and its GPCR, RFXP1-4, are Gs-coupled and exert anti-hypertrophic and anti-fibrotic effects^[227]. In cardiac fibroblasts, PGI₂-IP-PKA axis activates CREB to inhibit Ang II-induced SMAD2 activation, attenuating proliferation^[228].

ROLE OF GPCRS IN MONOCYTE/MACROPHAGE WITH RESPECT TO RV FAILURE

Macrophage features in the inflammation associated with heart failure, with resident macrophages being described as protective, while recruited being pathogenic^[191]. Increasing activity of the transcription factor KLF4 in resident macrophages to aid their survival or inhibiting MCP-1-CCR2 activity of recruited monocytes, has been suggested as a potential therapy^[191]. Macrophage polarization in PH is thought to contribute to cardiac and pulmonary inflammation-induced damage and remodeling. M1 macrophage phenotype is considered pro-inflammatory (versus the M2 phenotype), is involved in resolving inflammation, but implicated in tissue fibrosis^[229]. Some studies in PH suggest that M2 macrophages are more damaging than M1. Antagonizing the CX3CR1 chemokine receptor reduces pathogenic M2 in favor of less damaging M1 phenotype^[90,230]. Most chemokine receptors activate G α_{i1} /G α_{i3} , which have been linked to promotion of polarization to M1 macrophage via increased LPS-TLR4-NF- κ B, in contrast to CX3CR1 signaling^[76]. An interesting development in macrophage polarization/anti-inflammatory responses are the 6 atypical chemokine receptors, ACKR1-6, which are “duds” unable to activate G-proteins, and exert anti-inflammatory effects^[229]. In particular, the atypical chemokine receptor, CCRL2 (tentatively ACKR5) polarizes in favor of M2 phenotype^[229]. Other GPCRs aiding polarizing to M2 phenotype, include lipoxinA4-activated FPR2, PGE₂-receptors, and adenosine A_{2A}/A_{2B}-receptors^[231-234]. GPCRs clearly critically control macrophage polarization and might well be employed to diminish macrophage-induced inflammation occurring in PH. The role of GPCRs in cardiac inflammation is clearly complex, and it should be mentioned that increasing recruitment of pro-angiogenic monocytes may be beneficial in ED, and is also under control of GPCRs^[235-238].

GPCRS, WHICH MIGHT BECOME CLINICAL TARGETS IN PH

GPCRs activating cAMP-PKA axis in ECs or VSMCs, such as PGI₂ and adenosine (A_{2B}AR), generally induce vasodilation, are often anti-inflammatory and protective in PH. Secondly, GPCRs, such as for apelin, PGI₂, opioids, which increase NO release from EC to promote vasodilation, are also usually protective. Thus, any signals increasing cAMP, cGMP, NO and inhibiting Ca²⁺ are usually protective^[178,179]. By contrast, any GPCR signaling increasing Ca²⁺ in VSMC, or decreasing NO, cAMP, cGMP, or increasing inflammation, are usually pathogenic in PH. One very potent anti-inflammatory agent is adenosine, which exerts powerful anti-inflammatory effects acting at A_{2A}AR, and clearly plays a protective role in PH^[111,239]. New drugs (such as AEA061) are positive allosteric modulators of A_{2A}AR, that activate receptors without binding to the normal agonist binding site, offer a therapeutic possibility of fewer side effects as they do not act at A₁, A_{2B} or A₃ARs^[239]. Activation of A_{2A}AR without activating A₁, A_{2B}, and A₃ARs has been an issue in developing anti-inflammatory therapies. Other potentially protective GPCRs include FPR2, an atypical chemokine receptor on macrophages, was reported to exert anti-inflammatory action^[229,240]. Other protective receptors in PH include ET-1 receptor ET_B^[241], angiotensin II type 2 receptor^[242], adiponectin-receptor^[36,243], mas1 (a receptor for angiotensin 1-7)^[244], and relaxin receptors^[245,246]. ET_B receptor is also protective in porto-pulmonary hypertension, a disease secondary to liver failure, but in which the same therapeutics, PGI₂-cGMP-PDE-ET-1 receptor antagonist therapies are utilized^[247,248].

GPCRs with pathogenic action, which could be antagonized such that the drugs would be protective could include the CaSR, calcium sensing receptor in EC^[12,249], the succinate GPR91 on cardiac myocytes^[205,206], thromboxane receptors^[250], serotonin receptors^[251], LTB₄ receptors^[252], shingosine-1-phosphate receptors^[13,253-255] amongst others.

CONCLUSION

Research has highlighted many examples of pathological GPCR signaling, which can be targets for novel PH therapeutics. In PH pre-clinical studies many targets have been identified, but only few are druggable [Tables 1 and 2]. GPCRs, by contrast, represent good targets for pharmacological strategies and in all likelihood present one of the best opportunities for therapeutic intervention in PH. The heart alone is estimated to express some 200 different GPCRs, suggesting significantly better therapeutics based on targeting GPCRs are possible. The challenge is to devise the best pharmacological cocktail for the PH patient. At the moment, while much has been published with respect to GPCR action in PH, much more clearly awaits discovery.

DECLARATIONS

Authors' contributions

Literature review: Strassheim D

Writing the manuscript: Strassheim D, Karoor V, Gerasimovskaya E

Organizing the manuscript: Karoor V

Experimental data presented in the manuscript: Stenmark K

Discussion of the manuscript: Verin A, Gerasimovskaya E

Material support: Gerasimovskaya E

Availability of data and materials

Not applicable.

Financial support and sponsorship

The study was supported by grants from National Institute of Health R01-HL-086783 (to Gerasimovskaya E).

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Strange G, Playford D, Stewart S, Deague JA, Nelson H, Kent A, Gabbay E. Pulmonary hypertension: prevalence and mortality in the Armadale echocardiography cohort. *Heart* 2012;98:1805-11.
2. Joppi R, Gerardi C, Bertele V, Garattini S. A disease looking for innovative drugs: the case of pulmonary arterial hypertension. *Eur J Intern Med* 2018;55:47-51.
3. Thomsen W, Frazer J, Unett D. Functional assays for screening GPCR targets. *Curr Opin Biotechnol* 2005;16:655-65.
4. Salazar NC, Chen J, Rockman HA. Cardiac GPCRs: GPCR signaling in healthy and failing hearts. *Biochim Biophys Acta* 2007;1768:1006-18.
5. Offermanns S, Simon MI. Organization of transmembrane signalling by heterotrimeric G proteins. *Cancer Surv* 1996;27:177-98.
6. Rajagopal S, Shenoy SK. GPCR desensitization: acute and prolonged phases. *Cell Signal* 2018;41:9-16.

7. Kach J, Sethakorn N, Dulin NO. A finer tuning of G-protein signaling through regulated control of RGS proteins. *Am J Physiol Heart Circ Physiol* 2012;303:H19-35.
8. Tan JL, Ravid S, Spudich JA. Control of nonmuscle myosins by phosphorylation. *Annu Rev Biochem* 1992;61:721-59.
9. Ikebe M, Hartshorne DJ. Phosphorylation of smooth muscle myosin at two distinct sites by myosin light chain kinase. *J Biol Chem* 1985;260:10027-31.
10. Amano M, Ito M, Kimura K, Fukata Y, Chihara K, Nakano T, Matsuura Y, Kaibuchi K. Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J Biol Chem* 1996;271:20246-9.
11. Yamamura A. Pathological function of Ca²⁺-sensing receptor in pulmonary arterial hypertension. *J Smooth Muscle Res* 2014;50:8-17.
12. Smith KA, Ayon RJ, Tang H, Makino A, Yuan JX. Calcium-sensing receptor regulates cytosolic [Ca (2+)] and plays a major role in the development of pulmonary hypertension. *Front Physiol* 2016;7:517.
13. Ota H, Beutz MA, Ito M, Abe K, Oka M, McMurtry IF. S1P(4) receptor mediates S1P-induced vasoconstriction in normotensive and hypertensive rat lungs. *Pulm Circ* 2011;1:399-404.
14. Aiello RJ, Bourassa PA, Zhang Q, Dubins J, Goldberg DR, De Lombaert S, Humbert M, Guignabert C, Cavaasin MA, McKinsey TA, Paralkar V. Tryptophan hydroxylase 1 inhibition impacts pulmonary vascular remodeling in two rat models of pulmonary hypertension. *J Pharmacol Exp Ther* 2017;360:267-79.
15. Bhat L, Hawkinson J, Cantillon M, Reddy DG, Bhat SR, Laurent CE, Bouchard A, Biernat M, Salvail D. RP5063, a novel, multimodal, serotonin receptor modulator, prevents Sugen 5416-hypoxia-induced pulmonary arterial hypertension in rats. *Eur J Pharmacol* 2017;810:83-91.
16. Liu Y, Ren W, Warburton R, Toksoz D, Fanburg BL. Serotonin induces Rho/ROCK-dependent activation of Smads 1/5/8 in pulmonary artery smooth muscle cells. *FASEB J* 2009;23:2299-306.
17. Mori MX, Itsuki K, Hase H, Sawamura S, Kurokawa T, Mori Y, Inoue R. Dynamics of receptor-operated Ca(2+) currents through TRPC channels controlled via the PI(4,5)P2-PLC signaling pathway. *Front Pharmacol* 2015;6:22.
18. Kong F, Ma L, Zou L, Meng K, Ji T, Zhang L, Zhang R, Jiao J. Alpha1-adrenergic receptor activation stimulates calcium entry and proliferation via TRPC6 channels in cultured human mesangial cells. *Cell Physiol Biochem* 2015;36:1928-38.
19. Tanabe S, Kreutz B, Suzuki N, Kozasa T. Regulation of RGS-RhoGEFs by Galpha12 and Galpha13 proteins. *Methods Enzymol* 2004;390:285-94.
20. Mahavadi S, Nalli A, Al-Shboul O, Murthy KS. Inhibition of MLC20 phosphorylation downstream of Ca²⁺ and RhoA: a novel mechanism involving phosphorylation of myosin phosphatase interacting protein (M-RIP) by PKG and stimulation of MLC phosphatase activity. *Cell Biochem Biophys* 2014;68:1-8.
21. Horinouchi T, Higa T, Aoyagi H, Nishiya T, Terada K, Miwa S. Adenylate cyclase/cAMP/protein kinase A signaling pathway inhibits endothelin type A receptor-operated Ca(2+)(+) entry mediated via transient receptor potential canonical 6 channels. *J Pharmacol Exp Ther* 2012;340:143-51.
22. Nalli AD, Kumar DP, Al-Shboul O, Mahavadi S, Kuemmerle JF, Grider JR, Murthy KS. Regulation of Gbetagamma-dependent PLC-beta3 activity in smooth muscle: inhibitory phosphorylation of PLC-beta3 by PKA and PKG and stimulatory phosphorylation of Galphai-GTPase-activating protein RGS2 by PKG. *Cell Biochem Biophys* 2014;70:867-80.
23. Huang J, Zhou H, Mahavadi S, Sriwai W, Murthy KS. Inhibition of Galphaq-dependent PLC-beta1 activity by PKG and PKA is mediated by phosphorylation of RGS4 and GRK2. *Am J Physiol Cell Physiol* 2007;292:C200-8.
24. Zieba BJ, Artamonov MV, Jin L, Momotani K, Ho R, Franke AS, Nepl RL, Stevenson AS, Khromov AS, Chrzanowska-Wodnicka M, Somlyo AV. The cAMP-responsive Rap1 guanine nucleotide exchange factor, Epac, induces smooth muscle relaxation by down-regulation of RhoA activity. *J Biol Chem* 2011;286:16681-92.
25. Moon MY, Kim HJ, Kim JG, Lee JY, Kim J, Kim SC, Choi IG, Kim PH, Park JB. Small GTPase Rap1 regulates cell migration through regulation of small GTPase RhoA activity in response to transforming growth factor-beta1. *J Cell Physiol* 2013;228:2119-26.
26. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 1999;399:601-5.
27. Nishida M, Watanabe K, Sato Y, Nakaya M, Kitajima N, Ide T, Inoue R, Kurose H. Phosphorylation of TRPC6 channels at Thr69 is required for anti-hypertrophic effects of phosphodiesterase 5 inhibition. *J Biol Chem* 2010;285:13244-53.
28. Murthy KS, Zhou H, Grider JR, Makhlof GM. Inhibition of sustained smooth muscle contraction by PKA and PKG preferentially mediated by phosphorylation of RhoA. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G1006-16.
29. Rolli-Derkinderen M, Sauzeau V, Boyer L, Lemichez E, Baron C, Henrion D, Loirand G, Pacaud P. Phosphorylation of serine 188 protects RhoA from ubiquitin/proteasome-mediated degradation in vascular smooth muscle cells. *Circ Res* 2005;96:1152-60.
30. Luykenaar KD, Welsh DG. Activators of the PKA and PKG pathways attenuate RhoA-mediated suppression of the KDR current in cerebral arteries. *Am J Physiol Heart Circ Physiol* 2007;292:H2654-63.
31. Omori K, Kotera J. Overview of PDEs and their regulation. *Circ Res* 2007;100:309-27.
32. Murthy KS. Contractile agonists attenuate cGMP levels by stimulating phosphorylation of cGMP-specific PDE5; an effect mediated by RhoA/PKC-dependent inhibition of protein phosphatase 1. *Br J Pharmacol* 2008;153:1214-24.
33. Luo L, Zheng W, Lian G, Chen H, Li L, Xu C, Xie L. Combination treatment of adipose-derived stem cells and adiponectin attenuates pulmonary arterial hypertension in rats by inhibiting pulmonary arterial smooth muscle cell proliferation and regulating the AMPK/BMP/Smad pathway. *Int J Mol Med* 2018;41:51-60.
34. Li HH, Hsu HH, Chang GJ, Chen IC, Ho WJ, Hsu PC, Chen WJ, Pang JS, Huang CC, Lai YJ. Prostanoid EP4 agonist L-902,688 activates PPARgamma and attenuates pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 2018;314:L349-L59.

35. Nakagawa Y, Kishida K, Kihara S, Funahashi T, Shimomura I. Adiponectin ameliorates hypoxia-induced pulmonary arterial remodeling. *Biochem Biophys Res Commun* 2009;382:183-8.
36. Isobe S, Kataoka M, Kawakami T, Fukuda K. Adiponectin in chronic thromboembolic pulmonary hypertension. *Circ J* 2018;82:1466-8.
37. Telli G, Tel BC, Yersal N, Korkusuz P, Gumusel B. Effect of intermedin/adrenomedullin2 on the pulmonary vascular bed in hypoxia-induced pulmonary hypertensive rats. *Life Sci* 2018;192:62-7.
38. Chawla S, Rahar B, Saxena S. S1P prophylaxis mitigates acute hypobaric hypoxia-induced molecular, biochemical, and metabolic disturbances: a preclinical report. *IUBMB Life* 2016;68:365-75.
39. Harada-Shiba M, Takamisawa I, Miyata K, Ishii T, Nishiyama N, Itaka K, Kangawa K, Yoshihara F, Asada Y, Hatakeyama K, Nagaya N, Kataoka K. Intratracheal gene transfer of adrenomedullin using polyplex nanomicelles attenuates monocrotaline-induced pulmonary hypertension in rats. *Mol Ther* 2009;17:1180-6.
40. Burnouf C, Pruniaux MP. Recent advances in PDE4 inhibitors as immunoregulators and anti-inflammatory drugs. *Curr Pharm Des* 2002;8:1255-96.
41. Tobin JV, Zimmer DP, Shea C, Germano P, Bernier SG, Liu G, Long K, Miyashiro J, Ranganath S, Jacobson S, Tang K, Im GJ, Sheppeck J 2nd, Moore JD, Sykes K, Wakefield J, Sarno R, Banijamali AR, Profy AT, Milne GT, Currie MG, Masferrer JL. Pharmacological characterization of IW-1973, a novel soluble guanylate cyclase stimulator with extensive tissue distribution, antihypertensive, anti-inflammatory, and antifibrotic effects in preclinical models of disease. *J Pharmacol Exp Ther* 2018;365:664-75.
42. Stelzner TJ, O'Brien RF, Yanagisawa M, Sakurai T, Sato K, Webb S, Zamora M, McMurtry IF, Fisher JH. Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension. *Am J Physiol* 1992;262:L614-20.
43. Humbert M, Labrune P, Sitbon O, Le Gall C, Callebert J, Herve P, Samuel D, Machado R, Trembath R, Drouet L, Launay JM, Simonneau G. Pulmonary arterial hypertension and type-I glycogen-storage disease: the serotonin hypothesis. *Eur Respir J* 2002;20:59-65.
44. Hood KY, Mair KM, Harvey AP, Montezano AC, Touyz RM, MacLean MR. Serotonin signaling through the 5-HT1B receptor and NADPH oxidase 1 in pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol* 2017;37:1361-70.
45. Christman BW, McPherson CD, Newman JH, King GA, Bernard GR, Groves BM, Loyd JE. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N Engl J Med* 1992;327:70-5.
46. Mahajan CN, Afolayan AJ, Eis A, Teng RJ, Konduri GG. Altered prostanoid metabolism contributes to impaired angiogenesis in persistent pulmonary hypertension in a fetal lamb model. *Pediatr Res* 2015;77:455-62.
47. Li H, Elton TS, Chen YF, Oparil S. Increased endothelin receptor gene expression in hypoxic rat lung. *Am J Physiol* 1994;266:L553-60.
48. Weir EK, Hong Z, Varghese A. The serotonin transporter: a vehicle to elucidate pulmonary hypertension? *Circ Res* 2004;94:1152-4.
49. Wort SJ, Woods M, Warner TD, Evans TW, Mitchell JA. Cyclooxygenase-2 acts as an endogenous brake on endothelin-1 release by human pulmonary artery smooth muscle cells: implications for pulmonary hypertension. *Mol Pharmacol* 2002;62:1147-53.
50. Chandra SM, Razavi H, Kim J, Agrawal R, Kundu RK, de Jesus Perez V, Zamanian RT, Quertermous T, Chun HJ. Disruption of the apelin-APJ system worsens hypoxia-induced pulmonary hypertension. *Arterioscler Thromb Vasc Biol* 2011;31:814-20.
51. Yang P, Maguire JJ, Davenport AP. Apelin, Elabela/Toddler, and biased agonists as novel therapeutic agents in the cardiovascular system. *Trends Pharmacol Sci* 2015;36:560-7.
52. Yang P, Read C, Kuc RE, Buonincontri G, Southwood M, Torella R, Upton PD, Crosby A, Sawiak SJ, Carpenter TA, Glen RC, Morrell NW, Maguire JJ, Davenport AP. Elabela/Toddler is an endogenous agonist of the apelin apj receptor in the adult cardiovascular system, and exogenous administration of the peptide compensates for the downregulation of its expression in pulmonary arterial hypertension. *Circulation* 2017;135:1160-73.
53. Fediuk J, Gutsol A, Nolette N, Dakshinamurti S. Thromboxane-induced actin polymerization in hypoxic pulmonary artery is independent of Rho. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L13-26.
54. Hinton M, Gutsol A, Dakshinamurti S. Thromboxane hypersensitivity in hypoxic pulmonary artery myocytes: altered TP receptor localization and kinetics. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L654-63.
55. Sikarwar AS, Hinton M, Santhosh KT, Chelikani P, Dakshinamurti S. Palmitoylation of Galphaq determines its association with the thromboxane receptor in hypoxic pulmonary hypertension. *Am J Respir Cell Mol Biol* 2014;50:135-43.
56. Santhosh KT, Elkhateeb O, Nolette N, Outbih O, Halayko AJ, Dakshinamurti S. Milrinone attenuates thromboxane receptor-mediated hyperresponsiveness in hypoxic pulmonary arterial myocytes. *Br J Pharmacol* 2011;163:1223-36.
57. Rondelet B, Van Beneden R, Kerbaul F, Motte S, Fesler P, McEntee K, Brimiouille S, Ketelslegers JM, Naeije R. Expression of the serotonin 1b receptor in experimental pulmonary hypertension. *Eur Respir J* 2003;22:408-12.
58. Milara J, Gabarda E, Juan G, Ortiz JL, Guijarro R, Martorell M, Morcillo EJ, Cortijo J. Bosentan inhibits cigarette smoke-induced endothelin receptor expression in pulmonary arteries. *Eur Respir J* 2012;39:927-38.
59. Falcetti E, Hall SM, Phillips PG, Patel J, Morrell NW, Haworth SG, Clapp LH. Smooth muscle proliferation and role of the prostacyclin (IP) receptor in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2010;182:1161-70.
60. Kuwano K, Hashino A, Asaki T, Hamamoto T, Yamada T, Okubo K, Kuwabara K. 2-[4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy]-N-(methylsulfonyl)acetamide (NS-304), an orally available and long-acting prostacyclin receptor agonist prodrug. *J Pharmacol Exp Ther* 2007;322:1181-8.
61. Schermuly RT, Pullamsetti SS, Breitenbach SC, Weissmann N, Ghofrani HA, Grimminger F, Nilius SM, Schror K, Kirchhuth JM, Seeger W, Rose F. Iloprost-induced desensitization of the prostacyclin receptor in isolated rabbit lungs. *Respir Res* 2007;8:4.
62. Gatfield J, Menyhart K, Wanner D, Gnerre C, Monnier L, Morrison K, Hess P, Iglarz M, Clozel M, Nayler O. Selexipag active metabolite ACT-333679 displays strong anticontractile and antiremodeling effects but low beta-arrestin recruitment and desensitization

- potential. *J Pharmacol Exp Ther* 2017;362:186-99.
63. Chen CH, Lin H, Hsu YH, Sue YM, Cheng TH, Chan P, Chen TH. The protective effect of prostacyclin on adriamycin-induced apoptosis in rat renal tubular cells. *Eur J Pharmacol* 2006;529:8-15.
 64. Chen HH, Chen TW, Lin H. Prostacyclin-induced peroxisome proliferator-activated receptor- α translocation attenuates NF- κ B and TNF- α activation after renal ischemia-reperfusion injury. *Am J Physiol Renal Physiol* 2009;297:F1109-18.
 65. Kimura TE, Duggirala A, Smith MC, White S, Sala-Newby GB, Newby AC, Bond M. The Hippo pathway mediates inhibition of vascular smooth muscle cell proliferation by cAMP. *J Mol Cell Cardiol* 2016;90:1-10.
 66. Singh S, Simpson RL, Bennett RG. Relaxin activates peroxisome proliferator-activated receptor gamma (PPARgamma) through a pathway involving PPARgamma coactivator 1alpha (PGC1alpha). *J Biol Chem* 2015;290:950-9.
 67. Xia J, Yang L, Dong L, Niu M, Zhang S, Yang Z, Wumaier G, Li Y, Wei X, Gong Y, Zhu N, Li S. Cefminox, a dual agonist of prostacyclin receptor and peroxisome proliferator-activated receptor-gamma identified by virtual screening, has therapeutic efficacy against hypoxia-induced pulmonary hypertension in rats. *Front Pharmacol* 2018;9:134.
 68. Falcetti E, Flavell DM, Staels B, Tinker A, Haworth SG, Clapp LH. IP receptor-dependent activation of PPARgamma by stable prostacyclin analogues. *Biochem Biophys Res Commun* 2007;360:821-7.
 69. Idris-Khodja N, Ouerd S, Trindade M, Gornitsky J, Rehman A, Barhoumi T, Offermanns S, Gonzalez FJ, Neves MF, Paradis P, Schiffrin EL. Vascular smooth muscle cell peroxisome proliferator-activated receptor gamma protects against endothelin-1-induced oxidative stress and inflammation. *J Hypertens* 2017;35:1390-401.
 70. Harrington LS, Moreno L, Reed A, Wort SJ, Desvergne B, Garland C, Zhao L, Mitchell JA. The PPARbeta/delta agonist GW0742 relaxes pulmonary vessels and limits right heart hypertrophy in rats with hypoxia-induced pulmonary hypertension. *PLoS One* 2010;5:e9526.
 71. Nemenoff R, Meyer AM, Hudish TM, Mozer AB, Snee A, Narumiya S, Stearman RS, Winn RA, Weiser-Evans M, Geraci MW, Keith RL. Prostacyclin prevents murine lung cancer independent of the membrane receptor by activation of peroxisomal proliferator-activated receptor gamma. *Cancer Prev Res (Phila)* 2008;1:349-56.
 72. Lee KS, Kim SR, Park SJ, Park HS, Min KH, Jin SM, Lee MK, Kim UH, Lee YC. Peroxisome proliferator activated receptor-gamma modulates reactive oxygen species generation and activation of nuclear factor-kappaB and hypoxia-inducible factor 1alpha in allergic airway disease of mice. *J Allergy Clin Immunol* 2006;118:120-7.
 73. Wang J, Fu X, Yang K, Jiang Q, Chen Y, Jia J, Duan X, Wang EW, He J, Ran P, Zhong N, Semenza GL, Lu W. Hypoxia inducible factor-1-dependent up-regulation of BMP4 mediates hypoxia-induced increase of TRPC expression in PSMCs. *Cardiovasc Res* 2015;107:108-18.
 74. Wang J, Weigand L, Lu W, Sylvester JT, Semenza GL, Shimoda LA. Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular Ca²⁺ in pulmonary arterial smooth muscle cells. *Circ Res* 2006;98:1528-37.
 75. Wang Y, Lu W, Yang K, Wang Y, Zhang J, Jia J, Yun X, Tian L, Chen Y, Jiang Q, Zhang B, Chen X, Wang J. Peroxisome proliferator-activated receptor gamma inhibits pulmonary hypertension targeting store-operated calcium entry. *J Mol Med (Berl)* 2015;93:327-42.
 76. Chen T, Zhou Q, Tang H, Bozkanat M, Yuan JX, Raj JU, Zhou G. miR-17/20 Controls prolyl hydroxylase 2 (PHD2)/hypoxia-inducible factor 1 (HIF1) to regulate pulmonary artery smooth muscle cell proliferation. *J Am Heart Assoc* 2016;5.
 77. Gusan S, Anand-Srivastava MB. cAMP attenuates the enhanced expression of Gi proteins and hyperproliferation of vascular smooth muscle cells from SHR: role of ROS and ROS-mediated signaling. *Am J Physiol Cell Physiol* 2013;304:C1198-209.
 78. Dai Y, Luo W, Chang J. Rho kinase signaling and cardiac physiology. *Curr Opin Physiol* 2018;1:14-20.
 79. Oka M, Homma N, Taraseviciene-Stewart L, Morris KG, Kraskauskas D, Burns N, Voelkel NF, McMurtry IF. Rho kinase-mediated vasoconstriction is important in severe occlusive pulmonary arterial hypertension in rats. *Circ Res* 2007;100:923-9.
 80. Zhao L, Sebkhia A, Ali O, Wojciak-Stothard B, Mamanova L, Yang Q, Wharton J, Wilkins MR. Simvastatin and sildenafil combine to attenuate pulmonary hypertension. *Eur Respir J* 2009;34:948-57.
 81. Yao J, Xiong M, Tang B, Chen G, Liang M, Ma X, Wang Z, Wu Z. Simvastatin attenuates pulmonary vascular remodelling by down-regulating matrix metalloproteinase-1 and -9 expression in a carotid artery-jugular vein shunt pulmonary hypertension model in rats. *Eur J Cardiothorac Surg* 2012;42:e121-7.
 82. Li X, Liu L, Tupper JC, Bannerman DD, Winn RK, Sebt SM, Hamilton AD, Harlan JM. Inhibition of protein geranylgeranylation and RhoA/RhoA kinase pathway induces apoptosis in human endothelial cells. *J Biol Chem* 2002;277:15309-16.
 83. Kawasaki Y, Yokobayashi E, Sakamoto K, Tenma E, Takaki H, Chiba Y, Otashiro T, Ishihara M, Yonezawa S, Sugiyama A, Natori Y. Angiostatin prevents IL-1beta-induced down-regulation of eNOS expression by inhibiting the NF- κ B cascade. *J Pharmacol Sci* 2015;129:200-4.
 84. Niwano K, Arai M, Koitabashi N, Hara S, Watanabe A, Sekiguchi K, Tanaka T, Iso T, Kurabayashi M. Competitive binding of CREB and ATF2 to cAMP/ATF responsive element regulates eNOS gene expression in endothelial cells. *Arterioscler Thromb Vasc Biol* 2006;26:1036-42.
 85. Niwano K, Arai M, Tomaru K, Uchiyama T, Ohyama Y, Kurabayashi M. Transcriptional stimulation of the eNOS gene by the stable prostacyclin analogue beraprost is mediated through cAMP-responsive element in vascular endothelial cells: close link between PGI₂ signal and NO pathways. *Circ Res* 2003;93:523-30.
 86. Steven S, Daiber A, Doppeide JF, Munzel T, Espinola-Klein C. Peripheral artery disease, redox signaling, oxidative stress - Basic and clinical aspects. *Redox Biol* 2017;12:787-97.
 87. Sebkhia A, Strange JW, Phillips SC, Wharton J, Wilkins MR. Phosphodiesterase type 5 as a target for the treatment of hypoxia-induced pulmonary hypertension. *Circulation* 2003;107:3230-5.

88. Paffett ML, Lucas SN, Campen MJ. Resveratrol reverses monocrotaline-induced pulmonary vascular and cardiac dysfunction: a potential role for atrogin-1 in smooth muscle. *Vascul Pharmacol* 2012;56:64-73.
89. Maclean MR, Johnston ED, McCulloch KM, Pooley L, Houslay MD, Sweeney G. Phosphodiesterase isoforms in the pulmonary arterial circulation of the rat: changes in pulmonary hypertension. *J Pharmacol Exp Ther* 1997;283:619-24.
90. Amsellem V, Abid S, Poupel L, Parpaleix A, Rodero M, Gary-Bobo G, Latiri M, Dubois-Rande JL, Lipskaia L, Combadiere C, Adnot S. Roles for the CX3CL1/CX3CR1 and CCL2/CCR2 chemokine systems in hypoxic pulmonary hypertension. *Am J Respir Cell Mol Biol* 2017;56:597-608.
91. Balabanian K, Foussat A, Dorfmueller P, Durand-Gasselino I, Capel F, Bouchet-Delbos L, Portier A, Marfaing-Koka A, Krzysiek R, Rimaniol AC, Simonneau G, Emilie D, Humbert M. CX(3)C chemokine fractalkine in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2002;165:1419-25.
92. Serag AR, Hazaa SM, Afifi IK, Ghoname NF, Serag AR. Regulated upon activation, normal T-cell expressed and secreted chemokine and interleukin-6 in rheumatic pulmonary hypertension, targets for therapeutic decisions. *Eur J Cardiothorac Surg* 2010;37:853-8.
93. Launay JM, Herve P, Callebort J, Mallat Z, Collet C, Doly S, Belmer A, Diaz SL, Hatia S, Cote F, Humbert M, Maroteaux L. Serotonin 5-HT2B receptors are required for bone-marrow contribution to pulmonary arterial hypertension. *Blood* 2012;119:1772-80.
94. Xu J, Morinaga H, Oh D, Li P, Chen A, Talukdar S, Mamane Y, Mancini JA, Nawrocki AR, Lazarowski E, Olefsky JM, Kim JJ. GPR105 ablation prevents inflammation and improves insulin sensitivity in mice with diet-induced obesity. *J Immunol* 2012;189:1992-9.
95. Gerasimovskaya EV, Ahmad S, White CW, Jones PL, Carpenter TC, Stenmark KR. Extracellular ATP is an autocrine/paracrine regulator of hypoxia-induced adventitial fibroblast growth. Signaling through extracellular signal-regulated kinase-1/2 and the Egr-1 transcription factor. *J Biol Chem* 2002;277:44638-50.
96. Woodward HN, Anwar A, Riddle S, Taraseviciene-Stewart L, Fragoso M, Stenmark KR, Gerasimovskaya EV. PI3K, Rho, and ROCK play a key role in hypoxia-induced ATP release and ATP-stimulated angiogenic responses in pulmonary artery vasa vasorum endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L954-64.
97. Bodin P, Burnstock G. Evidence that release of adenosine triphosphate from endothelial cells during increased shear stress is vesicular. *J Cardiovasc Pharmacol* 2001;38:900-8.
98. Tackett BC, Sun H, Mei Y, Maynard JP, Cheruvu S, Mani A, Hernandez-Garcia A, Vigneswaran N, Karpen SJ, Thevananthar S. P2Y2 purinergic receptor activation is essential for efficient hepatocyte proliferation in response to partial hepatectomy. *Am J Physiol Gastrointest Liver Physiol* 2014;307:G1073-87.
99. Burnstock G, Knight GE. The potential of P2X7 receptors as a therapeutic target, including inflammation and tumour progression. *Purinergic Signal* 2018;14:1-18.
100. Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. *Nature* 2014;509:310-7.
101. Stachon P, Peikert A, Michel NA, Hergeth S, Marchini T, Wolf D, Dufner B, Hoppe N, Ayata CK, Grimm M, Cicko S, Schulte L, Reinohl J, von zur Muhlen C, Bode C, Idzko M, Zirikli A. P2Y6 deficiency limits vascular inflammation and atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 2014;34:2237-45.
102. Zhang Z, Wang Z, Ren H, Yue M, Huang K, Gu H, Liu M, Du B, Qian M. P2Y(6) agonist uridine 5'-diphosphate promotes host defense against bacterial infection via monocyte chemoattractant protein-1-mediated monocytes/macrophages recruitment. *J Immunol* 2011;186:5376-87.
103. Bar I, Guns PJ, Metallo J, Cammarata D, Wilkin F, Boeynants JM, Bult H, Robaye B. Knockout mice reveal a role for P2Y6 receptor in macrophages, endothelial cells, and vascular smooth muscle cells. *Mol Pharmacol* 2008;74:777-84.
104. Visovatti SH, Hyman MC, Goonewardena SN, Anyanwu AC, Kanthi Y, Robichaud P, Wang J, Petrovic-Djergovic D, Rattan R, Burant CF, Pinsky DJ. Purinergic dysregulation in pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 2016;311:H286-98.
105. Kylhammar D, Bune LT, Radegran G. P2Y(1) and P2Y(1)(2) receptors in hypoxia- and adenosine diphosphate-induced pulmonary vasoconstriction in vivo in the pig. *Eur J Appl Physiol* 2014;114:1995-2006.
106. Nijmeh H, Balasubramaniam V, Burns N, Ahmad A, Stenmark KR, Gerasimovskaya EV. High proliferative potential endothelial colony-forming cells contribute to hypoxia-induced pulmonary artery vasa vasorum neovascularization. *Am J Physiol Lung Cell Mol Physiol* 2014;306:L661-71.
107. Karmouty-Quintana H, Zhong H, Acero L, Weng T, Melicoff E, West JD, Hemnes A, Grenz A, Eltzschig HK, Blackwell TS, Xia Y, Johnston RA, Zeng D, Belardinelli L, Blackburn MR. The A2B adenosine receptor modulates pulmonary hypertension associated with interstitial lung disease. *FASEB J* 2012;26:2546-57.
108. Karmouty-Quintana H, Weng T, Garcia-Morales LJ, Chen NY, Pedroza M, Zhong H, Molina JG, Bunge R, Bruckner BA, Xia Y, Johnston RA, Loebe M, Zeng D, Seethamraju H, Belardinelli L, Blackburn MR. Adenosine A2B receptor and hyaluronan modulate pulmonary hypertension associated with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2013;49:1038-47.
109. Karmouty-Quintana H, Philip K, Acero LF, Chen NY, Weng T, Molina JG, Luo F, Davies J, Le NB, Bunge I, Volcik KA, Le TT, Johnston RA, Xia Y, Eltzschig HK, Blackburn MR. Deletion of ADORA2B from myeloid cells dampens lung fibrosis and pulmonary hypertension. *FASEB J* 2015;29:50-60.
110. Xu MH, Gong YS, Su MS, Dai ZY, Dai SS, Bao SZ, Li N, Zheng RY, He JC, Chen JF, Wang XT. Absence of the adenosine A2A receptor confers pulmonary arterial hypertension and increased pulmonary vascular remodeling in mice. *J Vasc Res* 2011;48:171-83.
111. Alencar AK, Pereira SL, Montagnoli TL, Maia RC, Kummerle AE, Landgraf SS, Caruso-Neves C, Ferraz EB, Tesch R, Nascimento JH, de Sant'Anna CM, Fraga CA, Barreiro EJ, Sudo RT, Zapata-Sudo G. Beneficial effects of a novel agonist of the adenosine A2A receptor on monocrotaline-induced pulmonary hypertension in rats. *Br J Pharmacol* 2013;169:953-62.

112. Shang P, He ZY, Chen JF, Huang SY, Liu BH, Liu HX, Wang XT. Absence of the Adenosine A2A receptor confers pulmonary arterial hypertension through RhoA/ROCK signaling pathway in mice. *J Cardiovasc Pharmacol* 2015;66:569-75.
113. Singh NK, Janjanam J, Rao GN. p115 RhoGEF activates the Rac1 GTPase signaling cascade in MCP1 chemokine-induced vascular smooth muscle cell migration and proliferation. *J Biol Chem* 2017;292:14080-91.
114. Norton CE, Broughton BR, Jernigan NL, Walker BR, Resta TC. Enhanced depolarization-induced pulmonary vasoconstriction following chronic hypoxia requires EGFR-dependent activation of NAD(P)H oxidase 2. *Antioxid Redox Signal* 2013;18:1777-88.
115. Wang Y, Xu X, Pan M, Jin T. ELMO1 directly interacts with gbetagamma subunit to transduce GPCR signaling to Rac1 activation in chemotaxis. *J Cancer* 2016;7:973-83.
116. Nguyen Dinh Cat A, Montezano AC, Burger D, Touyz RM. Angiotensin II, NADPH oxidase, and redox signaling in the vasculature. *Antioxid Redox Signal* 2013;19:1110-20.
117. Li Y, Pagano PJ. Microvascular NADPH oxidase in health and disease. *Free Radic Biol Med* 2017;109:33-47.
118. Rastogi R, Geng X, Li F, Ding Y. NOX activation by subunit interaction and underlying mechanisms in disease. *Front Cell Neurosci* 2016;10:301.
119. Paulsen CE, Carroll KS. Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery. *Chem Rev* 2013;113:4633-79.
120. Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT, Semenza GL. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. *J Clin Invest* 1999;103:691-6.
121. Tan Q, Kerestes H, Percy MJ, Pietrofesa R, Chen L, Khurana TS, Christofidou-Solomidou M, Lappin TR, Lee FS. Erythrocytosis and pulmonary hypertension in a mouse model of human HIF2A gain of function mutation. *J Biol Chem* 2013;288:17134-44.
122. Zhang L, Xiong W, Li N, Liu H, He H, Du Y, Zhang Z, Liu Y. Estrogen stabilizes hypoxia-inducible factor 1alpha through G protein-coupled estrogen receptor 1 in eutopic endometrium of endometriosis. *Fertil Steril* 2017;107:439-47.
123. Li M, Liu Y, Jin F, Sun X, Li Z, Liu Y, Fang P, Shi H, Jiang X. Endothelin-1 induces hypoxia inducible factor 1alpha expression in pulmonary artery smooth muscle cells. *FEBS Lett* 2012;586:3888-93.
124. De Francesco EM, Pellegrino M, Santolla MF, Lappano R, Ricchio E, Abonante S, Maggiolini M. GPER mediates activation of HIF1alpha/VEGF signaling by estrogens. *Cancer Res* 2014;74:4053-64.
125. Ji R, Chou CL, Xu W, Chen XB, Woodward DF, Regan JW. EP1 prostanoid receptor coupling to G i/o up-regulates the expression of hypoxia-inducible factor-1 alpha through activation of a phosphoinositide-3 kinase signaling pathway. *Mol Pharmacol* 2010;77:1025-36.
126. Kasama H, Sakamoto Y, Kasamatsu A, Okamoto A, Koyama T, Minakawa Y, Ogawara K, Yokoe H, Shiiba M, Tanzawa H, Uzawa K. Adenosine A2b receptor promotes progression of human oral cancer. *BMC Cancer* 2015;15:563.
127. Lee SJ, No YR, Dang DT, Dang LH, Yang VW, Shim H, Yun CC. Regulation of hypoxia-inducible factor 1alpha (HIF-1alpha) by lysophosphatidic acid is dependent on interplay between p53 and Kruppel-like factor 5. *J Biol Chem* 2013;288:25244-53.
128. Lee HY, Lee T, Lee N, Yang EG, Lee C, Lee J, Moon EY, Ha J, Park H. Src activates HIF-1alpha not through direct phosphorylation of HIF-1alpha specific prolyl-4 hydroxylase 2 but through activation of the NADPH oxidase/Rac pathway. *Carcinogenesis* 2011;32:703-12.
129. Du J, Xu R, Hu Z, Tian Y, Zhu Y, Gu L, Zhou L. PI3K and ERK-induced Rac1 activation mediates hypoxia-induced HIF-1alpha expression in MCF-7 breast cancer cells. *PLoS One* 2011;6:e25213.
130. Chang TC, Huang CJ, Tam K, Chen SF, Tan KT, Tsai MS, Lin TN, Shyue SK. Stabilization of hypoxia-inducible factor-1 {alpha} by prostacyclin under prolonged hypoxia via reducing reactive oxygen species level in endothelial cells. *J Biol Chem* 2005;280:36567-74.
131. Takabuchi S, Hirota K, Oda S, Nishi K, Oda T, Shingu K, Adachi T, Fukuda K. Opioid receptor stimulation does not affect cellular hypoxia-induced gene responses mediated by hypoxia-inducible factor 1 in cultured cell lines. *J Anesth* 2005;19:263-5.
132. Griguer CE, Oliva CR, Kelley EE, Giles GI, Lancaster JR, Jr., Gillespie GY. Xanthine oxidase-dependent regulation of hypoxia-inducible factor in cancer cells. *Cancer Res* 2006;66:2257-63.
133. Xia L, Mo P, Huang W, Zhang L, Wang Y, Zhu H, Tian D, Liu J, Chen Z, Zhang Y, Chen Z, Hu H, Fan D, Nie Y, Wu K. The TNF-alpha/ROS/HIF-1-induced upregulation of FoxM1 expression promotes HCC proliferation and resistance to apoptosis. *Carcinogenesis* 2012;33:2250-9.
134. Lee G, Won HS, Lee YM, Choi JW, Oh TI, Jang JH, Choi DK, Lim BO, Kim YJ, Park JW, Puigserver P, Lim JH. Oxidative dimerization of PHD2 is responsible for its inactivation and contributes to metabolic reprogramming via HIF-1alpha activation. *Sci Rep* 2016;6:18928.
135. Fukai K, Nakamura A, Hoshino A, Nakanishi N, Okawa Y, Ariyoshi M, Kaimoto S, Uchihashi M, Ono K, Tateishi S, Ikeda K, Ogata T, Ueyama T, Matoba S. Pyk2 aggravates hypoxia-induced pulmonary hypertension by activating HIF-1alpha. *Am J Physiol Heart Circ Physiol* 2015;308:H951-9.
136. Yang T, Li ZN, Chen G, Gu Q, Ni XH, Zhao ZH, Ye J, Meng XM, Liu ZH, Xiong CM, He JG. Increased levels of plasma CXCL12alpha are associated with right ventricular function in patients with idiopathic pulmonary arterial hypertension. *Heart Lung* 2014;43:322-7.
137. McCullagh BN, Costello CM, Li L, O'Connell C, Codd M, Lawrie A, Morton A, Kiely DG, Condliffe R, Elliot C, McLoughlin P, Gaine S. Elevated plasma CXCL12alpha is associated with a poorer prognosis in pulmonary arterial hypertension. *PLoS One* 2015;10:e0123709.
138. Kazimierczyk R, Blaszcak P, Jasiewicz M, Knapp M, Ptaszynska-Kopczynska K, Sobkowicz B, Waszkiewicz E, Grzywna R, Musial WJ, Kaminski KA. Increased platelet content of SDF-1alpha is associated with worse prognosis in patients with pulmonary arterial hypertension. *Platelets* 2018; doi: 10.1080/09537104.2018.1457780.

139. Wei L, Zhang B, Cao W, Xing H, Yu X, Zhu D. Inhibition of CXCL12/CXCR4 suppresses pulmonary arterial smooth muscle cell proliferation and cell cycle progression via PI3K/Akt pathway under hypoxia. *J Recept Signal Transduct Res* 2015;35:329-39.
140. Barst RJ. PDGF signaling in pulmonary arterial hypertension. *J Clin Invest* 2005;115:2691-4.
141. Wang Y, Bai Y, Qin L, Zhang P, Yi T, Teesdale SA, Zhao L, Pober JS, Tellides G. Interferon-gamma induces human vascular smooth muscle cell proliferation and intimal expansion by phosphatidylinositol 3-kinase dependent mammalian target of rapamycin raptor complex 1 activation. *Circ Res* 2007;101:560-9.
142. Little PJ. GPCR responses in vascular smooth muscle can occur predominantly through dual transactivation of kinase receptors and not classical Galphaq protein signalling pathways. *Life Sci* 2013;92:951-6.
143. Dahal BK, Heuchel R, Pullamsetti SS, Wilhelm J, Ghofrani HA, Weissmann N, Seeger W, Grimminger F, Schermuly RT. Hypoxic pulmonary hypertension in mice with constitutively active platelet-derived growth factor receptor-beta. *Pulm Circ* 2011;1:259-68.
144. Burch ML, Getachew R, Osman N, Febbraio MA, Little PJ. Thrombin-mediated proteoglycan synthesis utilizes both protein-tyrosine kinase and serine/threonine kinase receptor transactivation in vascular smooth muscle cells. *J Biol Chem* 2013;288:7410-9.
145. McKean JS, Murray F, Gibson G, Shewan DA, Tucker SJ, Nixon GF. The cAMP-producing agonist beraprost inhibits human vascular smooth muscle cell migration via exchange protein directly activated by cAMP. *Cardiovasc Res* 2015;107:546-55.
146. Viedt C, Vogel J, Athanasiou T, Shen W, Orth SR, Kubler W, Kreuzer J. Monocyte chemoattractant protein-1 induces proliferation and interleukin-6 production in human smooth muscle cells by differential activation of nuclear factor-kappaB and activator protein-1. *Arterioscler Thromb Vasc Biol* 2002;22:914-20.
147. Ashton AW, Cheng Y, Helisch A, Ware JA. Thromboxane A2 receptor agonists antagonize the proangiogenic effects of fibroblast growth factor-2: role of receptor internalization, thrombospondin-1, and alpha(v)beta3. *Circ Res* 2004;94:735-42.
148. Gao Y, Yokota R, Tang S, Ashton AW, Ware JA. Reversal of angiogenesis in vitro, induction of apoptosis, and inhibition of AKT phosphorylation in endothelial cells by thromboxane A(2). *Circ Res* 2000;87:739-45.
149. Ashton AW, Ware JA. Thromboxane A2 receptor signaling inhibits vascular endothelial growth factor-induced endothelial cell differentiation and migration. *Circ Res* 2004;95:372-9.
150. Strassheim D, Riddle SR, Burke DL, Geraci MW, Stenmark KR. Prostacyclin inhibits IFN-gamma-stimulated cytokine expression by reduced recruitment of CBP/p300 to STAT1 in a SOCS-1-independent manner. *J Immunol* 2009;183:6981-8.
151. Ren J, Zhang Y, Cai H, Ma H, Zhao D, Zhang X, Li Z, Wang S, Wang J, Liu R, Li Y, Qian J, Wei H, Niu L, Liu Y, Xiao L, Ding M, Jiang S. Human GPR4 and the notch signaling pathway in endothelial cell tube formation. *Mol Med Rep* 2016;14:1235-40.
152. Kerr BA, West XZ, Kim YW, Zhao Y, Tischenko M, Cull RM, Phares TW, Peng XD, Bernier-Latmani J, Petrova TV, Adams RH, Hay N, Naga Prasad SV, Byzova TV. Stability and function of adult vasculature is sustained by Akt/Jagged1 signalling axis in endothelium. *Nat Commun* 2016;7:10960.
153. Lin CH, Lilly B. Notch signaling governs phenotypic modulation of smooth muscle cells. *Vascul Pharmacol* 2014;63:88-96.
154. Chakravarti B, Yang J, Ahlers-Dannen KE, Luo Z, Flaherty HA, Meyerholz DK, Anderson ME, Fisher RA. Essentiality of regulator of G protein signaling 6 and oxidized Ca(2+)/calmodulin-dependent protein kinase II in Notch signaling and cardiovascular development. *J Am Heart Assoc* 2017;6.
155. Sangphech N, Osborne BA, Palaga T. Notch signaling regulates the phosphorylation of Akt and survival of lipopolysaccharide-activated macrophages via regulator of G protein signaling 19 (RGS19). *Immunobiology* 2014;219:653-60.
156. Ma YX, Wu ZQ, Feng YJ, Xiao ZC, Qin XL, Ma QH. G protein coupled receptor 50 promotes self-renewal and neuronal differentiation of embryonic neural progenitor cells through regulation of notch and wnt/beta-catenin signalings. *Biochem Biophys Res Commun* 2015;458:836-42.
157. Feng B, Li K, Zhong H, Ren G, Wang H, Shang Y, Bai M, Liang J, Wang X, Fan D. RhoE promotes metastasis in gastric cancer through a mechanism dependent on enhanced expression of CXCR4. *PLoS One* 2013;8:e81709.
158. Collett GP, Goh XF, Linton EA, Redman CW, Sargent IL. RhoE is regulated by cyclic AMP and promotes fusion of human BeWo choriocarcinoma cells. *PLoS One* 2012;7:e30453.
159. Yue X, Lin X, Yang T, Yang X, Yi X, Jiang X, Li X, Li T, Guo J, Dai Y, Shi J, Wei L, Youker KA, Torre-Amione G, Yu Y, Andrade KC, Chang J. Rnd3/RhoE modulates hypoxia-inducible factor 1alpha/vascular endothelial growth factor signaling by stabilizing hypoxia-inducible factor 1alpha and regulates responsive cardiac angiogenesis. *Hypertension* 2016;67:597-605.
160. Parker AR, Ayars AG, Altman MC, Henderson WR, Jr. Lipid mediators in aspirin-exacerbated respiratory disease. *Immunol Allergy Clin North Am* 2016;36:749-63.
161. Mitchell JA, Kirkby NS. Eicosanoids, prostacyclin and cyclooxygenase in the cardiovascular system. *Br J Pharmacol* 2018; doi: 10.1111/bph.14167.
162. Fink MP, O'Sullivan BP, Menconi MJ, Wollert PS, Wang H, Youssef ME, Fleisch JH. A novel leukotriene B4-receptor antagonist in endotoxin shock: a prospective, controlled trial in a porcine model. *Crit Care Med* 1993;21:1825-37.
163. Qian J, Tian W, Jiang X, Tamosiuniene R, Sung YK, Shuffle EM, Tu AB, Valenzuela A, Jiang S, Zamanian RT, Fiorentino DF, Voelkel NF, Peters-Golden M, Stenmark KR, Chung L, Rabinovitch M, Nicolls MR. Leukotriene B4 activates pulmonary artery adventitial fibroblasts in pulmonary hypertension. *Hypertension* 2015;66:1227-39.
164. Ee MT, Kantores C, Ivanovska J, Wong MJ, Jain A, Jankov RP. Leukotriene B4 mediates macrophage influx and pulmonary hypertension in bleomycin-induced chronic neonatal lung injury. *Am J Physiol Lung Cell Mol Physiol* 2016;311:L292-302.
165. Tian W, Jiang X, Tamosiuniene R, Sung YK, Qian J, Dhillon G, Gera L, Farkas L, Rabinovitch M, Zamanian RT, Inayatullah M, Fridlib M, Rajadas J, Peters-Golden M, Voelkel NF, Nicolls MR. Blocking macrophage leukotriene b4 prevents endothelial injury and reverses pulmonary hypertension. *Sci Transl Med* 2013;5:200ra117.

166. Tabata T, Ono S, Song C, Noda M, Suzuki S, Tanita T, Fujimura S. Role of leukotriene B4 in monocrotaline-induced pulmonary hypertension. *Nihon Kyobu Shikkan Gakkai Zasshi* 1997;35:160-6.
167. Albertini M, Clement MG. In pigs, inhaled nitric oxide (NO) counterbalances PAF-induced pulmonary hypertension. *Prostaglandins Leukot Essent Fatty Acids* 1994;51:357-62.
168. Ono S, Voelkel NF. PAF antagonists inhibit monocrotaline-induced lung injury and pulmonary hypertension. *J Appl Physiol* (1985) 1991;71:2483-92.
169. Smallbone BW, Taylor NE, McDonald JW. Effects of L-652,731, a platelet-activating factor (PAF) receptor antagonist, on PAF- and complement-induced pulmonary hypertension in sheep. *J Pharmacol Exp Ther* 1987;242:1035-40.
170. Lu A, Zuo C, He Y, Chen G, Piao L, Zhang J, Xiao B, Shen Y, Tang J, Kong D, Alberti S, Chen D, Zuo S, Zhang Q, Yan S, Fei X, Yuan F, Zhou B, Duan S, Yu Y, Lazarus M, Su Y, Breyer RM, Funk CD, Yu Y. EP3 receptor deficiency attenuates pulmonary hypertension through suppression of Rho/TGF-beta1 signaling. *J Clin Invest* 2015;125:1228-42.
171. Toki S, Zhou W, Goleniewska K, Reiss S, Dulek DE, Newcomb DC, Lawson WE, Peebles RS, Jr. Endogenous PGI2 signaling through IP inhibits neutrophilic lung inflammation in LPS-induced acute lung injury mice model. *Prostaglandins Other Lipid Mediat* 2018;136:33-43.
172. Zhou W, Hashimoto K, Goleniewska K, O'Neal JF, Ji S, Blackwell TS, Fitzgerald GA, Egan KM, Geraci MW, Peebles RS, Jr. Prostaglandin I2 analogs inhibit proinflammatory cytokine production and T cell stimulatory function of dendritic cells. *J Immunol* 2007;178:702-10.
173. Miyata M, Ueno Y, Sekine H, Ito O, Sakuma F, Koike H, Nishio S, Nishimaki T, Kasukawa R. Protective effect of beraprost sodium, a stable prostacyclin analogue, in development of monocrotaline-induced pulmonary hypertension. *J Cardiovasc Pharmacol* 1996;27:20-6.
174. Pickworth J, Rothman A, Iremonger J, Casbolt H, Hopkinson K, Hickey PM, Gladson S, Shay S, Morrell NW, Francis SE, West JD, Lawrie A. Differential IL-1 signaling induced by BMPR2 deficiency drives pulmonary vascular remodeling. *Pulm Circ* 2017;7:768-76.
175. Good RB, Gilbane AJ, Trinder SL, Denton CP, Coghlan G, Abraham DJ, Holmes AM. Endothelial to mesenchymal transition contributes to endothelial dysfunction in pulmonary arterial hypertension. *Am J Pathol* 2015;185:1850-8.
176. Nelin LD, White HA, Jin Y, Trittman JK, Chen B, Liu Y. The Src family tyrosine kinases src and yes have differential effects on inflammation-induced apoptosis in human pulmonary microvascular endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2016;310:L880-8.
177. Yu XH, Tang ZB, Liu LJ, Qian H, Tang SL, Zhang DW, Tian GP, Tang CK. Apelin and its receptor APJ in cardiovascular diseases. *Clin Chim Acta* 2014;428:1-8.
178. Wu Q, Wang HY, Li J, Zhou P, Wang QL, Zhao L, Fan R, Wang YM, Xu XZ, Yi DH, Yu SQ, Pei JM. Kappa-opioid receptor stimulation improves endothelial function in hypoxic pulmonary hypertension. *PLoS One* 2013;8:e60850.
179. Zhou Y, Wang Y, Wang X, Tian X, Zhang S, Yang F, Guo H, Fan R, Feng N, Jia M, Gu X, Wang Y, Li J, Pei J. The protective effects of kappa-opioid receptor stimulation in hypoxic pulmonary hypertension involve inhibition of autophagy through the AMPK-MTOR pathway. *Cell Physiol Biochem* 2017;44:1965-79.
180. Zhang J, Liu Q, Fang Z, Hu X, Huang F, Tang L, Zhou S. Hypoxia induces the proliferation of endothelial progenitor cells via upregulation of Apelin/APLNR/MAPK signaling. *Mol Med Rep* 2016;13:1801-6.
181. Zhang H, Gong Y, Wang Z, Jiang L, Chen R, Fan X, Zhu H, Han L, Li X, Xiao J, Kong X. Apelin inhibits the proliferation and migration of rat PASMCs via the activation of PI3K/Akt/mTOR signal and the inhibition of autophagy under hypoxia. *J Cell Mol Med* 2014;18:542-53.
182. Zhang J, Liu Q, Hu X, Fang Z, Huang F, Tang L, Zhou S. Apelin/APJ signaling promotes hypoxia-induced proliferation of endothelial progenitor cells via phosphoinositide-3 kinase/Akt signaling. *Mol Med Rep* 2015;12:3829-34.
183. Zheng H, Fu G, Dai T, Huang H. Migration of endothelial progenitor cells mediated by stromal cell-derived factor-1alpha/CXCR4 via PI3K/Akt/eNOS signal transduction pathway. *J Cardiovasc Pharmacol* 2007;50:274-80.
184. Foresta C, De Toni L, Di Mambro A, Garolla A, Ferlin A, Zuccarello D. The PDE5 inhibitor sildenafil increases circulating endothelial progenitor cells and CXCR4 expression. *J Sex Med* 2009;6:369-72.
185. Yaoita N, Shirakawa R, Fukumoto Y, Sugimura K, Miyata S, Miura Y, Nochioka K, Miura M, Tatebe S, Aoki T, Yamamoto S, Satoh K, Kimura T, Shimokawa H, Horiuchi H. Platelets are highly activated in patients of chronic thromboembolic pulmonary hypertension. *Arterioscler Thromb Vasc Biol* 2014;34:2486-94.
186. Diebold I, Djordjevic T, Hess J, Gorlach A. Rac-1 promotes pulmonary artery smooth muscle cell proliferation by upregulation of plasminogen activator inhibitor-1: role of NFkappaB-dependent hypoxia-inducible factor-1alpha transcription. *Thromb Haemost* 2008;100:1021-8.
187. Banerjee I, Fuseler JW, Price RL, Borg TK, Baudino TA. Determination of cell types and numbers during cardiac development in the neonatal and adult rat and mouse. *Am J Physiol Heart Circ Physiol* 2007;293:H1883-91.
188. Sun F, Lu Z, Zhang Y, Geng S, Xu M, Xu L, Huang Y, Zhuang P, Zhang Y. Stagedependent changes of beta2adrenergic receptor signaling in right ventricular remodeling in monocrotaline-induced pulmonary arterial hypertension. *Int J Mol Med* 2018;41:2493-504.
189. Wang S, Wu J, You J, Shi H, Xue X, Huang J, Xu L, Jiang G, Yuan L, Gong X, Luo H, Ge J, Cui Z, Zou Y. HSF1 deficiency accelerates the transition from pressure overload-induced cardiac hypertrophy to heart failure through endothelial miR-195a-3p-mediated impairment of cardiac angiogenesis. *J Mol Cell Cardiol* 2018;118:193-207.
190. Zhao Y, Yan M, Chen C, Gong W, Yin Z, Li H, Fan J, Zhang XA, Wang DW, Zuo H. MiR-124 aggravates failing hearts by suppressing CD151-facilitated angiogenesis in heart. *Oncotarget* 2018;9:14382-96.

191. Liao X, Shen Y, Zhang R, Sugi K, Vasudevan NT, Alaiti MA, Sweet DR, Zhou L, Qing Y, Gerson SL, Fu C, Wynshaw-Boris A, Hu R, Schwartz MA, Fujioka H, Richardson B, Cameron MJ, Hayashi H, Stamler JS, Jain MK. Distinct roles of resident and nonresident macrophages in nonischemic cardiomyopathy. *Proc Natl Acad Sci U S A* 2018;115:E4661-9.
192. Renaud-Gabardos E, Tatin F, Hantelys F, Lebas B, Calise D, Kunduzova O, Masri B, Pujol F, Sicard P, Valet P, Roncalli J, Chaufour X, Garmy-Susini B, Parini A, Prats AC. Therapeutic benefit and gene network regulation by combined gene transfer of apelin, FGF2, and SERCA2a into ischemic heart. *Mol Ther* 2018;26:902-16.
193. Dewachter C, Belhaj A, Rondelet B, Vercruyssen M, Schraufnagel DP, Rummelink M, Brimioulle S, Kerbaul F, Naeije R, Dewachter L. Myocardial inflammation in experimental acute right ventricular failure: effects of prostacyclin therapy. *J Heart Lung Transplant* 2015;34:1334-45.
194. Wu L, Gao L, Zhang D, Yao R, Huang Z, Du B, Wang Z, Xiao L, Li P, Li Y, Liang C, Zhang Y. C1QTNF1 attenuates angiotensin II-induced cardiac hypertrophy via activation of the AMPK pathway. *Free Radic Biol Med* 2018;121:215-30.
195. Chien PT, Lin CC, Hsiao LD, Yang CM. Induction of HO-1 by carbon monoxide releasing molecule-2 attenuates thrombin-induced COX-2 expression and hypertrophy in primary human cardiomyocytes. *Toxicol Appl Pharmacol* 2015;289:349-59.
196. Ramos-Kuri M, Rapti K, Mehel H, Zhang S, Dhandapany PS, Liang L, Garcia-Carranca A, Bobe R, Fischmeister R, Adnot S, Lebeche D, Hajjar RJ, Lipskaia L, Chemaly ER. Dominant negative Ras attenuates pathological ventricular remodeling in pressure overload cardiac hypertrophy. *Biochim Biophys Acta* 2015;1853:2870-84.
197. Cortes R, Rivera M, Rosello-Lleti E, Martinez-Dolz L, Almenar L, Azorin I, Lago F, Gonzalez-Juanatey JR, Portoles M. Differences in MEF2 and NFAT transcriptional pathways according to human heart failure aetiology. *PLoS One* 2012;7:e30915.
198. Aguero J, Ishikawa K, Hadri L, Santos-Gallego C, Fish K, Hammoudi N, Chaanine A, Torquato S, Naim C, Ibanez B, Pereda D, Garcia-Alvarez A, Fuster V, Sengupta PP, Leopold JA, Hajjar RJ. Characterization of right ventricular remodeling and failure in a chronic pulmonary hypertension model. *Am J Physiol Heart Circ Physiol* 2014;307:H1204-15.
199. Huang J, Chen L, Yao Y, Tang C, Ding J, Fu C, Li H, Ma G. Pivotal role of regulator of G-protein signaling 12 in pathological cardiac hypertrophy. *Hypertension* 2016;67:1228-36.
200. Ramadas N, Rajaraman B, Kuppuswamy AA, Vedantham S. Early growth response-1 (EGR-1) - a key player in myocardial cell injury. *Cardiovasc Hematol Agents Med Chem* 2014;12:66-71.
201. van der Feen DE, Dickinson MG, Bartelds B, Borgdorff MA, Sietsma H, Levy M, Berger RM. Egr-1 identifies neointimal remodeling and relates to progression in human pulmonary arterial hypertension. *J Heart Lung Transplant* 2016;35:481-90.
202. Dickinson MG, Kowalski PS, Bartelds B, Borgdorff MA, van der Feen D, Sietsma H, Molema G, Kamps JA, Berger RM. A critical role for Egr-1 during vascular remodelling in pulmonary arterial hypertension. *Cardiovasc Res* 2014;103:573-84.
203. Lemon DD, Harrison BC, Horn TR, Stratton MS, Ferguson BS, Wempe MF, McKinsey TA. Promiscuous actions of small molecule inhibitors of the protein kinase D-class IIa HDAC axis in striated muscle. *FEBS Lett* 2015;589:1080-8.
204. Vega RB, Harrison BC, Meadows E, Roberts CR, Papst PJ, Olson EN, McKinsey TA. Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. *Mol Cell Biol* 2004;24:8374-85.
205. Yang L, Yu D, Mo R, Zhang J, Hua H, Hu L, Feng Y, Wang S, Zhang WY, Yin N, Mo XM. The succinate receptor GPR91 is involved in pressure overload-induced ventricular hypertrophy. *PLoS One* 2016;11:e0147597.
206. Yang L, Yu D, Fan HH, Feng Y, Hu L, Zhang WY, Zhou K, Mo XM. Triggering the succinate receptor GPR91 enhances pressure overload-induced right ventricular hypertrophy. *Int J Clin Exp Pathol* 2014;7:5415-28.
207. Deschamps AM, Murphy E. Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats. *Am J Physiol Heart Circ Physiol* 2009;297:H1806-13.
208. Bopassa JC, Eghbali M, Toro L, Stefani E. A novel estrogen receptor GPER inhibits mitochondria permeability transition pore opening and protects the heart against ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2010;298:H16-23.
209. Nunn C, Zou MX, Sobiesiak AJ, Roy AA, Kirshenbaum LA, Chidiac P. RGS2 inhibits beta-adrenergic receptor-induced cardiomyocyte hypertrophy. *Cell Signal* 2010;22:1231-9.
210. Lee KN, Lu X, Nguyen C, Feng Q, Chidiac P. Cardiomyocyte specific overexpression of a 37 amino acid domain of regulator of G protein signalling 2 inhibits cardiac hypertrophy and improves function in response to pressure overload in mice. *J Mol Cell Cardiol* 2017;108:194-202.
211. Sjogren B, Parra S, Atkins KB, Karaj B, Neubig RR. Digoxin-mediated upregulation of RGS2 protein protects against cardiac injury. *J Pharmacol Exp Ther* 2016;357:311-9.
212. Takimoto E, Koitabashi N, Hsu S, Ketner EA, Zhang M, Nagayama T, Bedja D, Gabrielson KL, Blanton R, Siderovski DP, Mendelsohn ME, Kass DA. Regulator of G protein signaling 2 mediates cardiac compensation to pressure overload and antihypertrophic effects of PDE5 inhibition in mice. *J Clin Invest* 2009;119:408-20.
213. Tokudome T, Kishimoto I, Horio T, Arai Y, Schwenke DO, Hino J, Okano I, Kawano Y, Kohno M, Miyazato M, Nakao K, Kangawa K. Regulator of G-protein signaling subtype 4 mediates antihypertrophic effect of locally secreted natriuretic peptides in the heart. *Circulation* 2008;117:2329-39.
214. Yang J, Maity B, Huang J, Gao Z, Stewart A, Weiss RM, Anderson ME, Fisher RA. G-protein inactivator RGS6 mediates myocardial cell apoptosis and cardiomyopathy caused by doxorubicin. *Cancer Res* 2013;73:1662-7.
215. Miao R, Lu Y, Xing X, Li Y, Huang Z, Zhong H, Huang Y, Chen AF, Tang X, Li H, Cai J, Yuan H. Regulator of G-protein signaling 10 negatively regulates cardiac remodeling by blocking mitogen-activated protein kinase-extracellular signal-regulated protein kinase 1/2 signaling. *Hypertension* 2016;67:86-98.
216. Li Y, Tang XH, Li XH, Dai HJ, Miao RJ, Cai JJ, Huang ZJ, Chen AF, Xing XW, Lu Y, Yuan H. Regulator of G protein signalling 14

- attenuates cardiac remodelling through the MEK-ERK1/2 signalling pathway. *Basic Res Cardiol* 2016;111:47.
217. Xiao Y, Liu Y, Liu J, Kang YJ. The association between myocardial fibrosis and depressed capillary density in rat model of left ventricular hypertrophy. *Cardiovasc Toxicol* 2018;18:304-1.
218. Jabs M, Rose AJ, Lehmann LH, Taylor J, Moll I, Sijmonsma TP, Herberich SE, Sauer SW, Poschet G, Federico G, Mogler C, Weis EM, Augustin HG, Yan M, Gretz N, Schmid RM, Adams RH, Grone HJ, Hell R, Okun JG, Backs J, Nawroth PP, Herzig S, Fischer A. Inhibition of endothelial notch signaling impairs fatty acid transport and leads to metabolic and vascular remodeling of the adult heart. *Circulation* 2018;137:2592-608.
219. Kumar R, Mickael C, Kassa B, Gebreab L, Robinson JC, Koyanagi DE, Sanders L, Barthel L, Meadows C, Fox D, Irwin D, Li M, McKeon BA, Riddle S, Dale Brown R, Morgan LE, Evans CM, Hernandez-Saavedra D, Bandeira A, Maloney JP, Bull TM, Janssen WJ, Stenmark KR, Tuder RM, Graham BB. TGF-beta activation by bone marrow-derived thrombospondin-1 causes Schistosoma- and hypoxia-induced pulmonary hypertension. *Nat Commun* 2017;8:15494.
220. Gong H, An S, Sassmann A, Liu M, Mastej V, Mittal M, Zhang W, Hong Z, Offermanns S, Rehman J, Malik AB. PAR1 scaffolds TGFbetaRII to downregulate TGF-beta signaling and activate ESC differentiation to endothelial cells. *Stem Cell Reports* 2016;7:1050-8.
221. Zhou L, Chen H, Mao X, Qi H, Baker PN, Zhang H. G-protein-coupled receptor 30 mediates the effects of estrogen on endothelial cell tube formation in vitro. *Int J Mol Med* 2017;39:1461-7.
222. Li Z, Cheng L, Liang H, Duan W, Hu J, Zhi W, Yang J, Liu Z, Zhao M, Liu J. GPER inhibits diabetes-mediated RhoA activation to prevent vascular endothelial dysfunction. *Eur J Cell Biol* 2016;95:100-13.
223. Glembocki CC, Irons CE, Krown KA, Murray SF, Sprenkle AB, Sei CA. Myocardial alpha-thrombin receptor activation induces hypertrophy and increases atrial natriuretic factor gene expression. *J Biol Chem* 1993;268:20646-52.
224. Ide J, Aoki T, Ishivata S, Glusa E, Strukova SM. Proteinase-activated receptor agonists stimulate the increase in intracellular Ca²⁺ in cardiomyocytes and proliferation of cardiac fibroblasts from chick embryos. *Bull Exp Biol Med* 2007;144:760-3.
225. Snead AN, Insel PA. Defining the cellular repertoire of GPCRs identifies a profibrotic role for the most highly expressed receptor, protease-activated receptor 1, in cardiac fibroblasts. *FASEB J* 2012;26:4540-7.
226. Nishida M, Sato Y, Uemura A, Narita Y, Tozaki-Saitoh H, Nakaya M, Ide T, Suzuki K, Inoue K, Nagao T, Kurose H. P2Y6 receptor-Galpha12/13 signalling in cardiomyocytes triggers pressure overload-induced cardiac fibrosis. *EMBO J* 2008;27:3104-15.
227. Samuel CS, Du XJ, Bathgate RA, Summers RJ. 'Relaxin' the stiffened heart and arteries: the therapeutic potential for relaxin in the treatment of cardiovascular disease. *Pharmacol Ther* 2006;112:529-52.
228. Chen Y, Yang S, Yao W, Zhu H, Xu X, Meng G, Zhang W. Prostacyclin analogue beraprost inhibits cardiac fibroblast proliferation depending on prostacyclin receptor activation through a TGF beta-Smad signal pathway. *PLoS One* 2014;9:e98483.
229. Salvi V, Sozio F, Sozzani S, Del Prete A. Role of atypical chemokine receptors in microglial activation and polarization. *Front Aging Neurosci* 2017;9:148.
230. Pullamsetti SS, Savai R. Macrophage regulation during vascular remodeling: implications for pulmonary hypertension therapy. *Am J Respir Cell Mol Biol* 2017;56:556-8.
231. Li Y, Cai L, Wang H, Wu P, Gu W, Chen Y, Hao H, Tang K, Yi P, Liu M, Miao S, Ye D. Pleiotropic regulation of macrophage polarization and tumorigenesis by formyl peptide receptor-2. *Oncogene* 2011;30:3887-99.
232. Csoka B, Selmeczy Z, Kosco B, Nemeth ZH, Pacher P, Murray PJ, Kepka-Lenhart D, Morris SM, Jr., Gause WC, Leibovich SJ, Hasko G. Adenosine promotes alternative macrophage activation via A2A and A2B receptors. *FASEB J* 2012;26:376-86.
233. Lin HH, Stacey M. G Protein-coupled receptors in macrophages. *Microbiol Spectr* 2016;4.
234. Eruslanov E, Daurkin I, Ortiz J, Vieweg J, Kusmartsev S. Pivotal advance: tumor-mediated induction of myeloid-derived suppressor cells and M2-polarized macrophages by altering intracellular PGE(2) catabolism in myeloid cells. *J Leukoc Biol* 2010;88:839-48.
235. Kruger A, Mayer A, Roch T, Schulz C, Lendlein A, Jung F. Angiogenically stimulated alternative monocytes maintain their pro-angiogenic and non-inflammatory phenotype in long-term co-cultures with HUVEC. *Clin Hemorheol Microcirc* 2014;58:229-40.
236. Presta M, Andres G, Leali D, Dell'Era P, Ronca R. Inflammatory cells and chemokines sustain FGF2-induced angiogenesis. *Eur Cytokine Netw* 2009;20:39-50.
237. Sidibe A, Ropraz P, Jemelin S, Emre Y, Poittevin M, Pocard M, Bradfield PF, Imhof BA. Angiogenic factor-driven inflammation promotes extravasation of human proangiogenic monocytes to tumours. *Nat Commun* 2018;9:355.
238. Dopheide JF, Geissler P, Rubrech J, Trumpp A, Zeller GC, Bock K, Dorweiler B, Dunschede F, Munzel T, Radsak MP, Espinola-Klein C. Inflammation is associated with a reduced number of pro-angiogenic Tie-2 monocytes and endothelial progenitor cells in patients with critical limb ischemia. *Angiogenesis* 2016;19:67-78.
239. Welihinda AA, Amento EP. Positive allosteric modulation of the adenosine A2a receptor attenuates inflammation. *J Inflamm (Lond)* 2014;11:37.
240. Dufton N, Hannon R, Brancalone V, Dalli J, Patel HB, Gray M, D'Acquisto F, Buckingham JC, Perretti M, Flower RJ. Anti-inflammatory role of the murine formyl-peptide receptor 2: ligand-specific effects on leukocyte responses and experimental inflammation. *J Immunol* 2010;184:2611-9.
241. Ivy DD, McMurtry IF, Colvin K, Imamura M, Oka M, Lee DS, Gebb S, Jones PL. Development of occlusive neointimal lesions in distal pulmonary arteries of endothelin B receptor-deficient rats: a new model of severe pulmonary arterial hypertension. *Circulation* 2005;111:2988-96.
242. Cha SA, Park BM, Kim SH. Angiotensin-(1-9) ameliorates pulmonary arterial hypertension via angiotensin type II receptor. *Korean J Physiol Pharmacol* 2018;22:447-56.

243. Weng M, Raher MJ, Leyton P, Combs TP, Scherer PE, Bloch KD, Medoff BD. Adiponectin decreases pulmonary arterial remodeling in murine models of pulmonary hypertension. *Am J Respir Cell Mol Biol* 2011;45:340-7.
244. Hemnes AR, Rathinasabapathy A, Austin EA, Brittain EL, Carrier EJ, Chen X, Fessel JP, Fike CD, Fong P, Fortune N, Gerszten RE, Johnson JA, Kaplowitz M, Newman JH, Piana R, Pugh ME, Rice TW, Robbins IM, Wheeler L, Yu C, Loyd JE, West J. A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. *Eur Respir J* 2018;51.
245. Tozzi CA, Poiani GJ, McHugh NA, Shakarjian MP, Grove BH, Samuel CS, Unemori EN, Riley DJ. Recombinant human relaxin reduces hypoxic pulmonary hypertension in the rat. *Pulm Pharmacol Ther* 2005;18:346-53.
246. Pintalhao M, Castro-Chaves P, Vasques-Novoa F, Goncalves F, Mendonca L, Fontes-Carvalho R, Lourenco P, Almeida P, Leite-Moreira A, Bettencourt P. Relaxin serum levels in acute heart failure are associated with pulmonary hypertension and right heart overload. *Eur J Heart Fail* 2017;19:218-25.
247. Imamura M, Vitello AM, Limbird JN, Ivy DD, Fallon MB, Carter EP. Endothelin-B receptor overexpression prevents hypoxic pulmonary hypertension in cirrhotic rats. *Chest* 2005;128:580S-1S.
248. Reichenberger F, Voswinckel R, Steveling E, Enke B, Kreckel A, Olschewski H, Grimminger F, Seeger W, Ghofrani HA. Sildenafil treatment for portopulmonary hypertension. *Eur Respir J* 2006;28:563-7.
249. Xiao R, Su Y, Feng T, Sun M, Liu B, Zhang J, Lu Y, Li J, Wang T, Zhu L, Hu Q. Monocrotaline induces endothelial injury and pulmonary hypertension by targeting the extracellular calcium-sensing receptor. *J Am Heart Assoc* 2017;6:pii: e004865.
250. West JD, Voss BM, Pavliv L, de Caestecker M, Hemnes AR, Carrier EJ. Antagonism of the thromboxane-prostanoid receptor is cardioprotective against right ventricular pressure overload. *Pulm Circ* 2016;6:211-23.
251. West JD, Carrier EJ, Bloodworth NC, Schroer AK, Chen P, Ryzhova LM, Gladson S, Shay S, Hutcheson JD, Merryman WD. Serotonin 2B receptor antagonism prevents heritable pulmonary arterial hypertension. *PLoS One* 2016;11:e0148657.
252. Tian W, Jiang X, Sung YK, Qian J, Yuan K, Nicolls MR. Leukotrienes in pulmonary arterial hypertension. *Immunol Res* 2014;58:387-93.
253. Pyne NJ, Pyne S. Sphingosine kinase 1: a potential therapeutic target in pulmonary arterial hypertension? *Trends Mol Med* 2017;23:786-98.
254. Sysol JR, Natarajan V, Machado RF. PDGF induces SphK1 expression via Egr-1 to promote pulmonary artery smooth muscle cell proliferation. *Am J Physiol Cell Physiol* 2016;310:C983-92.
255. Bhavanam NP, Failla A, Cho Y, Lockey RF, Koliputi N. Commentary: the sphingosine kinase 1/sphingosine-1-phosphate pathway in pulmonary arterial hypertension. *Front Pharmacol* 2015;6:229.

Review

Open Access



Thrombospondins and remodeling of the tumor microenvironment

Olga Stenina-Adognravi, Santoshi Muppala, Jasmine Gajeton

Department of Molecular Cardiology, Cleveland Clinic, Cleveland, 44195, USA.

Correspondence to: Dr. Olga Stenina-Adognravi, Department of Molecular Cardiology, Cleveland Clinic, Cleveland, 44195, USA.
E-mail: stenino@ccf.org

How to cite this article: Stenina-Adognravi O, Muppala S, Gajeton J. Thrombospondins and remodeling of the tumor microenvironment. *Vessel Plus* 2018;2:30. <http://dx.doi.org/10.20517/2574-1209.2018.40>

Received: 30 May 2018 **First Decision:** 21 Aug 2018 **Revised:** 5 Sep 2018 **Accepted:** 11 Sep 2018 **Published:** 10 Oct 2018

Science Editor: Alexander D. Verin **Copy Editor:** Cui Yu **Production Editor:** Zhong-Yu Guo

Abstract

Vascular remodeling defines cancer growth and aggressiveness. Although cancer cells produce pro-angiogenic signals, the fate of angiogenesis critically depends on the cancer microenvironment. Composition of the extracellular matrix (ECM) and tumor inflammation determine whether a cancer will remain dormant, will be recognized by the immune system and eliminated, or whether the tumor will develop and lead to the spread and metastasis of cancer cells. Thrombospondins (TSPs), a family of ECM proteins that has long been associated with the regulation of angiogenesis and cancer, regulate multiple physiological processes that determine cancer growth and spreading, from angiogenesis to inflammation, metabolic changes, and properties of ECM. Here, we sought to review publications that describe various functions of TSPs that link these proteins to regulation of cancer growth by modulating multiple physiological and pathological events that prevent or support tumor development. In addition to its direct effects on angiogenesis, TSPs have important roles in regulation of inflammation, immunity, ECM properties and composition, and glucose and insulin metabolism. Furthermore, TSPs have distinct roles as regulators of remodeling in tissues and tumors, such that the pathways activated by a single TSP can interact and influence each other. The complex nature of TSP interactions and functions, including their different cell- and tissue-specific effects, may lead to confusing results and controversial conclusions when taken out of the context of interdisciplinary and holistic approaches. However, studies of TSP functions and roles in different systems of the organism offer an integrative view of tumor remodeling and a potential for finding therapeutic targets that would modulate multiple complementary processes associated with cancer growth.

Keywords: Thrombospondin, angiogenesis, inflammation, cancer

INTRODUCTION

The studies of cancer initiation and progression have focused on the molecular and cellular signaling



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



events that lead to changes in cancer cell differentiation, proliferation, and apoptosis, all of which initiate uncontrolled growth and spreading. The roles of immunity and of the response of the whole organism to cancer cells have been historically appreciated, but the role of the microenvironment in the initiation, progression, and spreading of cancer has become a more active field of study only recently. It has been accepted that cancer cells are constantly forming in a healthy body^[1,2] but they do not survive. They may remain dormant for years due to the healthy microenvironment, which does not support the tumor growth. The tumor microenvironment, which prevents or promotes cancer growth, consists of stromal and vascular cells, immune and inflammatory cells. Additionally, the extracellular matrix (ECM) and secreted signals that these cells generate promote or restrict cancer cell division and migration^[3,4]. The progression of cancer depends on the complex interplay between the tumor cells and the tumor microenvironment. Targeting the components of the tumor microenvironment is now a recognized powerful tool of cancer therapy and prevention of spreading and recurrence.

The development of tumor vasculature depends on angiogenesis, accompanied by inflammation, which is an important factor in predicting tumor vascularization, growth, and spreading. Targeting the tumor vasculature has been an active approach in finding new therapeutic targets for many years^[5] but, unfortunately, has not fulfilled the expectations of cancer therapies due to significant side effects and adverse events in tumors in response to hypoxia^[6]. It has become clear that we have a limited appreciation of pathological processes associated with the tumor microenvironment and, as a result, inadequate understanding of potential therapies that may improve the microenvironment and restrict tumor growth.

Tumor angiogenesis and the recruitment of immune and inflammatory cells into the tumor rely on the composition of ECM^[7]. One important event that occurs during tumor progression is the stiffening of the ECM, caused by the deposition of collagen and fibronectin, leading to increased proliferation and tumor advancement^[8]. Cancer-associated fibroblasts are important contributors of ECM stiffening^[9]. Tumor ECM is also modified by vascular and blood cells that release proteases and chemoattractants and deposit ECM to promote angiogenesis, additional recruitment of vascular and inflammatory cells, and inflammation^[10].

Tumor inflammation is closely associated with the tumor aggressiveness and metastasis^[11]. Activated cancer and vascular cells produce chemoattractants and pro-inflammatory signals to recruit inflammatory cells from blood. The accumulation of inflammatory cells in a tumor is an important prognostic index that has been successfully used to evaluate the aggressiveness of cancer in conjunction with other indexes that describe the proliferation rate of cancer cells and their migratory potential. CD68, a marker of macrophages is one of the 16 markers evaluated in Oncotype DX, a clinical test that is used to make therapeutic decisions and predict the aggressiveness of breast cancer^[12,13]. The recruitment and retention of inflammatory cells in tumors depends on the ECM composition^[14].

This article reviews the contribution of thrombospondins (TSPs), a family of secreted ECM proteins, in regulation of the cancer microenvironment and the initiation and progression of tumor growth that is defined by the vascular and ECM remodeling and inflammatory response. It is becoming clear that TSPs affect every pathological process associated with cancer advancement and are key protein regulators of the tissue remodeling that occurs with cancer growth.

TSPS AND CANCER

The ECM is complex and ever-changing. All the cells in a tumor constantly remodel the ECM and deposit growth factors, proteases, pro-inflammatory and chemoattractant proteins into ECM. The composition of ECM defines whether it will support cancer growth, angiogenesis, and inflammation by providing specific growth- and migration-promoting signals and changing the physical properties of the tissue. ECM contains structural components, e.g., collagens, and secreted proteins that define the interactions of the cells

with the structural elements of the ECM. One of the influential groups of proteins that regulates the interactions between structural proteins and cells are TSPs. This family of proteins consists of five members in humans (TSP-1 - TSP-5). The five members of the family share a high level of homology and a number of properties^[15]. However, they have unique domains and protein sequences that render each of the five TSPs distinct in their interactions with the ligands in ECM and with cell surface receptors. As a result, TSPs have distinct functions in tissue remodeling and regulation of cancer growth^[16]. Furthermore, a strong and repeated association of TSP expression or suppression in various cancers highlights their role in cancer regulation^[17].

TSP-3, TSP-4, AND TSP-5

TSPs of group B (TSP-3, TSP-4, and TSP-5) are evolutionarily older proteins with fewer domains than in the group A TSP proteins^[18]. Group B TSPs are important in embryonic development^[16,19] and participate in the activation of embryonic tissue remodeling programs^[20]. TSP-3 and TSP-5 are involved in the regulation of growth plate organization and limb length^[21]. Complete deletion of TSP-3 and TSP-5 leads to reduced limb length, which signifies their prominent role in skeletal growth^[21]. Not surprisingly, TSP-3 is also linked to cancer angiogenesis, metastasis and invasion in osteosarcoma patients^[22].

TSP-4 is one of the highly upregulated genes (in the top 1%) in several types of cancer, e.g., gastric cancer^[23-25] and breast cancer^[26-28]. Its expression is upregulated in stromal tissue of invasive breast and gastric adenoma cancers^[20,29]. A recent study suggests that the loss of miR-142, resulting in high expression of TSP-4, enhances hepatocellular carcinoma (HCC) invasion and progression. Therefore, targeting TSP-4 may be an important strategy to treat HCC^[30]. Increased expression of TSP-4 in ECM promotes invasion of the breast cancer cells^[20]. Another study stated that TSP-4 mRNA expression in fibroblasts was stimulated by cancer cells, suggesting that TSP-4 is an important novel marker in the detection of diffuse-type gastric adenocarcinomas^[29]. Flexible heteroarotinoid compounds coordinate growth, apoptosis and differentiation of cancer cells. One of the compounds of this group, SHetA2, inhibits angiogenic effects by decreasing the secretion of TSP-4, along with vascular endothelial growth factor A and fibroblast growth factor, in ovarian and renal cancers^[31].

TSP-4 promotes cancer angiogenesis and growth in mouse models of breast cancer^[32]. Knocking out TSP-4 in mice resulted in smaller tumors with decreased numbers of endothelial cells and lower levels of angiogenesis markers. Conversely, a P387 variant of TSP-4 that is a more active variant of TSP-4 in cellular effects and interactions with ligands^[32], had increased cancer angiogenesis and tumor growth^[32]. Although the vascular cells appear to be the main source of TSP-4 in breast cancers^[32,33], *in vivo*, the cancer cells themselves also produce small amounts of TSP-4 that appear to be sufficient to support angiogenesis and cancer growth even in TSP-4 deficient animals^[32,33]. Complete deletion of TSP-4, in both the host and the cancer cells, is required in order to document effects on tumor growth^[33]. In addition to these effects in tumors, TSP-4 promotes adhesion and migration of leukocytes^[34]. Thus, TSP-4 is a pro-angiogenic^[32] and a pro-inflammatory protein^[35] that supports tumor growth by activating multiple complementary pathways.

TSP-4 mediates the effects of transforming growth factor beta (TGF- β), a master regulator of ECM and inflammation, on angiogenesis^[33]. The direct roles of TSP-4 in ECM regulation remain poorly understood. However, it is clear that TSP-4 regulates collagen production and can prevent fibrosis in tissues^[36].

On the other hand, TSP-4 serves as a tumor suppressor in colorectal cancer and suppresses *in vitro* tumor colony formation^[37]. Epigenetic profiling studies revealed that hypermethylation of the TSP-4 promoter leads to its inactivation and loss of TSP-4 tumor suppressor function in cutaneous T cell lymphoma^[38]. The opposite effects of TSPs on cancer cells and on the cancer microenvironment are a recurrent theme when studying the roles of TSPs in cancer regulation, leading to controversial findings that are difficult to

explain. Ultimately, the results from *in vivo* studies, where TSPs levels have been manipulated, should be considered to help define physiological roles for individual TSPs in cancer regulation.

TSP-1 AND TSP-2

The more recently developed group of TSPs, which includes TSP-1 and TSP-2 and is termed “group A”, appeared in evolution along with the development of the cardiovascular system^[19,39]. These two proteins have several C-terminal domains homologous to group B proteins but differ from group B TSPs in their N-terminal protein parts^[40]. The newly acquired in evolution domains of TSP have important roles in regulating vascular tissue remodeling: they harbor sequences associated with the anti-angiogenic action of these two proteins^[41,42] and with the inhibition of metalloproteinases (MMP)^[43,44]. One of the N-terminal domains in TSP-1 contains a sequence that binds TGF- β : TSP-1 is a major activator of latent TGF- β ^[45-47].

The anti-angiogenic properties of TSP-1 and TSP-2 have been studied for over 30 years^[48]. TSP-1 and TSP-2 inhibit endothelial cell proliferation, migration, and apoptosis^[49-52]. The decreased expression of TSP-1 and TSP-2 in tumors and surrounding tissues has been reported^[53-55], and animal studies confirmed their anti-angiogenic and tumor-preventing action^[56]. Furthermore, TSP-1 expression has been associated with cancer dormancy^[57,58]; merely suppressing or overexpressing TSP-1 is enough to reverse the patterns of tumor growth in specific anatomical areas with differential expression of TSP-1^[59-63].

In addition to the direct effects of TSP-1 and TSP-2 on endothelial cells, the regulation of angiogenesis may be an indirect consequence of regulation of MMP activity^[44], binding of growth factors and regulation of their availability and activity^[64], and regulation of functions of the immune and inflammatory cells^[65,66].

The effects of TSP-1 on cancer cells are sometimes inconsistent with its anti-angiogenic and anti-cancer effects observed in *in vivo* studies. TSP-1 has adhesive properties that support cancer cell growth^[67-69], promote metastatic properties of breast cancer cells^[70], facilitate the invasion of squamous cell carcinoma^[71], breast cancer cells^[72] and melanoma^[73], may increase proliferation of cancer cells^[74], and decrease cancer cell apoptosis^[75].

INTEGRATIVE APPROACH TO UNDERSTANDING TSP ROLES IN CANCER

These contradictory effects of TSPs on cultured cancer cells and on the fate of a tumor *in vivo* have not been explained. To better understand their significance, these contradictory effects should be considered in a context of complex relationships between the cancer cells and the entire organism: prevention of a tumor growth not only relies on the cancer cell properties alone but also requires a concerted response of the body that involves the activation of immune responses, the recognition of cancer cells, and clearance of these cells. Tumor development occurs only when multiple body systems fail to eliminate cancer cells from the system. Cancer cells are constantly forming in different tissues and also circulate in blood^[76] but fail to attach and initiate a tumor growth when the microenvironment (including ECM of tissues), regulation of angiogenesis, and responses of the immune system are normal^[77,78]. Dysregulation of metabolic, immune, and tissue remodeling processes is what leads a single cancer cell to progress to a tumor rather than being recognized, killed and eliminated. The physiological balance of cancer cell attachment, proliferation and mobility versus their recognition, apoptosis, and elimination define the fate of each cancer cell that forms in the body.

The *in vivo* effects of TSP-1 and TSP-2, suggest that these proteins activate a whole-organism anti-angiogenic and anti-cancer program that ultimately leads to a decrease in cancer growth or to cancer cell dormancy^[58]. Thus, the positive effects of TSP-1 and TSP-2 on cancer cell proliferation could be considered

as a part of this program, which initiates anti-cancer defense in multiple body systems. For example, increased proliferation of cancer cells due to TSP-1 signaling may render the cancer cells more susceptible to the elimination by natural killer cells^[79]. Furthermore, TSP-1 signaling may facilitate activation of p53, a regulator of apoptosis^[80,81]. Promoting cancer cell proliferation and invasion may result in better responses from T-cells due to expression of cancer-specific antigens and their circulation in blood. Similar to the therapeutic approaches designed by humans, e.g., chemotherapy and radiation treatment of cancers, the natural body responses may be the most efficient when the cancer cells are rapidly growing. Understanding why TSPs have cell-specific responses and seemingly contradictory effects would explain how they protect from cancers in the case of TSP-1 or promote cancer growth in the case of TSP-4. Better understanding these complex and sometimes contradictory properties of TSPs will only be possible by developing an integrative approach and more holistic view of the pathological and physiological processes regulated by these proteins, considering the fact that they affect multiple organ systems.

Newly developed integrative approaches to cancer therapies have pushed the field to better understand the causes of cancer and the mechanisms, by which tumors grow and spread. As a result, inflammation and the metabolic changes have become the focus of many studies that investigate how the cancer microenvironment is regulated^[82,83]. A growing body of evidence connects increased levels of blood glucose and insulin and chronic inflammation with cancer initiation and progression^[84,85]. While the association of diabetes and cancer has been known for many years^[86-89], recent studies suggested that even post-prandial elevations in blood glucose and/or insulin increases the risk of cancer. The glycemic load (GL, a measure of the increase in post-prandial blood glucose caused by food) and/or the high dietary glycemic index (GI, another index that estimates the effect of foods on post-prandial blood glucose) were associated with a risk of breast cancer^[90-94]; with lung cancer^[95]; prostate cancer^[93,96], especially with its aggressive form^[97]; endometrial cancer^[93,98]; ovarian cancer^[93]; and digestive tract cancers (esophageal, stomach, colorectal, liver, gallbladder, and pancreatic)^[93,96,99-103]. The emerging evidence stresses the importance of diets low in GI and GL and reduction of carbohydrates in diets as a part of healthy nutrition and lifestyle to prevent cancer development and recurrence^[104-106]. The connection between chronic inflammation and cancer has been known for a long time: e.g., an association between the hepatitis and the liver cancer has been well recognized and studied^[107,108], the existence of cancers caused by pancreatitis and Crohn's disease has been known and accepted^[109,110], and the connection between the infection with *Helicobacter pylori* and stomach cancer has been confirmed^[111,112]. Diabetes, pre-diabetes, and metabolic syndrome are associated with chronic inflammation^[113-116] and can be induced by the chronic inflammation in growing adipose tissue^[117-119] and pancreas^[120-122]. Thus, metabolic dysfunction appears to increase the risk of cancer directly (due to an increased blood glucose and insulin) and by increasing the inflammation. TSP-1, that normally restrains angiogenesis and prevents the growth of a tumor, is downregulated by high blood glucose levels in many tissues^[123,124], thus, providing a link between the elevated blood glucose and cancer. TSP-1 has been shown to be downregulated by a microRNA, miR-467, in response to hyperglycemia^[124]. Inhibition of miR-467 using an antagonist effectively inhibited hyperglycemia-induced breast cancer growth in mice^[125]. Furthermore, decreased levels of TSP-1 are associated with higher inflammation in tissues, probably due to its ability to stimulate phagocytosis in macrophages and to promote the resolution of inflammation^[66,126]. Therefore, increasing the levels of TSP-1 may stop or prevent the growth of tumors in multiple complementary ways by decreasing cancer angiogenesis and promoting the resolution of cancer inflammation.

ANTI-CANCER TSP-BASED APPROACHES

The functions of TSP-2, TSP-3, and TSP-4 in regulation of cancer growth are not well enough understood to identify potential therapeutic approaches based on the regulation of expression of these proteins or on their specific ligands and cell surface receptors. However, TSP-1, a TSP family member discovered and purified from platelets^[127] 40 years ago, has been a target for developing strategies to modulate its levels or to

take advantage of its interactions with ligands in ECM and on the cell surface.

Multiple attempts to use TSP-1 fragments to inhibit cancer growth have been described in the literature. Adenovirus-mediated gene therapy containing an antiangiogenic fragment of TSP-1 inhibited the growth of the human leukemia xenograft in mice^[128]. Gene therapy with a fragment of TSP-1 inhibited the growth of human breast carcinoma, MDA-MB-435, *in vivo* in mice^[129]. The delivery of the fragment together with p53 resulted in a synergistic effect and decreased the cancer growth more than the TSP-1 fragment or p53 administered separately. Linear and cyclic peptide TSP-1 mimetics have been tested in anti-angiogenesis therapies^[130-135].

The interaction of TSP-1 with CD47 was shown to mediate multiple effects of TSP-1^[136]. Targeting this interaction, with the goal of increasing angiogenesis, led to an unexpected outcome - angiogenesis inhibition^[137]. The peptide, designed to block the interaction of TSP-1 with CD47, named TAX2, increased the binding of TSP-1 to CD36 and disrupted vascular endothelial growth factor receptor 2 activation and subsequent downstream NO signaling. This peptide was also tested in experimental animal cancer models and inhibited angiogenesis and growth of melanoma^[137], pancreatic carcinoma^[137] and neuroblastoma^[138]. It was also effective in preventing the spread of melanoma^[139]. The 4N1 peptide, based on the sequence of TSP-1 domain that binds CD47, was successfully used in a mouse model as an anti-leukemia agent^[140]. The interaction of TSP-1 with CD47 was found to be important in multiple processes related to tumor growth. For example, blocking the signaling through CD47 conferred protection of normal tissue to irradiation through activation of autophagy pathways^[141,142]. Modulation of the anti-tumor immunity by CD47 in T cells by this pathway has been described^[143]. Thus, this TSP-1-CD47 interaction appears to be a valuable therapeutic target.

One of the cell-specific effects of TSP-1, mediated by its interaction with CD47, limits cell survival in response to radiation^[144], suggesting that antagonizing this interaction would provide a selective radioprotection for normal cells and tissues. Another tissue- and cell-specific approach targeted a miRNA regulating TSP-1 production: miR-467 increases in a cell- and tissue-specific manner in response to hyperglycemia and silences the production of TSP-1^[124]. Thus, antagonizing this miRNA slows down the growth of certain cancers without affecting TSP-1 production in response to high glucose in other tissues^[125].

Some unexpected outcomes from using anti-TSP-1 strategies highlight the complexity of TSP-1 interactions and its functions. The domains involved in regulating angiogenesis, TGF- β activation, and MMP inhibition are localized in N-terminal part of TSP-1, while interaction with CD47 depends on the C-terminal domain of the protein. However, based on the results of peptide studies, the domains are functionally associated, such that blocking the interaction with one receptor also changes the interactions of distant domains with other receptors and ligands^[64,145]. Due to the multiple cell-specific functions, the effects of TSPs on various cells types that are involved in tumor progression should be also taken in the account when considering pharmacological interventions that target the expressions of TSPs or block their interactions with their ligands.

ECM proteins appear to be good targets for therapy because of their extracellular localization and relatively easy availability for drugs. However, very few ECM proteins have become successful therapeutic targets. Most ECM proteins, including TSPs, have a complex multi-domain structure with a number of ligands on the ECM and cell surface. The combined effect of TSP interactions with other ligands and receptors may not only depend on their protein levels in tissues but also on the availability of ligands and receptors on the cell surface. Ultimately, the systemic effects caused by inhibiting TSPs or regulating their production should be considered. Successful strategies need to be based on tissue- and cell-specific evidence such that

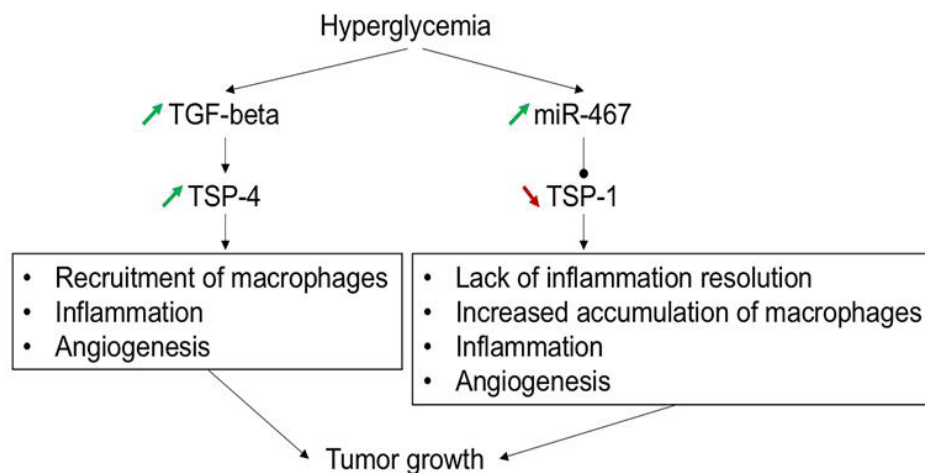


Figure 1. Hyperglycemia promotes cancer growth by regulating thrombospondin (TSP)-1- and TSP-4-dependent pathways. Upregulation of TGF- β in response to hyperglycemia leads to upregulation of TSP-4. TSP-4 is a pro-angiogenic protein that also promotes recruitment of macrophages and other leukocytes into tissues and increases local inflammation. Upregulation of miR-467 in a tissue-specific manner blocks TSP-1 production. In the absence of the anti-angiogenic pressure of TSP-1, cancer angiogenesis is increased. In the absence of TSP-1, the resolution of inflammation is impaired. Increased inflammation and angiogenesis promote cancer growth in the absence of TSP-1. TSP-1 and TSP-4 pathways converge and complement each other to promote the tumor growth

interactions do not alter the functions or expression of TSPs elsewhere.

Interactions between TSP pathways further complicate the final outcomes. For example, studies of the effects of hyperglycemia on breast cancer suggest that TSP-1-dependent pathways may synergize with TSP-4-dependent pathways. Higher expression of miR-467 in response to high glucose was associated with inhibition of TSP-1 production^[123,124,146] [Figure 1]. In addition to its anti-angiogenic effects, TSP-1 is a regulator of inflammation and functions of macrophages^[147]. TSP-1 is known to regulate the production of cytokines by macrophages^[148-150], to stimulate micropinocytosis^[151], motility^[152], to activate toll-like receptor 4 pathway in macrophages^[153], and to promote the resolution of inflammation^[150,154]. Hyperglycemia changes the levels of multiple ECM proteins, including the master ECM regulator TGF- β ^[155,156]. Higher levels of TGF- β have been detected in the cancers of diabetic patients^[157,158], and blocking TGF- β signaling leads to better outcomes in animal models^[159-161]. It was reported recently that increased levels of TGF- β led to increased production of TSP-4. Unlike TSP-1, TSP-4 is pro-angiogenic^[162,163] and increases accumulation of macrophages and other leukocytes in tissues *via* increased recruitment into tissues^[34,35]. Increased levels of TSP-4 combined with decreased levels of TSP-1 would promote a pro-angiogenic and inflammatory microenvironment leading to tumor growth [Figure 1].

In addition to functional interaction, TSP pathways interact at the mechanistic level. For example, TSP-1 activates TGF- β ^[45,47,164] and is downregulated by TGF- β ^[163] but TSP-4 is a mediator of the TGF- β effects^[163] and, in turn, modulates the expression of one of the TGF- β receptors, beta-glycan^[165], thus, controlling TGF- β signaling [Figure 2].

CONCLUSION

TSPs become available to many cell types after they are secreted and incorporated into ECM. They have multiple interactions and functions, which depend on the availability of specific cell surface receptors on each cell type at any given moment. The final outcome of modulating TSP levels is determined by a combined effect from their actions in multiple cell types and organs, from the tumor itself to the immune system and vasculature.

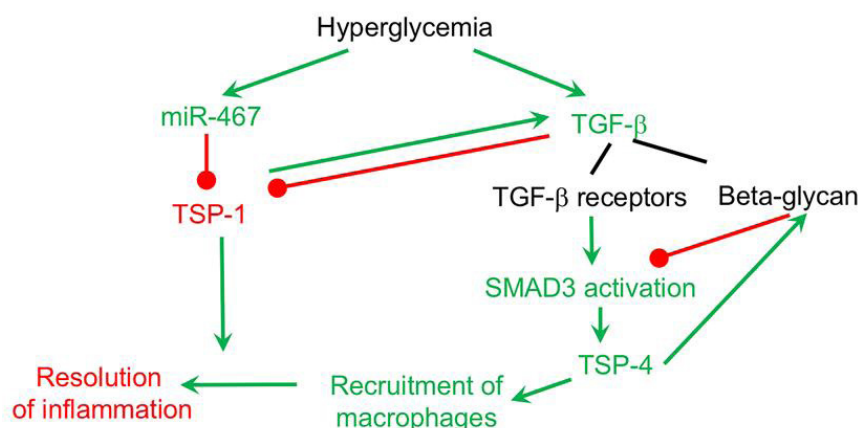


Figure 2. Interaction of hyperglycemia-regulated thrombospondin (TSP) pathways. TSP-4 increases recruitment of macrophages, while TSP-1 is needed for the resolution of inflammation. In response to hyperglycemia, TSP-1 levels are downregulated by increased levels of miR-467. TSP-4 is upregulated as a result of upregulation of TGF-β and activation of SMAD3. Upregulation of TSP-4 result in increased recruitment of macrophages into the tumor. In the absence of TSP-1 and resolution of inflammation, the accumulation of macrophages increases. In a feedback loop, TSP-4 increases the levels of an inhibitory TGF-β receptor beta-glycan. TGF-β further decreases the production of TSP-1. In a feedback loop, TSP-1 is an activator of TGF-β. Green arrow and text = upregulation in response to hyperglycemia; red arrow and text = downregulation in response to hyperglycemia. TGF-β: transforming growth factor beta

While multiple targets may potentiate the effects of modulation of TSP expression and functions, the complexity of TSP interactions requires an unbiased testing of the effects of potential anti-cancer therapies in *in vivo* models. When the interactions and mechanisms are dissected and understood, TSPs may become desirable targets for the integrative anti-cancer approaches.

DECLARATIONS

Authors' contributions

Analyzed the literature, collected the references: Muppala S, Gajeton J, Stenina-Adognravi O

Prepared a draft of the manuscript: Muppala S, Gajeton J

Prepared the final version of the manuscript: Stenina-Adognravi O

Availability of data and materials

Not applicable.

Financial support and sponsorship

The work on this review has been supported by funds from NIH to Olga Stenina-Adognravi (RO1 HL117216 and RO1 CA177771) and from the American Heart Association to Jasmine Gajeton/Olga Stenina-Adognravi (17PRE33660475).

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* 2013;19:1423-37.
2. Wang M, Zhao J, Zhang L, Wei F, Lian Y, et al. Role of tumor microenvironment in tumorigenesis. *J Cancer* 2017;8:761-73.
3. Bremnes RM, Al-Shibli K, Donnem T, Sirera R, Al-Saad S, et al. The role of tumor-infiltrating immune cells and chronic inflammation at the tumor site on cancer development, progression, and prognosis: emphasis on non-small cell lung cancer. *J Thorac Oncol* 2011;6:824-33.
4. Belli C, Trapani D, Viale G, D'Amico P, Duso BA, et al. Targeting the microenvironment in solid tumors. *Cancer Treat Rev* 2018;65:22-32.
5. Schaaf MB, Garg AD, Agostinis P. Defining the role of the tumor vasculature in antitumor immunity and immunotherapy. *Cell Death Dis* 2018;9:115.
6. Barker HE, Paget JT, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. *Nat Rev Cancer* 2015;15:409-25.
7. Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. *EMBO Rep* 2014;15:1243-53.
8. LaValley DJ, Zanotelli MR, Bordeleau F, Wang W, Schwager SC, et al. Matrix stiffness enhances VEGFR-2 internalization, signaling, and proliferation in endothelial cells. *Converg Sci Phys Oncol* 2017;3:4.
9. Ng CF, Frieboes HB. Model of vascular desmoplastic multispecies tumor growth. *J Theor Biol* 2017;430:245-82.
10. Neve A, Cantatore FP, Maruotti N, Corrado A, Ribatti D. Extracellular matrix modulates angiogenesis in physiological and pathological conditions. *Biomed Res Int* 2014; doi: 10.1155/2014/756078.
11. Yang L, Lin PC. Mechanisms that drive inflammatory tumor microenvironment, tumor heterogeneity, and metastatic progression. *Semin Cancer Biol* 2017;47:185-95.
12. Medrek C, Pontén F, Jirstrom K, Leandersson K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. *BMC Cancer* 2012;12:306.
13. Barcenas CH, Raghavendra A, Sinha AK, Syed MP, Hsu L, et al. Outcomes in patients with early-stage breast cancer who underwent a 21-gene expression assay. *Cancer* 2017;123:2422-31.
14. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb Perspect Biol* 2011; doi: 10.1101/cshperspect.a005058.
15. Adams JC, Lawler J. The thrombospondins. *Cold Spring Harb Perspect Biol* 2011; doi: 10.1101/cshperspect.a009712.
16. Tan K, Lawler J. The interaction of thrombospondins with extracellular matrix proteins. *J Cell Commun Signal* 2009;3:177-87.
17. Stenina-Adognravi O. Invoking the power of thrombospondins: regulation of thrombospondins expression. *Matrix Biol* 2014;37:69-82.
18. Hellewell AL, Gong X, Schärich K, Christofidou ED, Adams JC. Modulation of the extracellular matrix patterning of thrombospondins by actin dynamics and thrombospondin oligomer state. *Biosci Rep* 2015; doi: 10.1042/BSR20140168.
19. Bentley AA, Adams JC. The evolution of thrombospondins and their ligand-binding activities. *Mol Biol Evol* 2010;27:2187-97.
20. McCart Reed AE, Song S, Kutasovic JR, Reid LE, Valle JM, et al. Thrombospondin-4 expression is activated during the stromal response to invasive breast cancer. *Virchows Arch* 2013;463:535-45.
21. Posey KL, Hankenson K, Veerisetty AC, Bornstein P, Lawler J, et al. Skeletal abnormalities in mice lacking extracellular matrix proteins, thrombospondin-1, thrombospondin-3, thrombospondin-5, and type IX collagen. *Am J Pathol* 2008;172:1664-74.
22. Dalla-Torre CA, Yoshimoto M, Lee CH, Joshua AM, de Toledo SR, et al. Effects of THBS3, SPARC and SPP1 expression on biological behavior and survival in patients with osteosarcoma. *BMC Cancer* 2006;6:237.
23. Cho JY, Lim JY, Cheong JH, Park YY, Yoon SL, et al. Gene expression signature-based prognostic risk score in gastric cancer. *Clin Cancer Res* 2011;17:1850-7.
24. D'Errico M, de Rinaldis E, Blasi MF, Viti V, Falchetti M, et al. Genome-wide expression profile of sporadic gastric cancers with microsatellite instability. *Eur J Cancer* 2009;45:461-9.
25. Singh D, Febbo PG, Ross K, Jackson DG, Manola J, et al. Gene expression correlates of clinical prostate cancer behavior. *Cancer Cell* 2002;1:203-9.
26. Ma XJ, Wang Z, Ryan PD, Isakoff SJ, Barmettler A, et al. A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. *Cancer Cell* 2004;5:607-16.
27. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 2012;486:346-52.
28. Turashvili G, Bouchal J, Baumforth K, Wei W, Dziechciarkova M, et al. Novel markers for differentiation of lobular and ductal invasive breast carcinomas by laser microdissection and microarray analysis. *BMC Cancer* 2007;7:55.
29. Förster S, Gretschel S, Jöns T, Yashiro M, Kemmner W. THBS4, a novel stromal molecule of diffuse-type gastric adenocarcinomas, identified by transcriptome-wide expression profiling. *Mod Pathol* 2011;24:1390-403.
30. Su F, Zhao J, Qin S, Wang R, Li Y, et al. Over-expression of thrombospondin 4 correlates with loss of miR-142 and contributes to migration and vascular invasion of advanced hepatocellular carcinoma. *Oncotarget* 2017;8:23277-88.
31. Myers T, Chenedza S, Lightfoot S, Pan Y, Dedmond D, et al. Flexible heteroarotinoid (Flex-Het) SHetA2 inhibits angiogenesis in vitro and in vivo. *Invest New Drugs* 2009;27:304-18.

32. Muppala S, Frolova E, Xiao R, Krukovets I, Yoon S, et al. Proangiogenic properties of thrombospondin-4. *Arterioscler Thromb Vasc Biol* 2015;35:1975-86.
33. Muppala S, Xiao R, Krukovets I, Verbovetsky D, Yendamuri R, et al. Thrombospondin-4 mediates TGF- β -induced angiogenesis. *Oncogene* 2017;36:5189-98.
34. Pluskota E, Stenina OI, Krukovets I, Szpak D, Topol EJ, et al. Mechanism and effect of thrombospondin-4 polymorphisms on neutrophil function. *Blood* 2005;106:3970-8.
35. Frolova EG, Pluskota E, Krukovets I, Burke T, Drumm C, et al. Thrombospondin-4 regulates vascular inflammation and atherogenesis. *Circ Res* 2010;107:1313-25.
36. Frolova EG, Sopko N, Blech L, Popovic ZB, Li J, et al. Thrombospondin-4 regulates fibrosis and remodeling of the myocardium in response to pressure overload. *FASEB J* 2012;26:2363-73.
37. Greco SA, Chia J, Inglis KJ, Cozzi SJ, Ramsnes I, et al. Thrombospondin-4 is a putative tumour-suppressor gene in colorectal cancer that exhibits age-related methylation. *BMC Cancer* 2010;10:494.
38. van Doorn R, Zoutman WH, Dijkman R, de Menezes RX, Commandeur S, et al. Epigenetic profiling of cutaneous T-cell lymphoma: promoter hypermethylation of multiple tumor suppressor genes including BCL7a, PTPRG, and p73. *J Clin Oncol* 2005;23:3886-96.
39. Adams JC, Monk R, Taylor AL, Ozbek S, Fascetti N, et al. Characterisation of drosophila thrombospondin defines an early origin of pentameric thrombospondins. *J Mol Biol* 2003;328:479-94.
40. Stenina-Adognravi O. Thrombospondins: old players, new games. *Curr Opin Lipidol* 2013;24:401-9.
41. Iruela-Arispe ML, Lombardo M, Krutzsch HC, Lawler J, Roberts DD. Inhibition of angiogenesis by thrombospondin-1 is mediated by 2 independent regions within the type 1 repeats. *Circulation* 1999;100:1423-31.
42. Lawler J. Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. *J Cell Mol Med* 2002;6:1-12.
43. Bein K, Simons M. Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. *J Biol Chem* 2000;275:32167-73.
44. Rodriguez-Manzanique JC, Lane TF, Ortega MA, Hynes RO, Lawler J, et al. Thrombospondin-1 suppresses spontaneous tumor growth and inhibits activation of matrix metalloproteinase-9 and mobilization of vascular endothelial growth factor. *Proc Natl Acad Sci U S A* 2001;98:12485-90.
45. Murphy-Ullrich JE, Schultz-Cherry S, Höök M. Transforming growth factor-beta complexes with thrombospondin. *Mol Biol Cell* 1992;3:181-8.
46. Schultz-Cherry S, Chen H, Mosher DF, Misenheimer TM, Krutzsch HC, et al. Regulation of transforming growth factor-beta activation by discrete sequences of thrombospondin 1. *J Biol Chem* 1995;270:7304-10.
47. Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, et al. Thrombospondin-1 is a major activator of TGF-beta1 in vivo. *Cell* 1998;93:1159-70.
48. Good DJ, Polverini PJ, Rastinejad F, Le Beau MM, Lemons RS, et al. A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. *Proc Natl Acad Sci U S A* 1990;87:6624-8.
49. Ichii T, Koyama H, Tanaka S, Shioi A, Okuno Y, Otani S, et al. Thrombospondin-1 mediates smooth muscle cell proliferation induced by interaction with human platelets. *Arterioscler Thromb Vasc Biol* 2002;22:1286-92.
50. Dawson DW, Pearce SF, Zhong R, Silverstein RL, Frazier WA, et al. CD36 mediates the in vitro inhibitory effects of thrombospondin-1 on endothelial cells. *J Cell Biol* 1997;138:707-17.
51. Guo N, Krutzsch HC, Inman JK, Roberts DD. Thrombospondin 1 and type I repeat peptides of thrombospondin 1 specifically induce apoptosis of endothelial cells. *Cancer Res* 1997;57:1735-42.
52. Vogel T, Guo NH, Krutzsch HC, Blake DA, Hartman J, et al. Modulation of endothelial cell proliferation, adhesion, and motility by recombinant heparin-binding domain and synthetic peptides from the type I repeats of thrombospondin. *J Cell Biochem* 1993;53:74-84.
53. Lawler J. The functions of thrombospondin-1 and -2. *Curr Opin Cell Biol* 2000;12:634-40.
54. Kazerounian S, Yee KO, Lawler J. Thrombospondins in cancer. *Cell Mol Life Sci* 2008;65:700-12.
55. Lawler J, Detmar M. Tumor progression: the effects of thrombospondin-1 and -2. *Int J Biochem Cell Biol* 2004;36:1038-45.
56. Gutierrez LS, Suckow M, Lawler J, Ploplis VA, Castellino FJ. Thrombospondin 1--a regulator of adenoma growth and carcinoma progression in the APC(Min/+) mouse model. *Carcinogenesis* 2003;24:199-207.
57. Almog N, Henke V, Flores L, Hlatky L, Kung AL, et al. Prolonged dormancy of human liposarcoma is associated with impaired tumor angiogenesis. *FASEB J* 2006;20:947-9.
58. Naumov GN, Bender E, Zurakowski D, Kang SY, Sampson D, et al. A model of human tumor dormancy: an angiogenic switch from the nonangiogenic phenotype. *J Natl Cancer Inst* 2006;98:316-25.
59. Giurati S, Ryeom S, Fan AC, Bachireddy P, Lynch RC, et al. Sustained regression of tumors upon MYC inactivation requires p53 or thrombospondin-1 to reverse the angiogenic switch. *Proc Natl Acad Sci U S A* 2006;103:16266-71.
60. Weinstat-Saslow DL, Zabrenetzky VS, VanHoutte K, Frazier WA, Roberts DD, et al. Transfection of thrombospondin 1 complementary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. *Cancer Res* 1994;54:6504-11.
61. Streit M, Riccardi L, Velasco P, Brown LF, Hawighorst T, et al. Thrombospondin-2: a potent endogenous inhibitor of tumor growth and angiogenesis. *Proc Natl Acad Sci U S A* 1999;96:14888-93.
62. Streit M, Velasco P, Brown LF, Skobe M, Richard L, et al. Overexpression of thrombospondin-1 decreases angiogenesis and inhibits the growth of human cutaneous squamous cell carcinomas. *Am J Pathol* 1999;155:441-52.
63. Sheibani N, Frazier WA. Thrombospondin 1 expression in transformed endothelial cells restores a normal phenotype and suppresses

- their tumorigenesis. *Proc Natl Acad Sci U S A* 1995;92:6788-92.
64. Resovi A, Pinessi D, Chiorino G, Taraboletti G. Current understanding of the thrombospondin-1 interactome. *Matrix Biol* 2014;37:83-91.
65. Lopez-Dee Z, Pidcock K, Gutierrez LS. Thrombospondin-1: multiple paths to inflammation. *Mediators Inflamm* 2011;2011:296069.
66. Kirsch T, Woywodt A, Klose J, Wyss K, Beese M, et al. Endothelial-derived thrombospondin-1 promotes macrophage recruitment and apoptotic cell clearance. *J Cell Mol Med* 2010;14:1922-34.
67. Tuszyński GP, Rothman V, Murphy A, Siegler K, Smith L, et al. Thrombospondin promotes cell-substratum adhesion. *Science* 1987;236:1570-3.
68. Chandrasekaran S, Guo NH, Rodrigues RG, Kaiser J, Roberts DD. Pro-adhesive and chemotactic activities of thrombospondin-1 for breast carcinoma cells are mediated by $\alpha\beta 1$ integrin and regulated by insulin-like growth factor-1 and CD98. *J Biol Chem* 1999;274:11408-16.
69. Albo D, Rothman VL, Roberts DD, Tuszyński GP. Tumour cell thrombospondin-1 regulates tumour cell adhesion and invasion through the urokinase plasminogen activator receptor. *Br J Cancer* 2000;83:298-306.
70. Yee KO, Connolly CM, Duquette M, Kazerounian S, Washington R, et al. The effect of thrombospondin-1 on breast cancer metastasis. *Breast Cancer Res Treat* 2009;114:85-96.
71. Pal SK, Nguyen CT, Morita KI, Miki Y, Kayamori K, et al. THBS1 is induced by TGF β 1 in the cancer stroma and promotes invasion of oral squamous cell carcinoma. *J Oral Pathol Med* 2016;45:730-9.
72. Horiguchi H, Yamagata S, Rong Qian Z, Kagawa S, Sakashita N. Thrombospondin-1 is highly expressed in desmoplastic components of invasive ductal carcinoma of the breast and associated with lymph node metastasis. *J Med Invest* 2013;60:91-6.
73. Borsotti P, Ghilardi C, Ostano P, Silini A, Dossi R, et al. Thrombospondin-1 is part of a slug-independent motility and metastatic program in cutaneous melanoma, in association with VEGFR-1 and FGF-2. *Pigment Cell Melanoma Res* 2015;28:73-81.
74. Filleur S, Volpert OV, Degeorges A, Volland C, Reiher F, et al. In vivo mechanisms by which tumors producing thrombospondin 1 bypass its inhibitory effects. *Genes Dev* 2001;15:1373-82.
75. Mirochnik Y, Kwiatek A, Volpert OV. Thrombospondin and apoptosis: molecular mechanisms and use for design of complementation treatments. *Curr Drug Targets* 2008;9:851-62.
76. Rejniak KA. Circulating tumor cells: when a solid tumor meets a fluid microenvironment. *Adv Exp Med Biol* 2016;936:93-106.
77. Houghton AN, Guevara-Patiño JA. Immune recognition of self in immunity against cancer. *J Clin Invest* 2004;114:468-71.
78. Disis ML. Immune regulation of cancer. *J Clin Oncol* 2010;28:4531-8.
79. Jung H, Hsiung B, Pestal K, Procyk E, Raulet DH. RAE-1 ligands for the NKG2D receptor are regulated by E2F transcription factors, which control cell cycle entry. *J Exp Med* 2012;209:2409-22.
80. Yamauchi M, Imajoh-Ohmi S, Shibuya M. Novel antiangiogenic pathway of thrombospondin-1 mediated by suppression of the cell cycle. *Cancer Sci* 2007;98:1491-7.
81. Dameron KM, Volpert OV, Tainsky MA, Bouck N. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* 1994;265:1582-4.
82. Wu Y, Zhou BP. Inflammation: a driving force speeds cancer metastasis. *Cell Cycle* 2009;8:3267-73.
83. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860-7.
84. Ryu TY, Park J, Scherer PE. Hyperglycemia as a risk factor for cancer progression. *Diabetes Metab J* 2014;38:330-6.
85. Pisani P. Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies. *Arch Physiol Biochem* 2008;114:63-70.
86. Joslin EP, Lombard HL, Burrows RE, Manning MD. Diabetes and cancer. *N Engl J Med* 1959;260:486-8.
87. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, et al. Diabetes and cancer: a consensus report. *CA Cancer J Clin* 2010;60:207-21.
88. Noto H, Tsujimoto T, Sasazuki T, Noda M. Significantly increased risk of cancer in patients with diabetes mellitus: a systematic review and meta-analysis. *Endocr Pract* 2011;17:616-28.
89. Barone BB, Yeh HC, Snyder CF, Peairs KS, Stein KB, et al. Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis. *JAMA* 2008;300:2754-64.
90. Larsson SC, Bergkvist L, Wolk A. Glycemic load, glycemic index and breast cancer risk in a prospective cohort of Swedish women. *Int J Cancer* 2009;125:153-7.
91. Dong JY, Qin LQ. Dietary glycemic index, glycemic load, and risk of breast cancer: meta-analysis of prospective cohort studies. *Breast Cancer Res Treat* 2016;156:287-94.
92. Sieri S, Pala V, Brighenti F, Agnoli C, Grioni S. High glycemic diet and breast cancer occurrence in the Italian EPIC cohort. *Nutr Metab Cardiovasc Dis* 2013;23:628-34.
93. Turati F, Galeone C, Gandini S, Augustin LS, Jenkins DJ, et al. High glycemic index and glycemic load are associated with moderately increased cancer risk. *Mol Nutr Food Res* 2015;59:1384-94.
94. Mullie P, Koechlin A, Boniol M, Autier P, Boyle P. Relation between breast cancer and high glycemic index or glycemic load: a meta-analysis of prospective cohort studies. *Crit Rev Food Sci Nutr* 2016;55:152-9.
95. Melkonian SC, Daniel CR, Ye Y, Pierzynski JA, Roth JA, et al. Glycemic index, glycemic load, and lung cancer risk in non-hispanic whites. *Cancer Epidemiol Biomarkers Prev* 2016;25:532-9.
96. Hu J, La Vecchia C, Augustin LS, Negri E, de Groh M, et al. Glycemic index, glycemic load and cancer risk. *Ann Oncol* 2013;24:245-51.
97. Hardin J, Cheng I, Witte JS. Impact of consumption of vegetable, fruit, grain, and high glycemic index foods on aggressive prostate

- cancer risk. *Nutr Cancer* 2011;63:860-72.
98. Nagle CM, Olsen CM, Ibiebele TI, Spurdle AB, Webb PM, et al. Glycemic index, glycemic load and endometrial cancer risk: results from the Australian national endometrial cancer study and an updated systematic review and meta-analysis. *Eur J Nutr* 2013;52:705-15.
 99. Larsson SC, Giovannucci EL, Wolk A. Prospective study of glycemic load, glycemic index, and carbohydrate intake in relation to risk of biliary tract cancer. *Am J Gastroenterol* 2016;111:891-6.
 100. Sieri S, Krogh V, Agnoli C, Ricceri F, Palli D, et al. Dietary glycemic index and glycemic load and risk of colorectal cancer: results from the EPIC-Italy study. *Int J Cancer* 2015;136:2923-31.
 101. Sieri S, Agnoli C, Pala V, Grioni S, Brighenti F, et al. Dietary glycemic index, glycemic load, and cancer risk: results from the EPIC-Italy study. *Sci Rep* 2017;7:9757.
 102. Abe H, Aida Y, Ishiguro H, Yoshizawa K, Miyazaki T, et al. Alcohol, postprandial plasma glucose, and prognosis of hepatocellular carcinoma. *World J Gastroenterol* 2013;19:78-85.
 103. Keum N, Yuan C, Nishihara R, Zoltick E, Hamada T, et al. Dietary glycemic and insulin scores and colorectal cancer survival by tumor molecular biomarkers. *Int J Cancer* 2017;140:2648-56.
 104. Dehghan M, Mente A, Zhang X, Swaminathan S, Li W, et al. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *Lancet* 2017;390:2050-62.
 105. Augustin LS, Kendall CW, Jenkins DJ, Willett WC, Astrup A, et al. Glycemic index, glycemic load and glycemic response: an international scientific consensus summit from the international carbohydrate quality consortium (ICQC). *Nutr Metab Cardiovasc Dis* 2015;25:795-815.
 106. Augustin LS, Libra M, Crispo A, Grimaldi M, De Laurentiis M, et al. Low glycemic index diet, exercise and vitamin D to reduce breast cancer recurrence (DEDiCa): design of a clinical trial. *BMC Cancer* 2017;17:69.
 107. Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology* 1995;21:650-5.
 108. De Vos Irvine H, Goldberg D, Hole DJ, McMenamin J. Trends in primary liver cancer. *Lancet* 1998;351:215-6.
 109. Kirkegård J, Mortensen FV, Cronin-Fenton D. Chronic pancreatitis and pancreatic cancer risk: a systematic review and meta-analysis. *Am J Gastroenterol* 2017;112:1366-72.
 110. Weedon DD, Shorter RG, Ilstrup DM, Huizenga KA, Taylor WF. Crohn's disease and cancer. *N Engl J Med* 1973;289:1099-103.
 111. Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, et al. Overexpression of interleukin-1 β induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* 2008;14:408-19.
 112. Correa P, Houghton J. Carcinogenesis of helicobacter pylori. *Gastroenterology* 2007;133:659-72.
 113. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;444:860-7.
 114. Lackey DE, Olefsky JM. Regulation of metabolism by the innate immune system. *Nat Rev Endocrinol* 2016;12:15-28.
 115. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* 2011;11:98-107.
 116. Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease. *Nat Rev Immunol* 2011;11:738-49.
 117. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor- α in human obesity and insulin resistance. *J Clin Invest* 1995;95:2409-15.
 118. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. *Front Endocrinol (Lausanne)* 2016;7:30.
 119. Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. *J Cell Biol* 2015;208:501-12.
 120. Montane J, Cadavez L, Novials A. Stress and the inflammatory process: a major cause of pancreatic cell death in type 2 diabetes. *Diabetes Metab Syndr Obes* 2014;7:25-34.
 121. Wang W, Guo Y, Liao Z, Zou DW, Jin ZD, et al. Occurrence of and risk factors for diabetes mellitus in Chinese patients with chronic pancreatitis. *Pancreas* 2011;40:206-12.
 122. Ewald N, Kaufmann C, Raspe A, Kloer HU, Bretzel RG, et al. Prevalence of diabetes mellitus secondary to pancreatic diseases (type 3c). *Diabetes Metab Res Rev* 2012;28:338-42.
 123. Bhattacharyya S, Marinic TE, Krukovets I, Hoppe G, Stenina OI. Cell type-specific post-transcriptional regulation of production of the potent antiangiogenic and proatherogenic protein thrombospondin-1 by high glucose. *J Biol Chem* 2008;283:5699-707.
 124. Bhattacharyya S, Sul K, Krukovets I, Nestor C, Li J, et al. Novel tissue-specific mechanism of regulation of angiogenesis and cancer growth in response to hyperglycemia. *J Am Heart Assoc* 2012;1:e005967.
 125. Krukovets I, Legerski M, Sul P, Stenina-Adognravi O. Inhibition of hyperglycemia-induced angiogenesis and breast cancer tumor growth by systemic injection of microRNA-467 antagonist. *FASEB J* 2015;29:3726-36.
 126. Jiménez B, Volpert OV, Crawford SE, Febbraio M, Silverstein RL, et al. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nat Med* 2000;6:41-8.
 127. Lawler JW, Slayter HS, Coligan JE. Isolation and characterization of a high molecular weight glycoprotein from human blood platelets. *J Biol Chem* 1978;253:8609-16.
 128. Liu P, Wang Y, Li YH, Yang C, Zhou YL, et al. Adenovirus-mediated gene therapy with an antiangiogenic fragment of thrombospondin-1 inhibits human leukemia xenograft growth in nude mice. *Leuk Res* 2003;27:701-8.
 129. Xu M, Kumar D, Stass SA, Mixson AJ. Gene therapy with p53 and a fragment of thrombospondin I inhibits human breast cancer in vivo. *Mol Genet Metab* 1998;63:103-9.
 130. Chan LY, Craik DJ, Daly NL. Cyclic thrombospondin-1 mimetics: grafting of a thrombospondin sequence into circular disulfide-rich frameworks to inhibit endothelial cell migration. *Biosci Rep* 2015; doi: 10.1042/BSR20150210.

131. Yap R, Veliceasa D, Emmenegger U, Kerbel RS, McKay LM, et al. Metronomic low-dose chemotherapy boosts CD95-dependent anti-angiogenic effect of the thrombospondin peptide ABT-510: a complementation antiangiogenic strategy. *Clin Cancer Res* 2005;11:6678-85.
132. Henkin J, Volpert OV. Therapies using anti-angiogenic peptide mimetics of thrombospondin-1. *Expert Opin Ther Targets* 2011;15:1369-86.
133. Haviv F, Bradley MF, Kalvin DM, Schneider AJ, Davidson DJ, et al. Thrombospondin-1 mimetic peptide inhibitors of angiogenesis and tumor growth: design, synthesis, and optimization of pharmacokinetics and biological activities. *J Med Chem* 2005;48:2838-46.
134. Huang H, Campbell SC, Bedford DF, Nelius T, Veliceasa D, et al. Peroxisome proliferator-activated receptor gamma ligands improve the antitumor efficacy of thrombospondin peptide ABT510. *Mol Cancer Res* 2004;2:541-50.
135. Punekar S, Zak S, Kalter VG, Dobransky L, Punekar I, et al. Thrombospondin 1 and its mimetic peptide ABT-510 decrease angiogenesis and inflammation in a murine model of inflammatory bowel disease. *Pathobiology* 2008;75:9-21.
136. Rogers NM, Sharifi-Sanjani M, Csányi G, Pagano PJ, Isenberg JS. Thrombospondin-1 and CD47 regulation of cardiac, pulmonary and vascular responses in health and disease. *Matrix Biol* 2014;37:92-101.
137. Jeanne A, Sick E, Devy J, Floquet N, Belloy N, et al. Identification of TAX2 peptide as a new unpredicted anti-cancer agent. *Oncotarget* 2015;6:17981-8000.
138. Jeanne A, Martiny L, Dedieu S. Thrombospondin-targeting TAX2 peptide impairs tumor growth in preclinical mouse models of childhood neuroblastoma. *Pediatr Res* 2017;81:480-8.
139. Jeanne A, Boulagnon-Rombi C, Devy J, Thérêt L, Fichel C, et al. Matricellular TSP-1 as a target of interest for impeding melanoma spreading: towards a therapeutic use for TAX2 peptide. *Clin Exp Metastasis* 2016;33:637-49.
140. Li G, Wu H, Cui L, Gao Y, Chen L, et al. CD47-retargeted oncolytic adenovirus armed with melanoma differentiation-associated gene-7/interleukin-24 suppresses in vivo leukemia cell growth. *Oncotarget* 2015;6:43496-507.
141. Maxhimer JB, Soto-Pantoja DR, Ridnour LA, Shih HB, Degraff WG, et al. Radioprotection in normal tissue and delayed tumor growth by blockade of CD47 signaling. *Sci Transl Med* 2009; doi: 10.1126/scitranslmed.3000139.
142. Soto-Pantoja DR, Miller TW, Pendrak ML, DeGraff WG, Sullivan C, et al. CD47 deficiency confers cell and tissue radioprotection by activation of autophagy. *Autophagy* 2012;8:1628-42.
143. Soto-Pantoja DR, Terabe M, Ghosh A, Ridnour LA, DeGraff WG, et al. CD47 in the tumor microenvironment limits cooperation between antitumor T-cell immunity and radiotherapy. *Cancer Res* 2014;74:6771-83.
144. Isenberg JS, Maxhimer JB, Hyodo F, Pendrak ML, Ridnour LA, et al. Thrombospondin-1 and CD47 limit cell and tissue survival of radiation injury. *Am J Pathol* 2008;173:1100-12.
145. Jeanne A, Schneider C, Martiny L, Dedieu S. Original insights on thrombospondin-1-related antireceptor strategies in cancer. *Front Pharmacol* 2015;6:252.
146. Krukovets I, Legerski M, Sul P, Stenina-Adognravi O. Inhibition of hyperglycemia-induced angiogenesis and breast cancer tumor growth by systemic injection of microRNA-467 antagonist. *FASEB J* 2015;29:3726-36.
147. Moura R, Tjwa M, Vandervoort P, Van Kerckhoven S, Holvoet P, et al. Thrombospondin-1 deficiency accelerates atherosclerotic plaque maturation in ApoE^{-/-} mice. *Circ Res* 2008;103:1181-9.
148. Xing T, Wang Y, Ding WJ, Li YL, Hu XD, et al. Thrombospondin-1 production regulates the inflammatory cytokine secretion in THP-1 cells through NF-κB signaling pathway. *Inflammation* 2017;40:1606-21.
149. Stein EV, Miller TW, Ivins-O'Keefe K, Kaur S, Roberts DD. Secreted thrombospondin-1 regulates macrophage interleukin-1β production and activation through CD47. *Sci Rep* 2016;6:19684.
150. Zhao Y, Xion, Z, Lechner EJ, Klenotic PA, Hamburg BJ, et al. Thrombospondin-1 triggers macrophage IL-10 production and promotes resolution of experimental lung injury. *Mucosal Immunol* 2013;7:440-8.
151. Csányi G, Feck DM, Ghoshal P, Singla B, Lin H, et al. CD47 and Nox1 mediate dynamic fluid-phase macropinocytosis of native LDL. *Antioxid Redox Signal* 2017;26:886-901.
152. Liu Z, Morgan S, Ren J, Wang Q, Annis DS, et al. Thrombospondin-1 (TSP1) contributes to the development of vascular inflammation by regulating monocytic cell motility in mouse models of abdominal aortic aneurysm. *Circ Res* 2015;117:129-41.
153. Li Y, Qi X, Tong X, Wang S. Thrombospondin 1 activates the macrophage toll-like receptor 4 pathway. *Cell Mol Immunol* 2013;10:506-12.
154. Peter MR, Jerkic M, Sotov V, Douda DN, Ardelean DS, et al. Impaired resolution of inflammation in the endoglin heterozygous mouse model of chronic colitis. *Mediators Inflamm* 2014; doi: 10.1155/2014/767185.
155. Wu L, Derynck R. Essential role of TGF-beta signaling in glucose-induced cell hypertrophy. *Dev Cell* 2009;17:35-48.
156. Wheeler SE, Lee NY. Emerging roles of transforming growth factor β signaling in diabetic retinopathy. *J Cell Physiol* 2017;232:486-9.
157. Melzer C, Hass R, von der Ohe J, Lehnert H, Ungefroren H. The role of TGF-β and its crosstalk with RAC1/RAC1b signaling in breast and pancreas carcinoma. *Cell Commun Signal* 2017;15:19.
158. Wintrob ZA, Hammel JP, Nimako GK, Gaile DP, Forrest A, et al. Dataset on growth factor levels and insulin use in patients with diabetes mellitus and incident breast cancer. *Data Brief* 2017;11:183-91.
159. Park SY, Kim MJ, Park SA, Kim JS, Min KN, et al. Combinatorial TGF-β attenuation with paclitaxel inhibits the epithelial-to-mesenchymal transition and breast cancer stem-like cells. *Oncotarget* 2015;6:37526-43.
160. Kim D, Lee AS, Jung YJ, Yang KH, Lee S, et al. Tamoxifen ameliorates renal tubulointerstitial fibrosis by modulation of estrogen receptor α-mediated transforming growth factor-β1/Smad signaling pathway. *Nephrol Dial Transplant* 2014;29:2043-53.
161. Park CY, Min KN, Son JY, Park SY, Nam JS, et al. An novel inhibitor of TGF-β type I receptor, IN-1130, blocks breast cancer lung me-

- tastasis through inhibition of epithelial-mesenchymal transition. *Cancer Lett* 2014;351:72-80.
162. Muppala S, Frolova E, Xiao R, Krukovets I, Yoon S, et al. Proangiogenic properties of thrombospondin-4. *Arterioscler Thromb Vasc Biol* 2015;35:1975-86.
163. Muppala S, Xiao R, Krukovets I, Verbovetsky D, Yendamuri R, et al. Thrombospondin-4 mediates TGF- β -induced angiogenesis. *Oncogene* 2017;36:5189-98.
164. Murphy-Ullrich JE, Poczatek M. Activation of latent TGF-beta by thrombospondin-1: mechanisms and physiology. *Cytokine Growth Factor Rev* 2000;11:59-69.
165. Frolova EG, Drazba J, Krukovets I, Kostenko V, Blech L, et al. Control of organization and function of muscle and tendon by thrombospondin-4. *Matrix Biol* 2014;37:35-48.

Meeting Abstracts

Open Access



Selected meeting abstracts of 2018 healthcare and cardiology conference

Bangkok, Thailand; September 18-19, 2018; Published: 16 Oct 2018

Correspondence to: Dr. Ahmed Ahmed Fouad Abdelwahab Ahmed, Consultant Cardiac Surgeon, Ain Shams University, 11566 Cairo, Egypt. E-mail: afouad38@yahoo.com

1. Evaluation of the role of ischemia reversal therapy in ischemic heart disease using SPECT myocardial perfusion imaging: a pilot study

Rohit Sane¹, Rahul Mandole²

¹Founder, Madhavbaug Cardiac Care Clinics and Hospital, Mumbai 400603, India.

²Department of R&D, Madhavbaug Cardiac Care Clinics and Hospital, Mumbai 400603, India.

Ischemic heart disease (IHD) incidence has increased in India. Ayurveda, a 3000-year-old Indian traditional medicine system along with allopathic medicine can provide a solution to improve myocardial perfusion in stable IHD patients. This pilot study involves ischemia reversal programme (IRP), an Ayurvedic treatment modality to aid IHD patients using SPECT-myocardial perfusion imaging (SPECT-MPI) for assessment of myocardial perfusion in IHD patients. The present open-label study involved fourteen IHD patients who underwent IRP (21 sittings in total, administered twice per week) in Madhavbaug clinics along with their standard care therapy. The inclusion criteria were patients with known IHD, age = 40-70 years, BMI > 20 kg/m², and stress test positive for inducible ischemia. SPECT-MPI was performed at enrolment and post-IRP (12-week follow-up) from December 2016 to September 2017. VO₂max and time to onset of ischemia after stress test were also recorded. Seattle angina questionnaire (SAQ) was telephonically completed by research coordinators. Observations from SPECT-MPI test showed a significant difference in summed stress score (13.5 ± 10.3 , baseline vs. 10.7 ± 10.1 , post-IRP; $P = 0.01$) as well as summed difference score (8.9 ± 6.2 , baseline vs. 6.2 ± 6.3 , post-IRP; $P = 0.03$) compared from baseline to post-IRP sittings. And we also observed the increase in VO₂max levels (12.8 ± 5.7 to 19.4 ± 7.8) and the time to onset of ischemia (370.7 ± 201.1 to 597.8 ± 201.9) was observed. SAQ scores showed significant improvement post-IRP (30.2 ± 3.6 to 32.7 ± 3.5). Findings of this study suggest an improvement in myocardial perfusion post-IRP in IHD patients and depict the positive role of IRP as an add-on to standard care therapy in IHD management.



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



2. Tachycardiomyopathy (a diagnosis not to be missed)

Gautam Singal

Interventional Cardiologist, Holy Family Hospital, Okhla, New Delhi 110025, India.

Cardiomyopathy is a myocardial disorder in which the heart muscle is structurally and functionally abnormal in the absence of coronary artery disease, hypertension, valvular disease, and congenital heart disease. Tachycardiomyopathy (TCMP) or arrhythmia induced cardiomyopathy is one such sub category and if adequately treated leads to improvement in LV function. It is defined as “Atrial and/or ventricular dysfunction - secondary to rapid and/or asynchronous/irregular myocardial contraction, partially or completely reversed after treatment of the causative arrhythmia”. Arrhythmia may be the sole reason for LV dysfunction or is the reason for exacerbation in a patient with concomitant heart disease. In our case a 45-year-old lady (post MVR 1994) presented in emergency with sudden worsening dyspnea. At the time of admission she was in acute LVF with her ECG showing atrial fibrillation with fast ventricular rate. Left ventricular ejection fraction on echocardiogram was 35% with good prosthetic valve function. She underwent coronary angiogram, which showed normal coronaries with fluoroscopy showing good prosthetic valve function after stabilization and other precipitating causes were ruled out. She was cardioverted to normal sinus rhythm and was discharged from the hospital on guideline directed medical therapy. She is on regular follow-up and has remained asymptomatic and arrhythmia-free and her LV function has improved to 55%. The incidence and prevalence of TCMP are uncertain. Atrial fibrillation (AF) is present in 10% to 50% of patients with HF; many patients with cardiomyopathy and AF have worsening symptoms and LV function solely due to poorly controlled ventricular rates. The mechanisms of TCMP are not fully defined but include subclinical ischemia, abnormalities in energy metabolism, redox stress and calcium overload. The possibility of TCMP should be considered when eliciting a history of any new diagnosis of LV dysfunction, if there is evidence of persistent or frequently occurring tachycardia and its timely diagnosis is important given the potential for near-complete recovery with appropriate treatment.

3. Comparison of outcomes of thrombolysis vs. re-preoperation for stuck prosthetic valve in mitral position - 10 year experience

Karthik Raman, Anbarasu Mohanraj, Ravi Agarwal, Ejaz Ahmed Sheriff, Kurian Valikapathalil Mathew Kurian, Rajan Sethuratnam

Department of CTVS, Madras Medical Mission, Chennai, Tamil Nadu 600037, India.

Aim: To analyse the outcomes of thrombolysis vs. re-preoperations for stuck prosthetic valves in mitral position.

Methods: From the time period of January 2005 till December 2015, a total of 36 patients had undergone thrombolysis and 31 patients had undergone re-preoperations for stuck prosthetic valves in mitral position in our institution. The follow up period was 225 patient-years in thrombolysis group and 208 patient-years in re-operative group. The prosthetic sizes were 25, 27, 29, 31 in mitral position. Mean functional class was 2.6 ± 0.8 in thrombolysis and 3.4 ± 0.9 in re-operation group. The peak and mean gradient was 37.5 ± 4.8 , 18.2 ± 3.56 in thrombolysis and 40 ± 2.7 , 20 ± 4.3 in re-operation group respectively. Transthoracic and transoesophageal echocardiographs along with fluoroscopy were done for all the patients. The causes of obstruction were pannus formation in 7, generation of thrombus in 50, and both pannus and thrombus in 10. Presence of pannus was an indication for surgery.

Results: The analysis of thrombolytic group is as follows: incidence of death was 2% per patient-year, freedom from Peripheral embolism $97.3\% \pm 1.8\%$, freedom from CNS bleeding $98.2\% \pm 3.8\%$, freedom from stroke $97.2\% \pm 2.6\%$, freedom from TIA $98.1\% \pm 2.8\%$, freedom from Coronary embolism 100%, freedom from major bleeding with transfusion $96.3\% \pm 4.8\%$, freedom from thrombolytic failure $95.4\% \pm 3.7\%$. The peak and mean gradient was 11.5 ± 4.8 , 5.2 ± 3.56 at the end of completed thrombolysis. The analysis of re-operative group are as follows: incidence of death was 3% per patient-year, freedom from Peripheral embolism $98.3\% \pm 4.8\%$, freedom from CNS bleeding $99.2\% \pm 2.8\%$, freedom from stroke $95.2\% \pm 3.5\%$, freedom from TIA 100%, freedom from Coronary embolism 100%, freedom from major bleeding with transfusion $94.3\% \pm 5.4\%$. The peak and mean gradient was 9.5 ± 4.8 , 4.2 ± 3.56 at the end of re-operation on the 7th post-op day, 10.5 ± 4.8 , 4.2 ± 1.56 at the end of first year and 11.5 ± 4.8 , 5.2 ± 3.75 , at the end of third year respectively.

Conclusion: Re-preoperation and thrombolysis are the widely accepted options for treatment of mechanical heart valve thrombosis and both seem to be equally effective. The percentage of embolic events and recurrent thrombosis are higher in thrombolysis group while we had almost comparable mortality in both the groups. Longer follow-up with a large group of patients is necessary for further results.

4. Outcome of coronary artery bypass grafting surgery in patients with low ejection fraction

Mohammed Aslam Hossain, Mayank Acharya, Dharmendra Joshi, Niraj Bhattarai, Satish Vaidya, Karan Rai, Sanaul Sarker, Samir Azam Sunny

Department of Cardiac Surgery, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka 1000, Bangladesh.

Aim: To analyze the outcome of coronary artery bypass grafting surgery in patients with low ejection fraction. Coronary artery bypass surgery is one of the most frequently performed among various surgeries. In recent years the mortality and morbidity related to the procedure has decreased even in the developing world. However a significant peri surgical morbimortality burden remains among patients with heart failure patients. The factors of this may be due to problems in wound healing, pulmonary complications, post perfusion syndrome, poor tissue oxygenation, acute liver and kidney injuries, prolonged ventilation, frequent cerebrovascular events, presence of other comorbid conditions, all of which affect the patient more than compared to a similar patient with moderate or normal preoperative ejection fraction.

Methods: Data were obtained and analyzed from 102 patients who underwent coronary artery bypass grafting surgery having a preoperative left ventricular ejection fraction $\leq 40\%$. A subgroup of patients with EF $\leq 30\%$ ($n = 28$) were also analyzed separately.

Results: The mean age of our patient was 57.9 ± 7.5 years of which 85 (83.3%) were males and 17 (16.7%) were females. 74 (72.5%) patients had LVEF 31-40 and 28 (27.5%) had ≤ 30 . Comparison of pre-operative LVEF and post-operative LVEF at 1 and 3 months revealed mean LVEF 33.15 against 37.63 ($P \leq 0.001$) at 1 month and 33.09 against 38.04 ($P \leq 0.001$) at 3 months. Other per- and post-operative outcome variables were also analyzed like arrhythmia, inotrope support time, bypass requirement, pulmonary complications, mechanical ventilation time, death and other variables.

Conclusion: Operations on patients with low ejection fraction warrant additional vigilance, albeit with proper precaution the outcome is favorable.

5. Rare case of multiple right atrial myxomata with acute pulmonary thromboembolism: a case report

Parin Chandrakant Sangoi, Ramkumar Rajaram, Vignesh Gomathinayagam, Ajit Mullasari

Department of Cardiology, Madras Medical Mission, Chennai 600037, India.

This is a case report of a 56-year-old morbidly obese female who was presented to our emergency room with complaints of acute onset progressive breathlessness since 3 days (NYHA class IV) with presyncope and palpitation but no history of chest pain. The patient was diagnosed with pulmonary thromboembolism with IVC - RA thrombus 4 years back. During examination she was found to be morbidly obese, with presence of skin tags and naevi in interscapular and left zygoma region. Her vitals were: HR 118/min, BP 100/70 mmHg and SPO₂ 85% in room air. Cardiopulmonary examination revealed good heart sounds with basal crepts. ECG showed Sinus tachycardia (+). Echo revealed a pedunculated right atrial (RA) mass attached to the lower intra atrial septum of size 2.9 cm × 1.5 cm protruding through the tricuspid valve in diastole. Another mass of size 1 cm × 2 cm was seen in Right Atrium with pedicle attached near IVC-RA junction. The patient also had severe Tricuspid Regurgitation with pulmonary hypertension. Further investigations revealed an elevated D - Dimer and CT PA confirmed RA mass with acute pulmonary embolism involving both the lungs. The patient was treated with low molecular weight heparin and follow-up echo showed decrease in Pulmonary artery pressure with improvement in her symptoms. The presence of morbid obesity, cushin-goid habitus, cutaneous skin tag in the left interscapular area, cutaneous neavi, multiple atrial myomata and pulmonary embolism together steered us towards the possible diagnosis of Carney's Complex. This case has been presented because the right atrial mass produces a diagnostic dilemma with differential diagnosis being thrombus (type A&B), primary tumor of heart commonly myxomas, lipomas, sarcomas and metastatic tumours. Hence it is important to differentiate the masses because treatment options and prognosis of each lesion vary.

6. Lectin-like oxidized-LDL receptor as the anti-atherosclerosis vaccine candidate

Valentina Yurina

Brawijaya University, Malang 65145, Indonesia.

Cardiovascular disease remains the most burdening health problems worldwide. The disease accounts for 31.43% mortality globally. Some of the major risk factors for the disease are hypertension (33%), hypercholesterolemia (19%), overweight (56%), and current smoking (19%). Hypercholesterolemia indicated by the elevated low-density lipoprotein (LDL) cholesterol and its oxidized products, which unlike the LDL cholesterol, is unrecognizable by its receptor. The oxidized LDL cholesterol (ox-LDL) is taken up by the scavenger receptors in the endothelial cells and macrophages. Lectin-like ox-LDL receptor-1 (LOX-1) remains as the major ox-LDL scavenger receptor in the endothelial cells. The LOX-1 participates in the early atherosclerosis phase by inducing the endothelial dysfunction. However, in the late stage of atherosclerosis, LOX-1 also plays a role in the smooth muscle cells apoptosis and foam cells formation. The LOX-1 expression is relatively low in the basal level, hence its expression is enhanced by several clinical conditions, such as hypertension, diabetes, obesity, atherosclerosis, and myocardial infarction. Several approaches demonstrated that the inhibition of LOX-1 reduces the endothelial dysfunction and atherosclerosis development. These studies indicated that LOX-1 inhibition is a promising candidate for atherosclerosis prevention. Our study demonstrated LOX-1 efficacy as the atherosclerosis vaccine candidate through DNA vaccination and subunit vaccination approach.

Correction

Open Access



Correction: Energetic metabolism in cardiomyocytes: molecular basis of heart ischemia and arrhythmogenesis

María Sofía Martínez¹, Andrés García¹, Eliana Luzardo¹, Mervin Chávez-Castillo¹, Luis Carlos Olivar¹, Juan Salazar¹, Manuel Velasco², Joselyn Joanna Rojas Quintero^{1,3}, Valmore Bermúdez^{1,4}

¹Endocrine and Metabolic Diseases Research Center, School of Medicine, University of Zulia, Maracaibo 4001, Venezuela.

²Department of Pharmacology, "JM Vargas" Medical School, Central University of Venezuela, Caracas 1051, Venezuela.

³Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA.

⁴Advanced Frontier Studies Research Group (ALEF), Simón Bolívar University, Cúcuta 54003, Colombia.

Correspondence to: Dr. María Sofía Martínez, Endocrine and Metabolic Diseases Research Center, School of Medicine, University of Zulia, Maracaibo 4001, Venezuela. E-mail: mmartinez@fmed.luz.edu.ve

How to cite this article: Martínez MS, García A, Luzardo E, Chávez-Castillo M, Olivar LC, Salazar J, Velasco M, Quintero JJR, Bermúdez V. *Vessel Plus* 2018;2:32. <http://dx.doi.org/10.20517/2574-1209.2018.68>

Received: 26 Sep 2018 **First Decision:** 26 Sep 2018 **Revised:** 11 Oct 2018 **Accepted:** 11 Oct 2018 **Published:** 23 Oct 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Cui Yu **Production Editor:** Huan-Liang Wu

The [original article](#) was published on 28 Dec 2017.

After the publication of the article named “Energetic metabolism in cardiomyocytes: molecular basis of heart ischemia and arrhythmogenesis”^[1], we found that the article by Stanley *et al.*^[2] was unwisely omitted from the fourth paragraph of the section titled “Fuel for myocardial contraction: the role of macromolecules”. This reference should be cited in the first, third and fourth sentences of this section, namely “The metabolic machinery of the heart utilizes oxygen up to 80%-90% of the maximum capacity of the electron transport chain; however, at a resting state, the heart operates at only 15%-25% of its maximum oxidative capacity”, “Cardiomyocytes show an elevated rate of ATP hydrolysis, which is strongly linked to oxidative phosphorylation. Because under non-ischemic conditions, over 95% of these cells’ ATP is produced in this process, it is indispensable in order to assure the full replenishment of the cardiomyocytes’ ATP content every 10 s, and thus maintain constant concentrations of this molecule, even under conditions of increased frequency or force of contractions” and “Of the total energy produced by ATP hydrolysis, approximately 60%-70% serves as fuel for contraction, while the remaining 30%-40% is used by the Ca²⁺ ATPase pumps in the smooth sarcoplasmic reticulum and other ion pumps”.

REFERENCES

1. Martínez MS, García A, Luzardo E, Chávez-Castillo M, Olivar LC, Salazar J, Velasco M, Quintero JJR, Bermúdez V. Energetic metabolism in cardiomyocytes: molecular basis of heart ischemia and arrhythmogenesis. *Vessel Plus* 2017;1:230-41.
2. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 2005;85:1093-129.



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Review

Open Access



Heart transplantation: a history lesson of Lazarus

Sanjeet Singh Avtaar Singh^{1,3}, Nicholas Banner², Colin Berry³, Nawwar Al-Attar¹

¹Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK.

²Heart Failure and Mechanical Circulatory Support, Harefield Hospital, Harefield UB9 6JH, UK.

³Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow G12 8QQ, UK.

Correspondence to: Sanjeet Singh Avtaar Singh, Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Glasgow G81 4DY, UK. E-mail: sanjeetsingh@nhs.net

How to cite this article: Singh SSA, Banner N, Berry C, Al-Attar N. Heart transplantation: a history lesson of Lazarus. *Vessel Plus* 2018;2:33. <http://dx.doi.org/10.20517/2574-1209.2018.28>

Received: 7 May 2018 **First Decision:** 25 Sep 2018 **Revised:** 26 Sep 2018 **Accepted:** 26 Sep 2018 **Published:** 24 Oct 2018

Science Editors: Mario F. L. Gaudino, Cristiano Spadaccio **Copy Editor:** Cai-Hong Wang **Production Editor:** Zhong-Yu Guo

Abstract

One of the notable advances in modern day medicine is organ transplantation. None more so than the heart. A complex interaction between physiology, surgery and immunology that spanned decades, involving the hard work of many pioneers in their fields. We revisit the contributions of the pioneers as well as marvel at the paradigm shifts in medicine that have made heart transplantation safe and reproducible with in excess of 3000 transplants done yearly today.

Keywords: Heart transplantation, history, immunosuppression

ORGAN TRANSPLANTATION AND ANCIENT HISTORY

Organ transplantation is arguably one of the greatest feats of modern medicine of the past century. Initially stemming from historical experimentation, it has become a mainstay of treatment for many chronic conditions and continues to do so in spite of improvements in device technology. Organ donation however underwent several challenges initially with cultural acceptance, ethics and legality, and political pressure. It has since evolved with the merging of improvements in the donation-allocation-procurement process, advances in technology, refinement of surgical technique, scientific breakthroughs in organ preservation, cognitive and methodical improvements in immunology and immunosuppression alongside expertise in managing adherent complications of organ transplantation.

In ancient civilisations, the practice of removal of organ/tissues for a multitude of reasons (beautification or therapeutic) was initiated. Hindu texts from 3 millennia ago provide detailed accounts of skin grafting from



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



fatty regions (buttocks) or protrusions (chin) for reconstruction of mutilated noses incurred during wars or punishments^[1].

One of the earliest records of organ transplantation, Bian Que, a reported clairvoyant during Han Dynasty in Ancient China reportedly performed an exchange of hearts. He felt that the attainment of balance was possible by exchanging organs between men of “strong will” but “weak spirit” with that of one with opposite traits by intoxicating a “patient” with fortified wine prior to “cutting their breasts removing their hearts and applying numinous medicine”^[2].

The New Testament describes several cases of auto-transplantation by today’s definition; Jesus of Nazareth reattached the ear of a servant after it had been cut by Simon Peter’s sword. It also describes how Saint Mark re-implanted an amputated hand of a soldier^[3]. Archaeological records have revealed that in the Bronze age, the term “trephination” was first revealed whereby bone segments were temporarily removed to decompress brain swelling^[4].

Jacopoda Varagine (348 AD) described the “miracle of the black leg” where a gangrenous leg of Justinian (Roman deacon) was replaced with that of a dead Ethiopian man^[5].

In 1688, Job van Meeneren successfully grafted a segment of bone from the skull of a dog to a defect in a human patient’s cranium^[6]. A Russian aristocrat had a fragment of canine skull tissue inserted during a repair after an injury. He had it explanted due to threats of excommunication from the church^[6]. Such accounts of events highlighted the initial inquisitiveness with the concept of transplantation.

THE PRE-TRANSPLANT ERA

Although organ transplantation had not taken place yet, the early 20th century witnessed the first skin and corneal transplants. The initial work behind corneal transplant is attributed to Franz Reisinger who experimented with “keratoplasty” in 1818^[7]. Twenty years later, Samuel Bigger performed the first successful corneal transplant in a gazelle. The first attempted corneal xenotransplantation on a human was performed in 1838 was unsuccessful. Improvements in antisepsis, anaesthesiology and surgical technique played a pivotal role, alongside ongoing animal experimentation. This subsequently led to the first successful human corneal transplant in 1905 by a Eduard Zirm (1887-1948) in Olmutz near Prague^[7]. The first successfully grafted tissue however was performed by Jacques-Louis Reverdin, who transplanted small detached skin grafts onto a wound and noted hastened granulating of wounds on 8th December 1869^[8]. Solid organ transplantation would follow a similar path with years of experimentation, before successful results were noted.

French president Marie François Sadi Carnot died from severed portal vein in 1894. This had a profound effect on a young surgeon, Alexis Carrel^[9]. He mastered vascular anastomotic suturing methods and introduced smaller needles. Carrel coated his needles, instruments and thread with petroleum jelly to reduce the thrombogenicity of the foreign material. He also perfected the concept of eversion thereby allowing blood within the vessels continuous endothelial contact. He also revolutionised antisepsis in surgery and pioneered methods of extracorporeal tissue preservation, by using salt solution at freezing point^[10].

In 1902, he successfully performed the first heterotopic kidney transplant by inserting a dog’s kidney into its own neck. He noted that the kidney began producing urine immediately^[9]. He later successfully transplanted organs, including kidneys, ovaries and thyroid glands between different dogs. In 1912, he became the first surgeon to win a Nobel Prize “in recognition of his work on vascular sutures and the transplantation of blood vessels and organs”^[11].

To prevent blood clotting Carrel coated his needles, instruments and thread with paraffin jelly and he used an everting technique, rolling back the cut vessel ends like cuffs and then stitching the turned-back ends

together to ensure that the blood would keep contact with the smooth inside of the vessel (endothelium). This and the use of strict asepsis to avoid infection allowed him to develop the techniques further by moving hearts, kidneys and spleens during experiments in dogs and also allowed other groups to begin experimentation in animal models of transplantation. Carrell famously noted that despite success in the technical aspects of transplantation, there were consistent hostile host responses to the foreign allografts especially during xenotransplantation^[12].

“Should an organ, extirpated from an animal and replanted into its owner by a certain technique, continue to functionate normally, and should it cease to functionate normally when transplanted into another animal by the same technique, the physiologic disturbance could not be considered as brought about by the organ but would be due to the influence of the host, that is, the biological factors”.

Despite Carrell's observations, between 1905-1910, several surgical peers such as M Princeteau, Mathieu Jaboulay and Ernst Unger in this era attempted xenotransplantation of rabbit, pig and macaque kidneys to humans with disastrous results^[13].

PRE-IMMUNOSUPPRESSION ERA

Leo Loeb first noted that the strength and timing of rejection in skin homografts on rodents was potentially caused by genetic disparity between donor and recipient and highlighted the involvement of lymphocytes in the 1930s^[14]. He theorised that this genetic disparity did not occur in identical twins thus they would accept exchanged skin grafts. Unfortunately, his findings were ridiculed due to his inbreeding of mice. Contemporaries such as Peter Medawar dismissed the importance of lymphocytes and adopted the humoral theory of rejection^[14]. The ensuing two decades were fraught with failed attempts of kidney transplantation in both human and animal models by Voronoy (1937), Simonsen (1953) and Dempster (1953) who even used radiation in organ transplant recipients^[15]. Medawar's renewed interest in transplant rejection brought him to the Burns Unit at Glasgow Royal Infirmary (Gibson and Medawar, 1943) with Thomas Gibson. He remained convinced that skin grafts in burn victims failed because of humoral rather than cellular immunity^[16]. His work with Rupert Billingham and Hugh Donald revealed that even fraternal twin cows accepted skin grafts, not just identical twin cows^[17]. Across the Atlantic, Ray Owen at the University of Wisconsin noted a hybrid of blood cell types in fraternal twins. He concluded that there was persistence of chimerism from the intrauterine transfer of stem cells which was probably responsible for this^[14]. Medawar, Billingham and Leslie Brent induced chimerism and homograft acceptance in mice by injecting inoculating intrauterine fetuses with donor strain spleen cell^[18]. This was ultimately successful and resulted in a Nobel Prize in 1966 for Peter Medawar. They later discovered that some of the immunocompetent cells from the splenic tissues “attacked” the lymphoid tissue of the host (Graft-Versus-Host-Disease), thereby proving the role of cellular immunity as first theorised by Loeb^[14].

Meanwhile, Joseph Murray and his team performing the first successful kidney transplant in 1954 using as a donor the recipient's identical twin bypassing the issues with immunity^[19]. This generated a lot of interest in the field of transplantation. Joan Main and Richmond Prehn attempted to recreate Medawar's stem cell inoculation. They radiated mice to allow induction of bone marrow from a donor. Murray's team used this method with poor outcomes as 11 of the 12 patients who underwent kidney transplantation with total body irradiation died within a month^[14]. The survivor maintained adequate function of his fraternal twin's kidney for 20 years thereby becoming the first successful non-identical twin kidney transplantation. Jean Hamburger and René Küss from Paris performed 4 successful transplants using total body irradiation without marrow inoculation^[15].

EARLY IMMUNOSUPPRESSION

Robert Schwartz and William Dameshek discovered that 6-mercaptopurine (6-MP), which was primarily used for treatment of malignancies, also reduced the antibody response of rabbits to bovine albumin^[14]. Roy Calne used 6-MP on canine kidney homografts and noted that it significantly prolonged survival^[20]. His findings however were not replicated when 3 kidney transplant recipients treated with 6-MP died. Calne began a research fellowship with Joseph Murray and despite the trend of total body irradiation, pursued work with 6-MP and later azathioprine^[14].

In 1963, at a National Research Council conference in Washington, the preliminary results of total body irradiation versus immunosuppressive drugs had reached equipoise with few patients surviving beyond 1 year. The practice of transplantation was questioned due to its poor long-term survival. Every represented centre demonstrated poor survival bar one. Thomas Starzl, combining azathioprine with prednisone achieved > 70% survival at 1-year follow up^[21]. He noted that large doses of prednisone could reverse early rejection that occurred and this could then be tapered down. This led to the formation of 50 new transplant centres in the United States alone that year^[16] and remained the mainstay of immunosuppression for the next 20 years. Immunosuppression also brought a new pathology, opportunistic infections and malignancy. Starzl himself noted that there were a high rate of bacterial, viral, fungal and protozoal infections found in post-mortem examination^[22].

Antilymphocyte serum (ALS) was first discovered by Elie Metchnikoff in 1899. In 1961, Byron Waksman identified that lymphocytic depletion could suppress delayed hypersensitivity reactions^[23]. Combining the two concepts, Michael Woodruff demonstrated that ALS administration alongside thoracic duct drainage via a fistula extended skin allograft survival in rodents, a finding later replicated by Medawar^[24,25]. In 1966, Polyclonal antilymphocyte globulin (ALG) was successfully synthesized from human leukocyte inoculated horses and became the staple of a triple regimen alongside steroids and azathioprine^[26].

HISTORY OF CARDIAC SURGERY AND TRANSPLANTATION

Unlike its other surgical counterparts, cardiac surgery was a relatively unknown subspecialty in the early 20th century. In 1881 at the Vienna Medical Society, Theodore Billroth once proclaimed.

“No surgeon who wished to preserve the respect of his colleagues would ever attempt to suture a wound of the heart”^[27].

The first cardiac procedure of the modern era was performed by Henry C. Dalton in St. Louis to repair a pericardial wound in a victim of a stabbing^[28]. In 1923, Elliot Carr Cutler and Samuel A. Levine successfully relieved a stenotic mitral valve in a 12-year-old girl. F. John Lewis, performed the first successful repair of an atrial septal defect in 1952 using hypothermia to protect the myocardium^[29]. C. Walton Lillehei performed 45 open heart surgeries utilizing a technique called controlled cross-circulation using parents of the children as “pump oxygenators”^[30].

The introduction of the cardiopulmonary bypass circuit revolutionised cardiac surgery. John Gibbon perfected the device in 1953 and subsequently performed the successfully performed an atrial septal defect closure^[31]. John Kirklin modified the pump and achieved relative success in small series of patients at the Mayo Clinic^[32]. However it was Richard DeWall’s cardiopulmonary bypass device with a disposable bubble oxygenator and simple pump action that enabled the correction of cardiac conditions under direct vision^[33].

The ensuing period saw numerous attempts to correct myocardial ischaemia until the Robert Hans Goetz successfully grafted the right internal mammary artery to the right coronary artery, thereby performing the first coronary artery bypass graft in 1960, much to the chagrin of the medical and surgical fraternity at the time^[34].

Inspired by the work of Carrel and Loeb, Frank C Mann identified 2 techniques for heterotopic cardiac transplantation^[35]. In his experimental model, he described using either a distal or proximal end of a divided carotid artery to supply blood to the aorta and assist circulation. The coronary sinus blood returned to the right atrium with both the vena cavae closed off and drained into the right ventricle. The pulmonary artery was anastomosed to the jugular vein. They noted that the pulse generated by the heart gradually faded with the longest lasting heart failing after 8 days.

Vladimir Demikhov, a visionary surgeon developed a mechanical device too large to be inserted entirely within the thorax of a dog, but it functioned as a substitute for the heart for as long as 5.5 h. Till 1946, intrathoracic transplantation had never been accomplished in a warm-blooded animal. The first issue encountered was ongoing nourishment of the heart using arterialised blood. He ligated of the aorta, venae cavae, azygos, and brachiocephalic and left subclavian arteries perfused the heart with arterialised blood was returned to the left atrium after passing through the pulmonary circuit and delivered by the left ventricle into the coronaries. He used this method in around 300 experiments and maintained the heart in good condition for up to 4 h^[36].

Despite multiple initial failures of intrathoracic transplant of the heart, one dog survived for 32 postoperative days. Perhaps his greatest achievement was a series of orthotopic heart transplants that he performed without hypothermia or the use of a cardiopulmonary bypass machine. He performed end-to-side anastomosis of the donor aorta, pulmonary artery and venae cavae to the corresponding recipient vessels and reattached the pulmonary veins to the recipients left atrium and closed off with a purse strings. He reported survival times of up to 15.5 h, thereby creating the first model of an orthotopic heart transplant providing the entirety of the pumping function^[37]. Demikhov's research was not published in English until 1962.

Interest in Frank C Mann's work was rekindled in 1951. Marcus et al created a technique using 3 dogs, a donor, a recipient and a receptacle for the donor heart when disconnected from the circulation^[38]. The final model was not too dissimilar to the cross-circulation utilized by Lillihei. This "interim parabiotic perfusion" was used to place the heart in the 2 previously mentioned configurations as described by Mann. In 1953, Marcus and associates managed to achieve a survival time of 48 h for heterotopic heart transplantation^[39]. Wilfred Neptune and colleagues were the first to utilise hypothermia with a heart-lung block and achieved a survival time of 6 h in a canine model^[40].

Webb, Howard and Neely produced 12 successful orthotopic heart transplants surviving as long as 7.5 h using a different method of anastomosing the pulmonary veins of the donor to recipient compared to Demikhov^[41]. The first involvement of British cardiac surgeons occurred in 1959 when Cass and Brock described a series of methods for autotransplantation while including leaving the recipients atria and septal crest behind to avoid pulmonary vein and vena cavae anastomosis^[42].

In 1960, Lower and Shumway published results of their experiments with orthotopic homotransplantations using an oxygenator and partial atrial preservation as described by Cass and Brock. They yielded excellent results with 5 of the 8 dogs experimented on surviving between 6-21 days^[43]. To date, the bi-atrial anastomosis is still noted as the Shumway Technique.

Shumway paid meticulous attention to surgical technique and myocardial protection using isotonic saline at 4 °C. In addition they introduced the concept of assistance time whereby the recipient dogs were left on the cardiopulmonary bypass for a short period of time to ease the heart into assuming the circulatory load^[44].

Shumway's group also described initial issues such as the incidence of complete atrioventricular block. On learning lessons from rejection in renal transplant patients, Reemtsma et al attempted to use methotrexate

for heterotopic heart transplantation in 21 canines, and prolonged survival up to 26 post-operative days^[45]. Blumenstock mimicked the findings of Reemtsma's group with one canine in their cohort surviving for up to 42 days in 1963^[46].

The first ethical dilemma faced by the fraternity was the concept of the donor as "the definition of irreversible coma" was only established in 1968 by an Ad Hoc Committee of Harvard Medical School on Brain Death^[47]. The likelihood of a potential donor dying at exactly the same time as a recipient needing a heart was minute and a decision was made that the team would not halt ventilation of the patient in January 1964, but instead would utilise a chimpanzee as a donor^[48]. A patient presented with a large thrombus that had embolised to the left side of the heart and placed on mechanical coronary perfusion. The chimpanzee heart was explanted and implanted into the patient. Despite initially beating well, it became apparent that the heart was not able to support the larger volume of a human circulation and the patient died within an hour of weaning from cardiopulmonary bypass.

Dr. Christiaan Barnard had worked alongside Shumway in Minnesota. He had also performed the first successful kidney transplant in South Africa to understand transplant immunology and geared for a heart transplantation. On 14th September 1967, Louis Washkansky was admitted in the Groote Schuur Hospital in Cape Town, South Africa. Dr. Velva Schrire (Chief Cardiologist) recommended Washkansky as the appropriate case for transplant. On 2nd December 1967, a 24-year-old female, Denise Ann Darvall was pronounced dead after sustaining a massive cerebral injury following a collision. Both patients were brought to theaters A and B where and mutual consent was obtained^[49].

"If you can't save my daughter, you must try and save this man." Edward Darvall (Denise's Father).

On 3rd December 1967, Dr. Christiaan Barnard performed the first successful human-to-human orthotopic heart transplantation. Her heart was taken via the Shumway technique with the heart cooled to 10 °C. He used a combination of local irradiation, azathioprine, prednisone, and actinomycin C as his immunosuppression regime. The post-operative course of the patient was very promising, he contracted Pseudomonas pneumonia and died on the 18th post-operative day^[50].

Dr. Adrian Kantrowitz and his team performed the 2nd heart transplant (the first in a paediatric patient) in Brooklyn. Kantrowitz was already well known for designing the first intra-aortic balloon pump and had conducted considerable laboratory experiments in puppy hearts believing that the immune system of a younger heart may offer less allogenic resistance. On 6th December 1967, He transplanted an anencephalic donor heart into a 3 week-old patient diagnosed with tricuspid atresia. He performed the operation in hypothermic conditions under circulatory arrest. Despite initial recovery into sinus rhythm, the recipient developed irreversible acidosis and died^[51].

Norman Shumway and his team performed their first heart transplant a month after Kantrowitz. The recipient developed chronic and progressive heart failure after "post-viral myocardial fibrosis" and coronary artery disease. The procedure was complicated by size mismatch with the donor heart being much smaller than the recipient's. The recipient received a combination of methylprednisolone and azathioprine preoperatively and post-operatively with the addition of prednisolone. However, the patient did not succumb to rejection. Shumway noted that in the initial post-operative period the patient was mildly hypotensive and oliguric into the second postoperative day despite administration of isoproterenol and temporary digitalization. The patient developed a consumptive coagulopathy before succumbing to multiorgan dysfunction and bronchopneumonia^[52].

Across the Atlantic, Dr. Donald Ross, who trained under Lord Russell Brock, performed the first heart transplant in the United Kingdom. The patient, a 45-year-old man, survived for 46 days before succumbing

to infection. He performed 2 more unsuccessful transplants before a moratorium was declared^[53].

Denton Cooley's group reported moderate success early on at Baylor with 7 of 10 patients surviving 4.5 months^[54]. To reduce the risk of rejection, they used blood-group compatibility, lymphocyte crossmatch studies (histocompatibility) as described by Dr. Paul Terasaki, and developed a matching system to predict the likelihood of a good outcome post-transplant^[55]. They also administered anti-lymphocyte globulin in addition to the other anti-rejection medications.

EARLY ISSUES WITH HEART TRANSPLANTATION

Within a year of the Barnard's feat, 102 heart transplantations were performed internationally^[56]. Shumway famously quipped "Suddenly heart transplants were being done in places where one would hesitate to have his atrial septal defect closed".

The early promise of heart transplantation however soon diminished as the number of transplants rapidly fell from 100 (1968) to 18 (1970), with many inexperienced units abandoning the procedure. Kantrowitz, who was on the review panel for the National Institute of Health agreed to support Shumway and his unit in their ongoing research^[57]. In 1971, they identified several identifiers of acute rejection^[58]:

- (1) Electrocardiographic findings: i. Increased QRS voltage; ii. Arrhythmia; iii. Right axis deviation; iv. ST-T wave changes;
- (2) Clinical Findings: i. Appearance of gallop rhythm; ii. Decreased precordial activity; iii. Hypotension;
- (3) Echocardiography findings: i. Increased thickness of left ventricular wall; ii. Increased right ventricular diameter.

Using the above-mentioned criteria, they successfully treated 57 of 60 patients with methylprednisolone, actinomycin D and ALG. As the experience of long-term survival in heart transplants increased, Shumway noted a condition he titled "chronic rejection"^[59]. It manifested as diffuse allograft vasculopathy and led to episodes of sick sinus syndrome or myocardial infarction, usually proving fatal.

In 1962, Dr. Souji Konno developed the catheter-type endomyocardial biopsy (EMB) allowing samples of myocardium of patients suspected of having intrinsic musculature abnormality to be taken using a biptome inserted via a peripheral vein or arterial cutdown^[60]. It was initially developed for diagnoses of cardiomyopathies as opposed to limited thoracotomy approaches. The biptome usually provided samples containing endocardium and myocardium, usually sufficient for microscopic examination.

In 1971, a young cardiothoracic surgeon, Dr. Philip Caves undertook a British American Research Fellowship to Stanford to work with Shumway. While here, he worked with instrument maker, Werner Schulz to create the Stanford-Caves Schulz biptome which transformed the management of heart transplant patients. There were 2 Stanford biptomes that differed in size and length. The longer and thinner biptome was used for left ventricular biopsy and the shorter and thicker one for right ventricular biopsy^[61,62]. The samples obtained were between 1-3 mm in diameter. He noted that changes seen in endomyocardial specimens matched those seen in grafts at post-mortem examinations. The samples taken from the endomyocardial surface were also free of post-operative inflammatory changes that complicated sub-epicardial samples taken during thoracotomy. Finally, he noted that the pathologic changes of cardiac allograft rejection were more prominent in the endomyocardial surface (as the graft came in direct contact with the host's circulation). Philip Caves also worked with Margaret Billingham who was a pathologist at Stanford. In 1974, they developed a standardised histological scale to pathologically grade the severity of cardiac rejection based on the extent of infiltrates^[63]. This was incorporated into routine practice and significantly improved the survival of heart transplant recipients at Stanford.

IMMUNOSUPPRESSION IN HEART TRANSPLANTATION

Ciclosporin

Another notable feat in transplantation during this era was the discovery of Ciclosporin A. In 1976, J.F Borel reported the immunosuppressive effects of a fungal metabolite (*Tolypocladium inflatum*) isolated from Swiss soil samples. He noted that skin graft rejection in mice and graft-versus-host disease in mice and rats were considerably delayed by cyclosporin A. He also noted that it had a direct antilymphocytic effect by targeting an early stage of mitogenic triggering of the immunocompetent lymphoid cell and lacked the myelosuppressive effects of cytostatic drugs used at the time^[64]. Roy Calne, who previously worked on azathioprine, conducted *in vivo* immunosuppression with ciclosporin A on porcine cardiac allografts. His group stated that “Ciclosporin A is more effective in suppressing rejection than any other drug that we have used in pigs with orthotopic cardiac allografts”^[65].

Terence English, a South African born surgeon who previously worked with Lord Russell Brock and Donald Ross, nearly abandoned medicine to be a mining engineer. He visited Stanford on advice of his friend Philip Caves in 1973^[66]. He was truly impressed with the outcomes of heart transplant recipients at the unit. In 1978, Terence English, sought approval from the Transplant Advisory Panel of the Department of Health but was informed that there were no funds for a transplant programme^[67]. Given the moratorium, the panel were not keen on “one-off” operations. He duly persisted but his initial attempt was unsuccessful as the donor had arrested prior to implantation and sustained an irreversible brain injury. He persevered and in July 1979, performed the first successful heart transplant in the United Kingdom. The recipient, Keith Castle lived for 5 and a half years^[68].

“He subsequently became the best possible advertisement for cardiac transplantation except for his inability to give up smoking” Sir Terence English on Keith Castle^[68].

Although initial reports on Ciclosporin were favourable, the improvements came with a price. Ciclosporin was nephrotoxic when used over a long period^[69]. Other side effects include hypertension, hepatotoxicity, gingival hyperplasia, hypertrichosis, involuntary tremor, and an increased risk of malignancy^[70]. With the improvements in survival after the initial transplantation, the recipients were at risk of nephrotoxicity and morbidities associated with immunosuppression primarily infections. These drawbacks however did not offset positive impact Ciclosporin offered over previous methods. Immunosuppression formed the initial challenges in cardiac transplantation with suboptimal immunosuppressive regimens either causing allograft rejection or infectious complications from over-immunosuppression.

A European Multicentre trial evaluating renal graft survival at 1-year showed that Ciclosporin alone as a first-line immunosuppressive agent was more effective than with azathioprine and steroids^[71]. Stanford’s group meanwhile reported 1 and 5-year survival rates of 83% and 55%, respectively using a 3-drug protocol of Ciclosporin A, azathioprine, and prednisone^[72].

Tacrolimus

Tacrolimus (Tradename: Prograf®, Astellas Pharma US, Inc. Northbrook, IL) a calcineurin inhibitor like Ciclosporin was discovered from a soil sample from the foot of Mount Tsukuba in Tokyo in 1984. It was cultured from an actinobacter, *Streptomyces tsukubaensis*^[73]. It suppresses interleukin-2 production associated with T-cell activation, thus inhibiting the differentiation and proliferation of cytotoxic T cells. Thomas Starzl once again led research into safety and efficacy of Tacrolimus at University of Pittsburgh Medical School^[74]. Tacrolimus had a more limited adverse effect profile and comparative studies suggest superiority over Ciclosporin in preventing allograft rejection while causing less antibody suppression^[75,76]. The pharmacokinetics were far more predictable than for micro-emulsion Ciclosporin^[77].

Numerous randomized controlled trials comparing tacrolimus to Ciclosporin have been done. Two multicentre studies comparing tacrolimus to oil-based Ciclosporin (Tradename: Sandimmune® Oral Solution, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey) showed no significant difference between the groups at 12 months. Graft survival, renal function and infection rates were not significantly different between the groups although more patients in the Ciclosporin group developed hypertension and hypercholesterolaemia^[78,79].

A micro-emulsion formulation of Ciclosporin (Tradename: Neoral® Oral Solution, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936) was developed and was shown to have a better bioavailability profile with more predictable pharmacokinetics compared to the oil-based preparations^[80]. A multicenter, randomized study of both preparations of Ciclosporin revealed fewer episodes of rejection requiring antilymphocyte antibodies and fewer study discontinuations for treatment failures in the micro-emulsion based Ciclosporin cohort of patients compared to those treated with oil-based Ciclosporin without any adverse events^[81].

When compared to tacrolimus, micro-emulsion based Ciclosporin (alongside cytolytic induction) and a tapered steroid regime showed equivalent patient and graft survival at 19 months. However, there was an increased incidence of biopsy proven acute rejection in the Ciclosporin group at 6 months. Tacrolimus was associated with a higher incidence of new-onset diabetes mellitus, lower rates of post-transplant hypertension and lower incidences of dyslipidaemia^[82]. Similar findings were noted in another trial without cytolytic induction^[83].

Mycophenolate mofetil

Another agent that is commonly used is [mycophenolate mofetil (MMF); CellCept, Roche Laboratories, Nutley, NJ]. It is an effective anti-proliferative agent that improves rejection and survival when used as part of combination therapy. Its active metabolite, mycophenolic acid, is a non-competitive inhibitor of inosine monophosphate dehydrogenase in the de novo pathway for purine synthesis^[84]. Therefore, MMF has some selectivity for lymphocytes over other cell types as lymphocytes rely on this pathway for DNA replication and proliferation. Studies have shown that heart transplant patients receiving MMF therapy had lower levels of C-reactive protein, circulating B lymphocytes, activated T lymphocytes and natural killer (NK) cells compared to patients receiving azathioprine^[85].

Mechanistic target of rapamycin inhibitors

Everolimus (Tradename: Certican, Novartis Pharma Schweiz AG, Bern, Switzerland) and Sirolimus (Tradename: Rapamune, Wyeth Europa Ltd., Maidenhead, UK) are mechanistic target of rapamycin inhibitors^[86]. They work by inhibiting proliferation signals by suppressing the cytokine-driven T-lymphocyte proliferation, resulting in an arrest of the cell cycle. Unlike the calcineurin inhibitors, they demonstrate little or no nephrotoxic side effects. Recent studies have even shown a reduction in the incidence of chronic allograft vasculopathy (CAV) with Everolimus as measured by IVUS among heart-transplant recipients after 1 year^[87,88]. Sirolimus however is linked to an increase in total cholesterol and triglyceride levels^[89,90]. Everolimus on the other hand, is linked with an increase in total cholesterol levels, without increased triglyceride levels, but a significant increase in HDL which may explain its attenuation of CAV^[91].

Cytolytic induction therapy

Cytolytic Induction therapy comprises of immunosuppressive drugs that have been introduced into clinical transplantation directed against human lymphoid cells. Several different forms of cytolytic induction therapy have been used as identified in [Table 1](#).

In heart transplantation especially, kidney dysfunction has been demonstrated to be risk factors for early death^[93,94]. Cytolytic induction allows post-operative renal recovery from a pre-renal aetiology without the

Table 1. Different types of cytolytic induction therapy available (adapted with permission from Wahlers^[92])

Substance	Origin	Dosages applied	Routes investigated	Monoclonal/polyclonal
Antilymphocyte-Globulin ATGAM	Horse	Various 7-14 days	IM	Polyclonal
Antithymocyte-Globulin Bieber-ATG Tecelac	Rabbit	1, 5-3 mg/kg per day 1-10 days	IM/IV	Polyclonal
OKT III antibody	Mouse	5-10 mg/day 4-14 days	IV	Monoclonal
BMA 031 antibody	Mouse	Experimental	IV	Monoclonal
Anti-LFA antibody	Synthetic	Experimental	IV	Monoclonal

negative impact of high nephrotoxic ciclosporin/Ciclosporin/tacrolimus levels. It effectively allows bridging of immunosuppression until a steady state is reached for the regular immunosuppression medications. Most centres use a combination of the abovementioned immunosuppressants to achieve adequate immunosuppression. In 2006, Kobashigawa led a trial comparing 3 different immunosuppression regimes, micro-emulsion Ciclosporin with MMF, tacrolimus with MMF or tacrolimus with sirolimus^[95].

The 343 heart transplant recipients in this trial were randomized to receive corticosteroids and on of the mentioned regimes. Cytolytic induction therapy was used for up to 5 days. The primary endpoint of moderate rejection or haemodynamic compromise rejection requiring treatment showed no significant difference between the three groups at 6 months and 1 year. The probability of treated rejection was significantly lower in both the tacrolimus groups compared with the micro-emulsion Ciclosporin/mycophenolate mofetil group. The tacrolimus/sirolimus group had more fungal infections and more impaired wound healing.

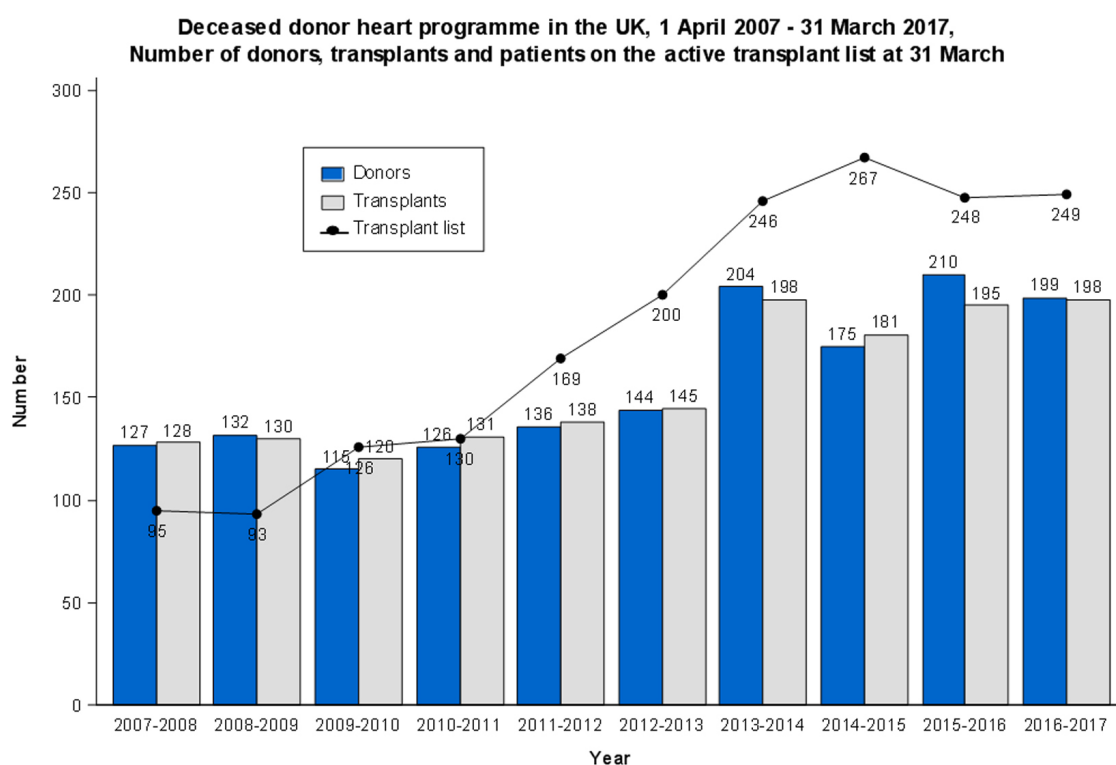
On the other hand, recent trials involving combinations with everolimus have shown promising results including reduced cytomegalovirus infections^[96], reduced cutaneous cancer incidence^[97], and CAV attenuation effects^[98].

CURRENT STATUS OF HEART TRANSPLANTATION

Heart transplantation is considered to be the “gold-standard treatment” for refractory advanced heart failure in carefully selected patients^[99-101]. A major limiting factor of transplantation is the emerging gap between the number of donors (available grafts) and the number of patients on the waiting list. This issue is apparent even in the neighbouring France^[102]. The utilization of marginal donors or expanded-criteria donors has steadily increased over the decades. Part of the decision-making process currently between physician, surgeon and patient includes discussing the potential options available. Currently, the choices include continued medical therapy (5% to 10% weekly mortality risk), mechanical circulatory support (10% to 15% operative risk), or a transplant which may or may not include a clause for marginal organs.

The “Standard Donor” or “Traditional Criteria” for a donor as suggested by Copeland^[103] is as follows: (1) age < 50 years; (2) echocardiogram showing no important segmental abnormalities or global hypokinesis, ejection fraction greater than 50%, and normal valves; (3) inotropes less than 15 µg/kg/min of dopamine; (4) donor to recipient weight ratio 1.5 to 0.7; (5) cold ischemic time less than 4 h; (6) no donor infection; (7) negative serology for hepatitis B, hepatitis C, and human immunodeficiency Virus; and (8) normal electrocardiogram or minor ST-T wave abnormalities, with no conduction system disease.

The rising number of patients listed for heart transplantation has resulted in an increased number of donors from beyond the “standard criteria” pool as a result of the undersupply of available organs. “Marginal Donors” as they are termed would, under conventional transplant guidelines, be declined as potential organ donors^[104]. Median waiting times in the UK for hearts on the non-urgent list is currently 1280 days and 26 days for the urgently listed^[105] [Figure 1].



Source: Transplant activity in the UK, 2016-2017, NHS Blood and Transplant

Figure 1. Deceased donor heart programme in the UK, 1 April 2007 - 31 March 2017, number of donors, transplants and patients on the active transplant list at 31 March

Forays into xenotransplantation as a potential pool of organs to solve the problem of donor-organ supply were also touted but to date, these remain in the experimental phase^[106].

The decision to accept a marginal donor organ is made on a recipient focused individualized basis rather than specific values, parameters or conditions [Figure 2].

The number of “standard donors” for kidney transplants were first notably reduced after the implementation of the compulsory wearing of seat belts in the United Kingdom which was approved by parliament in 1982 and became law on 1 February 1983^[107]. Other legislations include zero-tolerance drinking-and-driving law resulting in fewer traffic accidents with fatal victims^[108]. During this time period the United Kingdom Transplant Support Service Authority demonstrated a 12% increase in the number of cardiac donors aged greater than 41 years between 1988 and 1995^[109]. The initial reluctance to use organs from older donors especially the heart was due to longstanding dogma that older hearts were thought to more susceptible to the catecholamine flood that accompanies brain death^[110]. Internationally, gun crime has also been closely associated with donor organ availability. Studies in Brazil have shown a direct correlation between urban violence and gun crime to organ donors^[111,112].

Initial studies exploring the extended age criteria showed no significant difference in terms of left ventricular function and the incidence of infection and rejection^[109,113]. The risk of dying on the waiting list outweighed that of receiving an organ from an older donor^[114].

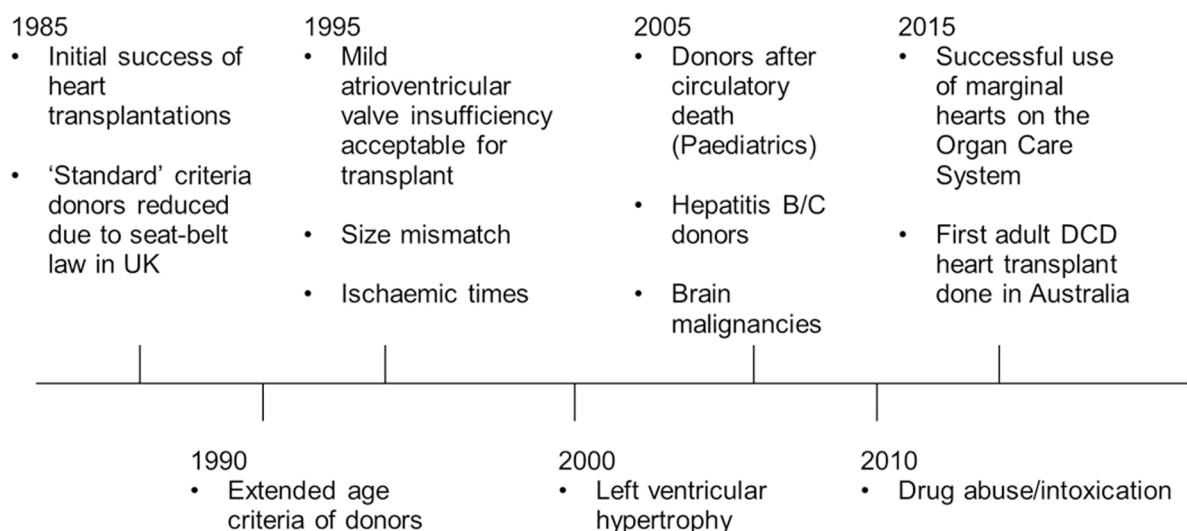


Figure 2. Timeline of events where modifications of "Standard criteria" toward more marginal donors were implemented

Some surgeons also opted to accept hearts with mild-to-moderate mitral or tricuspid insufficiency or secundum-type atrial septal defects as these could be repaired immediately or post-operatively with good results^[115].

As the understanding of myocardial protection improved, the use of mildly hypertrophic left ventricles with short ischaemic times were also proposed with the caveat that there were no ECG changes^[116].

Patients with underlying malignancies were previously never considered donor candidates. However, the risk of metastasis from a primary intracranial tumour is low. A German study in one of the earliest studies evaluating the outcomes of recipients receiving organs from donors with intracranial malignancies showed good follow up outcomes of more than 5 years^[117].

Transplantation also requires commitment from the patients and health care providers as it involves a long-term programme of treatment including pharmacological immunosuppression and regular surveillance^[118]. Clinical decisions therefore should consider a patient's ability to adhere to the demands of ongoing treatment. Alternatives to transplantation include the use of Ventricular Assist Devices (VADs). These are however limited in the National Health Service (NHS) due to the limited health care funding. In North America, the Food and Drug Administration recently approved VADs as destination therapy^[119]. In its current form, heart transplantation confers a significant survival advantage with a 1-year survival of 84.5% and a 5-year survival of 72.5% which is significantly improved as compared to the 76.9% 1-year survival and 62.7% 5-year survival in the 1980s^[120,121].

PRIMARY DIAGNOSTIC INDICATIONS FOR TRANSPLANT

The most frequent indications for heart transplantation in adults are chronic heart failure secondary to dilated cardiomyopathy or ischaemic heart disease^[118]. There is also a significant number of patients (approximately 3%) with adult congenital heart disease who present with advanced heart failure in adulthood^[122]. These patients are slightly more complex to manage both surgically (due to the abnormal anatomy, complex adhesions) and medically (due to human leucocyte antigen sensitisation, potentially elevated pulmonary vascular resistance secondary to univentricular circulations and erythrocytosis secondary to cyanosis)^[118,122]. Coronary artery disease is the most important contributor to heart failure with a population-attributable risk of 65% in men and 48% in women^[123]. Most of the patients however can be classified into ischaemic or non-ischaemic cardiomyopathies.

DECLARATIONS

Authors' contributions

Wrote, drafted, and edited the manuscript: Singh SSA
Supervised: Banner N
Reviewed and edited the manuscript: Berry C, Al-Attar N

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Bergan A. Ancient myth, modern reality: a brief history of transplantation. *J Biocommun* 1997;24:2-9.
2. Salguero CP. Buddhism & Medicine in East Asian history. *Religion Compass* 2014;8:239-50.
3. Linden PK. History of solid organ transplantation and organ donation. *Crit Care Clin* 2009;25:165-84.
4. Goodrich JT. The ancient art of trepanation--a Greek Bronze Age "hole in the skull". *World Neurosurg* 2014;81:296-7.
5. Gutkind L. Many sleepless nights: the world of organ transplantation. Norton & Company; 1988.
6. Hewitt CW, Gordon CR, Lee WPA. Transplantation of composite tissue allografts. New York: Springer; 2008.
7. Crawford AZ, Patel DV, McGhee CNJ. A brief history of corneal transplantation: from ancient to modern. *Oman J Ophthalmol* 2013;6:S12-7.
8. Davis JS. Address of the president: the story of plastic surgery. *Ann Surg* 1941;113:641-56.
9. Merchant J, Tan SY. Alexis Carrel (1873-1944): pioneer of vascular surgery and organ transplantation. *Singapore Med J* 2013;54:602-3.
10. Aida L. Alexis Carrel (1873-1944): visionary vascular surgeon and pioneer in organ transplantation. *J Med Biogr* 2014;22:172-5.
11. Sade RM. Transplantation at 100 years: Alexis Carrel, pioneer surgeon. *Ann Thorac Surg* 2005;80:2415-8.
12. Shayan H. Organ transplantation: from myth to reality. *J Invest Surg* 2001;14:135-8.
13. Cooper DKC, Kemp E, Platt JL, White DJG. Xenotransplantation. Berlin: Springer; 1997.
14. Barker CF, Markmann JF. Historical overview of transplantation. *Cold Spring Harb Perspect Med* 2013;3:a014977.
15. Dempster WJ. Kidney homotransplantation. *Br J Surg* 1953;40:447-65.
16. Brent LB. A history of transplantation immunology. Los Angeles Press; 2005.
17. Anderson D, Billingham RE, Lampkin GH, Medawar PB. The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattle. *Heredity* 1951;5:379.
18. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature* 1953;172:603-6.
19. Harrison JH, Merrill JP, Murray JE. Renal homotransplantation in identical twins. *Surg Forum* 1956;6:432-6.
20. Calne RY. The rejection of renal homografts. Inhibition in dogs by 6-mercaptopurine. *Lancet* 1960;1:417-8.
21. Hamilton D, Barker CF, Starzl TE. A history of organ transplantation. Pittsburgh Press; 2012.
22. Hill RB Jr, Dahrling BE, II, Starzl TE, Rifkind D. Death after transplantation. *Am J Med* 1967;42:327-34.
23. Waksman BH, Arbouys S, Arnason BG. The use of specific "lymphocyte" antisera to inhibit hypersensitive reactions of the "delayed" type. *J Exp Med* 1961;114:997-1022.
24. Woodruff MF, Anderson NA. Effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocytic serum on

- the survival of skin homografts in rats. *Nature* 1963;200:702.
25. Levey RH, Medawar PB. Nature and mode of action of antilymphocytic antiserum. *Proc Natl Acad Sci U S A* 1966;56:1130-7.
 26. Starzl TE, Marchioro TL, Porter KA, Iwasaki Y, Cerilli GJ. The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal homotransplantation. *Surg Gynecol Obstet* 1967;124:301-8.
 27. Weisse AB. *Medical odysseys*. New Jersey Press; 1991.
 28. Weisse AB. Cardiac surgery: a century of progress. *Tex Heart Inst J* 2011;38:486-90.
 29. Lewis FJ, Taufic M. Closure of atrial septal defects with the aid of hypothermia; experimental accomplishments and the report of one successful case. *Surgery* 1953;33:52-9.
 30. Lillehei CW, Varco RL, Cohen M, Warden HE, Patton C, et al. The first open-heart repairs of ventricular septal defect, atrioventricular communis, and tetralogy of Fallot using extracorporeal circulation by cross-circulation: a 30-year follow-up. *Ann Thorac Surg* 1986;41:4-21.
 31. Gibbon JH Jr. The development of the heart-lung apparatus. *Am J Surg* 1978;135:608-19.
 32. Kirklin JW, Dushane JW, Patrick RT, Donald DE, Hetzel PS, et al. Intracardiac surgery with the aid of a mechanical pump-oxygenator system (gibbon type): report of eight cases. *Proc Staff Meet Mayo Clin* 1955;30:201-6.
 33. Dewart RA, Gott VL, Lillehei CW, Read RC, Varco RL, et al. A simple, expendable, artificial oxygenator for open heart surgery. *Surg Clin North Am* 1956:1025-34.
 34. Konstantinov IE. Robert H. Goetz: the surgeon who performed the first successful clinical coronary artery bypass operation. *Ann Thorac Surg* 2000;69:1966-72.
 35. Mann FC, Priestley JT, Markowitz JJ, Yater WM. Transplantation of the intact mammalian heart. *Arch Surg* 1933;26:219-24.
 36. Shumacker HB Jr. A surgeon to remember: notes about Vladimir Demikhov. *Ann Thorac Surg* 1994;58:1196-8.
 37. Cooper DK. Experimental development of cardiac transplantation. *BMJ* 1968;4:174-81.
 38. Marcus E, Wong SN, Luisada AA. Homologous heart grafts; transplantation of the heart in dogs. *Surg Forum* 1951:212-7.
 39. Cooper DK. Transplantation of the heart and both lungs. I. Historical review. *Thorax* 1969;24:383-90.
 40. Picicche M, Carpentier A. *Dawn and evolution of cardiac procedures*. New York; 2013.
 41. Webb WR, Howard HS, Neely WA. Practical methods of homologous cardiac transplantation. *J Thorac Surg* 1959;37:361-6.
 42. Cass MH, Brock R. Heart excision and replacement. *Guys Hosp Rep* 1959;108:285-90.
 43. Lower RR, Shumway NE. Studies on orthotopic homotransplantation of the canine heart. *Surg Forum* 1960;11:18-9.
 44. Lower RR, Stofer RC, Shumway NE. Homovital transplantation of the heart. *J Thorac Cardiovasc Surg* 1961;41:196-204.
 45. Reemtsma K, Williamson WE Jr, Iglesias F, Pena E, Sayegh SF, et al. Studies in homologous canine heart transplantation: prolongation of survival with a folic acid antagonist. *Surgery* 1962;52:127-33.
 46. Blumenstock DA, Hechtman HB, Collins JA, Jaretzki A 3rd, Hosbein JD, et al. Prolonged survival of orthotopic homotransplants of the heart in animals treated with methotrexate. *J Thorac Cardiovasc Surg* 1963;46:616-25.
 47. A definition of irreversible coma. Report of the Ad Hoc Committee of the Harvard Medical School to examine the definition of brain death. *Jama* 1968;205:337-40.
 48. Hardy JD, Kurrus FD, Chavez CM, Neely WA, Eraslan S, et al. Heart transplantation in man. Developmental studies and report of a case. *Jama* 1964;188:1132-40.
 49. Kalra A, Seth S, Hote M, Airan B. The story of heart transplantation: from cape town to cape comorin. *J Pract Cardiovasc Sci* 2016;2:120-5.
 50. Barnard CN. The operation. A human cardiac transplant: an interim report of a successful operation performed at Groote Schuur Hospital, Cape Town. *S Afr Med J* 1967;41:1271-4.
 51. Kantrowitz A, Haller JD, Joos H, Cerruti MM, Carstensen HE. Transplantation of the heart in an infant and an adult. *Am J Cardiol* 1968;22:782-90.
 52. Stinson EB, Dong E, Schroeder JS, Harrison DC, Shumway NE. Initial clinical experience with heart transplantation. *Am J Cardiol* 1968;22:791-803.
 53. Cooley DA. In memoriam: Donald N. Ross (1922-2014). *Tex Heart Inst J* 2014;41:456-7.
 54. Cooley DA, Bloodwell RD, Hallman GL, Leachman RD, Nora JJ, et al. Cardiac transplantation: general considerations and results. *Ann Surg* 1969;169:892-905.
 55. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969;280:735-9.
 56. Patterson C, Patterson KB. The history of heart transplantation. *Am J Med Sci* 1997;314:190-7.
 57. DiBardino DJ. The history and development of cardiac transplantation. *Tex Heart Inst J* 1999;26:198-205.
 58. Griep RB, Stinson EB, Dong E, Clark DA, Shumway NE. Acute rejection of the allografted human heart: diagnosis and treatment. *Ann Thorac Surg* 1971;12:113-26.
 59. Clark DA, Stinson EB, Griep RB, Schroeder JS, Shumway NE, et al. Cardiac transplantation in man: VI. prognosis of patients selected for cardiac transplantation. *Ann Intern Med* 1971;75:15-21.
 60. Nishikawa T, Sekiguchi M, Ishibashi-Ueda H. More than 50 years after Konno's development of the endomyocardial biopsy. *Int Heart J* 2017;58:840-6.
 61. Caves PK, Stinson EB, Graham AF, Billingham ME, Grehl TM, et al. Percutaneous transvenous endomyocardial biopsy. *JAMA* 1973;225:288-91.

62. Melvin KR, Mason JW. Endomyocardial biopsy: its history, techniques and current indications. *CMAJ* 1982;126:1381-6.
63. Caves P, Billingham M, Stinson E, Shumway N. Serial transvenous biopsy of the transplanted human heart improved management of acute rejection episodes. *Lancet* 1974;303:821-6.
64. Borel JF, Feurer C, Gubler HU, Stahelin H. Biological effects of cyclosporin A: a new antilymphocytic agent. *Agents Actions* 1976;6:468-75.
65. Calne RY, White DJG, Rolles K, Smith DP, Herbertson BM. Prolonged survival of pig orthotopic heart grafts treated with cyclosporin A. *Lancet* 1978;311:1183-5.
66. Morris T. *The matter of the heart*. London; 2017.
67. Newton C. Interview: Sir Terence English. *Bull Roy Coll Surg Engl* 2015;97:289-91.
68. English T. *Follow your star*. Indiana; 2011.
69. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. *NEJM* 1984;311:699-705.
70. Patel JK, Kobashigawa JA. Tacrolimus in heart transplant recipients. *BioDrugs* 2007;21:139-43.
71. European Multicentre Trial G. Cyclosporin in cadaveric renal transplantation: one-year follow-up of a multicentre trial. *Lancet* 1983;2:986-9.
72. Starnes VA, Shumway NE. Heart transplantation--Stanford experience. *Clin Transpl* 1987;7:11.
73. Fung JJ. Tacrolimus and transplantation: a decade in review. *Transplantation* 2004;77:S41-3.
74. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, et al. FK 506 for liver, kidney, and pancreas transplantation. *Lancet* 1989;2:1000-4.
75. Jurcevic S, Dunn MJ, Crisp S, Busing K, Rinaldi M, et al. A new enzyme-linked immunosorbent assay to measure anti-endothelial antibodies after cardiac transplantation demonstrates greater inhibition of antibody formation by tacrolimus compared with cyclosporine. *Transplantation* 1998;65:1197-202.
76. Behr TM, Richter K, Fischer P, Spes CH, Meiser B, et al. Incidence of humoral rejection in heart transplant recipients treated with tacrolimus or cyclosporine A. *Transplant Proc* 1998;30:1920-1.
77. Wang CH, Ko WJ, Chou NK, Wang SS. Therapeutic drug monitoring of tacrolimus in cardiac transplant recipients: a comparison with cyclosporine neoral. *Transplant Proc* 2004;36:2386-7.
78. Reichart B, Meiser B, Vigano M, Rinaldi M, Martinelli L, et al. European Multicenter Tacrolimus (FK506) heart pilot study: one-year results--European Tacrolimus Multicenter Heart Study Group. *J Heart Lung Transplant* 1998;17:775-81.
79. Taylor DO, Barr ML, Radovancevic B, Renlund DG, Mentzer RM Jr, et al. A randomized, multicenter comparison of tacrolimus and cyclosporine immunosuppressive regimens in cardiac transplantation: decreased hyperlipidemia and hypertension with tacrolimus. *J Heart Lung Transplant* 1999;18:336-45.
80. Cooney GF, Jeevanandam V, Choudhury S, Feutren G, Mueller EA, et al. Comparative bioavailability of neoral and sandimmune in cardiac transplant recipients over 1 year. *Transplant Proc* 1998;30:1892-4.
81. Eisen HJ, Hobbs RE, Davis SF, Carrier M, Mancini DM, et al. Safety, tolerability, and efficacy of cyclosporine microemulsion in heart transplant recipients: a randomized, multicenter, double-blind comparison with the oil-based formulation of cyclosporine--results at 24 months after transplantation. *Transplantation* 2001;71:70-8.
82. Grimm M, Rinaldi M, Yonan NA, Arpesella G, Arizón Del Prado JM, et al. Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients - a large European trial. *Am J Transplant* 2006;6:1387-97.
83. Kobashigawa JA, Patel J, Furukawa H, Moriguchi JD, Yeatman L, et al. Five-year results of a randomized, single-center study of tacrolimus vs microemulsion cyclosporine in heart transplant patients. *J Heart Lung Transplant* 2006;25:434-9.
84. Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology* 2000;47:85-118.
85. Eisen HJ, Kobashigawa J, Keogh A, Bourge R, Renlund D, et al. Three-year results of a randomized, double-blind, controlled trial of mycophenolate mofetil versus azathioprine in cardiac transplant recipients. *J Heart Lung Transplant* 2005;24:517-25.
86. MacKeigan JP, Krueger DA. Differentiating the mTOR inhibitors everolimus and sirolimus in the treatment of tuberous sclerosis complex. *Neuro-Oncology* 2015;17:1550-9.
87. Kobashigawa JA, Pauly DF, Starling RC, Eisen H, Ross H, et al. Cardiac allograft vasculopathy by intravascular ultrasound in heart transplant patients: substudy from the everolimus versus mycophenolate mofetil randomized, multicenter trial. *JACC Heart Fail* 2013;1:389-99.
88. Andreassen AK, Andersson B, Gustafsson F, Eiskjaer H, Radegran G, et al. Everolimus initiation and early calcineurin inhibitor withdrawal in heart transplant recipients: a randomized trial. *Am J Transplant* 2014;14:1828-38.
89. Wlodarczyk Z, Vitko S, Salmela K, Czajkowski Z, Margreiter R. Lipid metabolism in renal transplant patients receiving tacrolimus/sirolimus combination therapy. *Transplant Proc* 2005;37:1871-3.
90. Lindenfeld J, Miller GG, Shakar SF, Zolty R, Lowes BD, et al. Drug therapy in the heart transplant recipient: part II: immunosuppressive drugs. *Circulation* 2004;110:3858-65.
91. Tenderich G, Fuchs U, Zittermann A, Muckelbauer R, Berthold HK, et al. Comparison of sirolimus and everolimus in their effects on blood lipid profiles and haematological parameters in heart transplant recipients. *Clin Transplant* 2007;21:536-43.
92. Wahlers T. Cytolytic induction therapy in heart and lung transplantation: the protagonist opinion. *Transplant Proc* 1998;30:1100-3.
93. Odum J, Wheat J, Laks H, Kobashigawa J, Gjertson D, et al. Peri-operative renal function and outcome after orthotopic heart transplantation. *J Heart Lung Transplant* 2006;25:162-6.

94. Wahlers T, Cremer J, Fleguth HG, Jurmann M, Herrmann G, et al. Adjusted triple drug immunosuppression and kidney function following heart transplantation. *Transplant Proc* 1989;21:2492-3.
95. Kobashigawa JA, Miller LW, Russell SD, Ewald GA, Zucker MJ, et al. Tacrolimus with mycophenolate mofetil (MMF) or sirolimus vs. cyclosporine with MMF in cardiac transplant patients: 1-year report. *Am J Transplant* 2006;6:1377-86.
96. Kobashigawa J, Ross H, Bara C, Delgado JF, Dengler T, et al. Everolimus is associated with a reduced incidence of cytomegalovirus infection following de novo cardiac transplantation. *Transpl Infect Dis* 2013;15:150-62.
97. Euvrard S, Boissonnat P, Roussoulières A, Kanitakis J, Decullier E, et al. Effect of everolimus on skin cancers in calcineurin inhibitor-treated heart transplant recipients. *Transpl Int* 2010;23:855-7.
98. Eisen HJ, Kobashigawa J, Starling RC, Pauly DF, Kfoury A, et al. Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial. *Am J Transplant* 2013;13:1203-16.
99. Mehra MR, Canter CE, Hannan MM, Semigran MJ, Uber PA, et al. The 2016 international society for heart lung transplantation listing criteria for heart transplantation: a 10-year update. *J Heart Lung Transplant* 2016;35:1-23.
100. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, et al. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016;37:2129-200.
101. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, et al. 2016 ACC/AHA/HFSA focused update on new pharmacological therapy for heart failure: an update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on clinical practice guidelines and the Heart Failure Society of America. *Circulation* 2016;134:e282-93.
102. Dorent R, Gandjbakhch E, Goéminne C, Ivanès F, Sebbag L, et al. Assessment of potential heart donors: a statement from the French heart transplant community. *Arch Cardiovasc Dis* 2017; doi: 10.1016/j.acvd.2017.12.001.
103. Copeland JG. Only optimal donors should be accepted for heart transplantation: protagonist. *J Heart Lung Transplant* 1995;14:1038-42.
104. Brock MV, Salazar JD, Cameron DE, Baumgartner WA, Conte JV. The changing profile of the cardiac donor. *J Heart Lung Transplant* 2001;20:1005-9.
105. NHS Blood and Transplant. Transplant Activity in the UK, 2016-2017. NHS Blood and Transplant; 2017.
106. Cooper DK, Keogh AM, Brink J, Corris PA, Klepetko W, et al. Report of the Xenotransplantation Advisory Committee of the International Society for Heart and Lung Transplantation: the present status of xenotransplantation and its potential role in the treatment of end-stage cardiac and pulmonary diseases. *J Heart Lung Transplant* 2000;19:1125-65.
107. Thompson JF, Wood RF, Cahill AP, Franklin PM, Morris PJ. Kidney transplantation and seat belt legislation. *BMJ (Clinical research ed)* 1983;287:1260-1.
108. Calil AM, Sallum EA, Domingues Cde A, Nogueira Lde S. Mapping injuries in traffic accident victims: a literature review. *Rev Lat Am Enfermagem* 2009;17:120-5.
109. Mercer P, Sharples L, Edmunds J, Gittins R, Baines J, et al. Evaluating the donor pool: impact of using hearts from donors over the age of 49 years. *Transplant Proc* 1997;29:3293-6.
110. Young JB. Age before beauty: the use of "older" donor hearts for cardiac transplantation. *J Heart Lung Transplant* 1999;18:488-91.
111. Silva SFR, Silva SL, Nascimento AC, Parente MM, Albuquerque CA, et al. Profile of organ donors in Ceará, northeastern Brazil, from 1998 to 2012. *Transplant Proc* 2014;46:1692-4.
112. Rodrigues SdLL, Ferraz Neto JB-HdE, Sardinha LAdC, Araujo S, Zambelli HJL, et al. Profile of effective donors from organ and tissue procurement services. *Rev Bras Ter Intensiva* 2014;26:21-7.
113. Drinkwater DC, Laks H, Blitz A, Kobashigawa J, Sabad A, et al. Outcomes of patients undergoing transplantation with older donor hearts. *J Heart Lung Transplant* 1996;15:684-91.
114. Bennett LE, Edwards EB, Hosenpud JD. Transplantation with older donor hearts for presumed "stable" recipients: an analysis of the Joint International Society for Heart and Lung Transplantation/United Network for Organ Sharing Thoracic Registry. *J Heart Lung Transplant* 1998;17:901-5.
115. Massad MG, Smedira NG, Hobbs RE, Hoercher K, Vandervoort P, et al. Bench repair of donor mitral valve before heart transplantation. *Ann Thorac Surg* 1996;61:1833-5.
116. Marelli D, Laks H, Fazio D, Moore S, Moriguchi J, et al. The use of donor hearts with left ventricular hypertrophy. *J Heart Lung Transplant* 2000;19:496-503.
117. Hornik L, Tenderich G, Wlost S, Zittermann A, Minami K, et al. Organs from donors with primary brain malignancy: the fate of cardiac allograft recipients. *Transplant Proc* 2004;36:3133-7.
118. Banner NR, Bonser RS, Clark AL, Clark S, Cowburn PJ, et al. UK guidelines for referral and assessment of adults for heart transplantation. *Heart* 2011;97:1520-7.
119. Stewart GC, Mehra MR. A history of devices as an alternative to heart transplantation. *Heart Fail Clin* 2014;10:S1-12.
120. Wilhelm MJ. Long-term outcome following heart transplantation: current perspective. *J Thorac Dis* 2015;7:549-51.
121. Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, et al. The registry of the international society for heart and lung transplantation: thirty-first official adult heart transplant report--2014; focus theme: retransplantation. *J Heart Lung Transplant* 2014;33:996-1008.

122. Burchill LJ. Heart transplantation in adult congenital heart disease. *Heart* 2016;102:1871-7.
123. Lund LH, Edwards LB, Dipchand AI, Goldfarb S, Kucheryavaya AY, et al. The registry of the International Society for Heart and Lung Transplantation: thirty-third adult heart transplantation report-2016; focus theme: primary diagnostic indications for transplant. *J Heart Lung Transplant* 2016;35:1158-69.

Review

Open Access



Risk factors for atherosclerosis and vascular calcification in patients with type 2 diabetes on long-term hemodialysis

Tatyana Archakova, Liudmila Nedosugova

Endocrinology Department, Medical Faculty, I.M. Sechenov First Moscow State Medical University, Moscow 119991, Russia.

Correspondence to: Dr. Liudmila Nedosugova, Endocrinology Department, Medical Faculty, I.M. Sechenov First Moscow State Medical University, Moscow 119991, Russia. E-mail: profmila@mail.ru

How to cite this article: Archakova T, Nedosugova L. Risk factors for atherosclerosis and vascular calcification in patients with type 2 diabetes on long-term hemodialysis. *Vessel Plus* 2018;2:34. <http://dx.doi.org/10.20517/2574-1209.2018.52>

Received: 8 Jul 2018 **First Decision:** 28 Aug 2018 **Revised:** 5 Sep 2018 **Accepted:** 10 Sep 2018 **Published:** 26 Oct 2018

Science Editor: Igor A. Sobenin **Copy Editor:** Cui Yu **Production Editor:** Zhong-Yu Guo

Abstract

Type 2 diabetes mellitus (DM) is a risk factor for the progression of cardiovascular mortality, exacerbated by the development of chronic renal failure secondary to diabetic nephropathy, which requires long-term hemodialysis (LTH). However, in the case of LTH cardiovascular mortality exceeds that in the general population, especially in patients with diabetes. The identification of risk factors for the progression of atherosclerosis and vascular calcification in patients with DM on LTH is of great importance for finding a more effective approach to the prevention of cardiovascular mortality in a given cohort of patients. The presented review contains analysis of current literature data on the evaluation of both traditional and non-traditional risk factors for cardiovascular morbidity in order to improve the effectiveness of therapeutic and diagnostic tactics.

Keywords: Diabetes mellitus, vascular calcification, diabetic nephropathy, cardiovascular mortality, haemodialysis

INTRODUCTION

According to the latest data, the number of patients with diabetes in the world over the past 10 years has more than doubled, and according to the forecasts of the International Diabetes Federation, by the year 2,040, 642 million people will suffer from diabetes. In the Russian Federation, as in all countries of the world, there is a high rate of increase in the incidence of diabetes. The most dangerous consequences of the global epidemic of diabetes are its systemic vascular complications: diabetic nephropathy (DN), retinopathy, atherosclerotic lesions^[1], which are the main cause of disability and mortality in patients with diabetes. According to the World Health Organization, more than 75% of patients with type 2 diabetes die due to vascular accidents^[2].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



It is well known that patients with diabetes often have clinically proven ischemic heart disease (IHD) and heart failure (HF) compared to a group of the same age without diabetes^[3-5].

According to the American Heart Association, DN ranks second among the leading causes of death in patients with type 2 diabetes^[6]. In the United States and Japan, DN is most common among all kidney diseases (35%-45%), eclipsing such kidney pathologies as glomerulonephritis, polycystic kidney disease, pyelonephritis, *etc.* In Europe, the prevalence of DN is less threatening, but the demand for extracorporeal treatment continues to grow^[7].

According to the National Kidney Foundation of Japan (1998) there is a high prevalence of cardiovascular morbidity in patients with chronic kidney disease (CKD), and cardiovascular mortality is higher in dialysis patients than in the general population^[8].

Patients with CKD and diabetes are considered the highest risk group for the development of cardiovascular complications^[9], which require appropriate preventive measures. The complex clinical profile of patients with diabetes on long-term hemodialysis (LTH) has prompted the search for new markers of cardiovascular risk, determined the tactics of therapy and the importance of prevention measures for cardiovascular complications. The purpose of the review is to analyze literature data on the evaluation of both traditional and non-traditional risk factors for cardiovascular morbidity in patients with diabetes on LTH, in order to find a more effective approach to therapeutic and diagnostic tactics.

ATHEROSCLEROSIS, KIDNEY DISEASE AND CARDIOVASCULAR DISEASES IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Atherosclerosis is a multifaceted disease, with traditional risk factors such as diabetes, obesity, dyslipidemia and hypertension, smoking and low physical activity.

Tanaka *et al.*^[10] showed that the low glomerular filtrate rate and proteinuria are independently associated with the development of atherosclerosis, which is facilitated by various pathogenetic mechanisms. CKD is a risk factor for the development of cardiovascular morbidity, and it also contributes to the development of dyslipidemia. Depending on the stage of the process, the nature of dyslipidemia in patients with CKD is different^[11]. In the initial stages of CKD hypertriglyceridemia (HTG) develops because the enzymatic breakdown of triglycerides decreases due to a reduction in lipoprotein lipase activity.

CKD is also characterized by a decrease in the concentration of high-density anti-atherogenic lipoproteins (HDL), due to a low concentration and a decrease in the activity of lecithin-cholesterol acyltransferase, which leads to a disruption in the synthesis and transport of HDL and their accelerated degradation^[12].

It was shown that in patients with significant proteinuria and nephrotic syndrome, lipid metabolism disorders are associated with the increase of low-density lipoproteins (LDL), HTG and hypercholesterolemia^[13]. Kato *et al.*^[14] showed that cardiovascular events are the main cause of death in patients on LTH. The index of intima-media thickness (IMT) was significantly higher in patients who died from cardiovascular diseases and correlated with age and calcification of the aorta, showing the importance of measuring the IMT index as a predictor of the progression of cardiovascular mortality in patients on LTH.

Gluba *et al.*^[15] demonstrated that end-stage renal disease and type 2 diabetes are associated with the accelerated development of atherosclerosis. It was shown that atherosclerosis in the carotid and coronary arteries is an independent prognostic factor of mortality in patients with end-stage of CKD^[16]. In DN and manifested atherosclerosis, altered LDL are detected, which undergo oxidative modification^[17].

MULTIPLE MODIFICATION OF LDL IN BLOOD PLASMA

Modification of LDL occurs due to glycation, desialylation and oxidation of lipid and protein components of LDL, which, as shown in the studies, can eventually lead to their aggregation and the formation of immune complexes (IC). As a result, LDL become atherogenic, which increases their capture by macrophages and subsequent development of atherosclerosis. It was shown that modified LDL (m-LDL) are characterized by numerous changes in carbohydrate, protein and lipid constituents^[18]. m-LDL, which were isolated from the blood of patients with IHD, are able to accumulate cholesterol and are atherogenic. It is indicated that m-LDL undergo changes in a particle of lipoprotein, undergo desialylation, increase electronegative charge and become smaller and denser^[19]. Desialylated LDL stimulate intracellular esterification of free cholesterol, which leads to the accumulation of cholesterol esters (cholesterol). When studying a number of chemical and physical parameters, a reliable inverse correlation was found between the atherogenic LDL and the content of sialic acid in them.

M-LDL are: cytotoxic for endothelial cells, chemotoxic for monocytes, inhibit the migration of macrophages; induce endothelial expression of macrophage colony-stimulating factor, which is the main regulator of macrophage survival, proliferation and differentiation; increase the synthesis of collagen in smooth muscle cells; inhibit lipopolysaccharide-induced expression of nuclear factor-kappaB; induce apoptosis; inhibit the release and/or functions of nitric oxide (vasospasm); increase the expression of adhesion molecules on blood vessels; increase the tissue factor activity in endothelial cells (which leads to thrombosis); cause the synthesis of a wide range of pro-inflammatory cytokines in macrophages; and cause an increase in circulating levels of antibodies^[20]. Multiple modification of lipoprotein particles involves the formation of antigens against which antibodies can be formed^[21]. It has been shown that in the blood of most patients with coronary atherosclerosis there are circulating IC, consisting of LDL and anti-LDL antibodies^[22]. An increase in the level of m-LDL and a high titer of antibodies to them in patients with diabetes with an existing cardiovascular pathology was revealed in comparison with the control group^[23].

Diabetes is a proven risk factor for developing atherosclerosis. Atherosclerotic vascular wall lesions progress in diabetes. Numerous data indicate that blood of type 2 diabetes mellitus (DM) patients contains m-LDL that undergo glycation. It provides non-enzymatic oxidation of LDL. It has been established that m-LDL in the blood of patients with diabetes induce intracellular accumulation of cholesterol, which is associated with a different modification, both with desialylation and non-enzymatic glycation^[21].

Lankin *et al.*^[24] estimated the effect of the carbonyl modification of LDL on the properties of particles that determine their increased atherogenicity, such as the formation of intermolecular crosslinks in ApoB100, the oxidability of LDL particles and their ability for subsequent aggregation. With concomitant diabetes, a sharp progression of atherosclerotic vascular lesions was noted. In addition, it has been shown that the level of lipohydroperoxides in plasma LDL in patients with diabetes is 3 times higher than in patients with ischemic heart disease; it has also been demonstrated that desialylation is observed in the serum of patients with diabetes.

Borodachev *et al.*^[25] showed that in diabetic patients, the content of low-density lipoprotein cholesterol increases, which produces a direct atherogenic effect, while m-LDL have a small size, a greater density than the native ones, and carry an electronegative charge, i.e., become desialylated (have a reduced content of sialic acid) and glycosylated.

Bucala *et al.*^[26] showed a significant increase in the level of the end products of glucose oxidation and the formation of m-LDL in the blood of patients with diabetes and CKD compared with a healthy control group.

Thus, hyperglycemia induces oxidative stress, which contributes to the accumulation of toxic products, which in turn leads to atherogenic modification of m-LDL, endothelial dysfunction and atherosclerosis progression in patients with diabetes.

NON-TRADITIONAL RISK FACTORS

Non-traditional risk factors in patients with diabetes on LTH, which play a huge role in the development of vascular calcification (VC) and cardiovascular pathology in this group of patients, should not be overlooked.

CKD is more associated with non-traditional risk factors. They include impaired calcium-phosphate metabolism, which can lead to VC. VC was found in patients receiving long-term dialysis, who also demonstrated impaired calcium-phosphate metabolism^[27]. Secondary hyperparathyroidism (SHPT), hypercalcemia and hyperphosphatemia are important links in the pathogenesis of VC in patients on LTH.

VC is a widespread complication of CKD and may lead to an increase in cardiovascular morbidity. VC is divided into two types according to the localization of calcifications: calcification of the inner membrane (intima) and the medial membrane (media)^[28]. Calcification of the intima is associated with an atherosclerotic process. Risk factors for the development of atherosclerosis have been discussed above. Calcification of the media (medial calcinosis, Menkberg sclerosis) is observed in patients on LTH in the absence of risk factors for atherosclerosis. The severity of VC may depend on many factors: the duration of hemodialysis therapy, the age and degree of disorders in calcium-phosphate metabolism^[29]. In the works of Rumberger *et al.*^[30] hemodynamic consequences of VC are presented: loss of elasticity of arteries, increase in pulse pressure, development of left ventricular hypertrophy (LVH), HF, lower coronary artery perfusion and myocardial ischemia, which are the main causes of death of the majority of patients with CKD. According to Ribeiro *et al.*^[31], the prevalence of calcification of the mitral and aortic valves (MVC and AVC) in patients on LTH is much higher than in the control group comparable in age and sex. Thus, according to the results of Kalpakian *et al.*^[32], coronary artery calcification (CAC) was found in 53%-92% of patients with CKD. Raggi *et al.*^[33] showed that CAC is a predictor of cardiovascular morbidity in elderly patients with CKD. The degree of CAC was associated with male sex, diabetes and an increase in calcium-phosphate ratio.

It was revealed that CAC was much more common among patients on LTH, in comparison with patients without CKD. A possible reason for this may be impaired calcium-phosphate metabolism, rather than traditional risk factors, as previously believed^[34,35].

Komaba *et al.*^[36], summarized the results of long-term follow-up study of patients on LTH, 38% of whom had diabetes and had elevated levels of calcium (Ca), phosphorus (P), and intact parathyroid hormone (iPTH). Patients with Ca, P and iPTH levels exceeding target ones showed the highest cardiovascular mortality.

Research by Bellasi *et al.*^[37] included patients with end-stage CKD. All patients underwent electron beam computed tomography (CT) for quantitative evaluation of CAC and calcification of the AVC on the Agatston scale. Calcification of heart valves was assessed by two-dimensional echocardiography (echo). As a result, the researchers concluded that patients who had valvular calcification or CAC had a higher risk of developing cardiovascular diseases. Lee *et al.*^[38] studied factors that are associated with the calcification of the aortic arch in patients on LTH. Calcification of the aortic arch was identified by X-ray. Patients were followed-up for 10 years. The increase in calcification was associated with age, higher levels of Ca and blood glucose. During the follow-up period, the authors found that the degree of calcification of the aortic arch was directly related to cardiovascular mortality. According to the data of instrumental studies, Volkov *et al.*^[39] have shown that coronary heart disease in 55.6% of patients and HF in 50.0% of patients on LTH. Combination of MVC and AVC was predominant. Valve calcification was more often observed in older patients, with longer dialysis treatment, more pronounced SHPT, inflammatory changes, and atherosclerosis. Calcification of

valves was combined with a greater frequency of IHD, HF and dilatation of the left atrium^[39]. Taking into account the research data, one can come to the conclusion that hyperphosphataemia, hypercalcemia and an increase in the level of calcium-phosphate ratio are risk factors for the development of VC.

Other potential mechanisms of VC are associated with fibroblast growth factor-23 (FGF-23) and the activity of the transmembrane Klotho protein, which play an important role in the systemic regulation of phosphate homeostasis^[40].

FGF-23 is a protein consisting of 251 amino acids (32 kDa molecular weight), which is secreted from osteocytes, mainly from osteoblasts^[41]. FGF-23 exerts its biological effects through the activation of FGF receptors. FGF1s receptors, binding to the Klotho protein, become 1000 times more sensitive to interaction with FGF-23 than other FGF receptors or Klotho protein alone. Klotho is a 130 kDa transmembrane protein. In the kidneys, FGF-23 induces phosphaturia, suppressing the expression of the sodium-phosphate cotransporter type IIa and IIc in the proximal tubule^[42].

The correlation between elevated levels of FGF-23 and adverse clinical outcomes in patients with CKD, such as cardiovascular morbidity and mortality^[43], has been shown. The relationship between an increase in the concentration of FGF-23 and the progression of CKD from stage I to V was revealed; a higher level of FGF-23 was observed in the group of patients on LTH. The same correlation was observed between the elevated FGF-23 level in the blood serum and the Pourcelot resistive index (according to Doppler ultrasound)^[44]. Jean *et al.*^[45] obtained data indicating that mortality in dialysis patients is directly correlated with the level of FGF-23. Basic research of Grabner *et al.*^[46] convincingly showed that FGF-23 can directly lead to the development of LVH. The study revealed that the increase in FGF-23 led to LVH of *de novo*, and a high level of FGF-23 caused an increase in the frequency of LVH irrespective of the presence or absence of hypertension. FGF-23 causes LVH independently of the Klotho coreceptor, which is expressed predominantly in the kidneys and parathyroid glands and is absent in the cardiomyocytes. High levels of FGF-23 were also independently associated with endothelial dysfunction.

Inaba *et al.*^[47] studied the effect of FGF-23 on the development of aorta and peripheral artery calcification in men on LTH suffering from diabetes, and without diabetes. It was shown that an elevated level of FGF-23 in plasma in type 2 diabetes is significantly correlated with VC compared to patients without diabetes.

Chan *et al.*^[48], confirmed the link between an increase in FGF-23 concentration in plasma, diabetes and the calcification of coronary arteries.

Gutiérrez *et al.*^[49] studied mortality associated with elevated levels of phosphorus and FGF-23 in patients on LTH. The researchers concluded that an elevated level of FGF-23 is independently correlated with mortality among patients on LTH. Studies put forward the main role of FGF-23 as a future biomarker of cardiovascular morbidity and mortality.

Hyperphosphatemia is one of the main risk factors for the development of cardiovascular pathology and mortality among patients with chronic renal failure.

Hyperphosphatemia directly associates with HF and cardiomyopathy, which can explain the direct correlation between phosphorus levels, cardiovascular morbidity and mortality. Hyperphosphatemia is associated with densification of the vascular wall, increased pulse wave velocity, LVH, decreased coronary blood flow and cardiovascular mortality^[50]. In recent years, it has been proven that HF is an active and regulated process (similar to bone mineralization), in which various bone-related proteins participate. In addition to decreasing arterial compliance and their increased stiffness, hyperphosphatemia is closely

involved in the mechanisms of development and progression of VC involving mineralization of vascular smooth muscle cells (VSMC) by phosphorus flux through sodium-dependent transporters, VSMC apoptosis. Cell death in the vascular wall leads to development of cell membrane debris and apoptotic cells, which can become the primary foci of apatite deposits. Increasing concentration of P and Ca, on the one hand, leads to the growth of apatite crystals by passive precipitation, and on the other, can apparently activate cellular and tissue mechanisms of calcification: suppressing differentiation of monocytes/macrophages in osteoblast-like cells with increased FGF-23 levels and change in Klotho protein expression. Consequently, hyperphosphatemia and rebalancing inducers and inhibitors of calcification, the presence of systemic inflammation and oxidative stress contribute to the medial calcification in CKD. Osteogenic mechanisms involve changes in the phenotype of vascular wall cells. Proteins characteristic for bone tissue (osteopontin, osteocalcin, bone morphogenetic protein-2 (Run 2), as well as ectopic foci of typical bone and cartilage tissue formation were found in VC foci^[51].

A study of Kestenbaum^[52], conducted among patients with CRF, showed that the presence of phosphorus in the blood serum exceeding 3.5 mg/dL (1.13 mmol/L) was associated with a significant increase of mortality risk, and for each increase of 1 mg/dL raised the risk of death by 18%.

The CKD Outcomes and Practice Patterns Study showed that hyperphosphatemia ($\text{PO}_4 > 6.1$ mg/dL) was associated with an increase in total and cardiovascular mortality by 1.18^[53].

In 10% of participants in the 15 years prospective study it was noted that the initial level of serum phosphorus had a tight association with the calcification of the coronary arteries^[54]. A close correlation of hyperphosphatemia and LVH has been identified, the development of which is a predictor of the CKD patients mortality.

When assessing the effect of elevated levels of PTH and calcium-phosphate product on cardiovascular mortality, Coen *et al.*^[55] concluded that the mortality of patients on LTH is higher due to non-traditional risk factors.

It was shown that hyperphosphatemia is an independent factor determining the unfavorable prognosis, accelerating the progression of IHD, aggravating systolic hypertension and LVH, increasing the risk of arrhythmia, as well as acute and congestive HF in patients on LTH^[56].

DIABETES MELLITUS AND VC

Type 2 diabetes is one of the main independent risk factors for the development of cardiovascular pathology that is the cause of death of more than 60% of patients with type 2 diabetes^[57]. In case of combined pathology (diabetes and atherosclerosis), the vascular wall is subject to changes that lead to a decrease in the effective lumen of the artery or thromboembolic complications.

However, in patients with diabetes in addition to atherosclerosis, calcification occurs. It was shown that the intensity of calcification increases in cases of diabetes, as confirmed by Peter Lanzer *et al.*^[58]. Scientists have concluded that Menkeberg sclerosis is x4.5 time more likely to be present in women and x1.8 in men with diabetes than in individuals of appropriate age and sex who do not suffer from diabetes. Pathogenesis of VC in diabetes is similar to pathogenetic processes occurring in CKD.

Ishimura *et al.*^[59] compared the factors influencing the calcification of peripheral vessels in patients on long-term dialysis suffering from type 2 diabetes and without type 2 diabetes. It was revealed that the prevalence of VC in patients with diabetes was higher than in patients without diabetes. DM often combines with

calcification of the arteries, the presence of which is a reliable marker of future cardiovascular events due to a combination of pathogenetic mechanisms in CKD and DM. There was also a high prevalence of CAC in patients with diabetes with CKD at stages 2-5^[60].

It was shown that patients with diabetes before dialysis have a greater risk of developing VC. CAC was calculated by CT. The prevalence of CAC and calcification of peripheral arteries were significantly higher in patients with diabetes and CKD at the pre-dialysis stage compared with the group of CKD stages 4-5 without diabetes^[61].

According to CT, the aortic calcification index was also significantly higher in patients with diabetes on hemodialysis than without diabetes^[62].

Qu *et al.*^[63] studied the importance of coronary Ca according to CT in the development of cardiovascular morbidity and mortality in patients with diabetes for 6 years. They found that in patients with diabetes and coronary calcification, a fourfold increase in mortality was noted. It was concluded that the risk of developing cardiovascular pathology increases with the presence of diabetes, age and VC.

CONCLUSION

Thus, the analysis of literature data indicates a high occurrence of diabetes, especially in the terminal stage of CKD patients on LTH. At the same time, the influence of both m-LDL and atherosclerosis to LDL (traditional risk factors in the development of cardiovascular morbidity and mortality in patients), as well as FGF-23 and the Kloth protein, and P-Ca ratio (non-traditional factors) that contribute to VC in diabetes mellitus, which is very important the study of these changes will allow developing more optimal approaches to the prevention of cardiovascular morbidity and mortality in patients with diabetes complicated by DN.

DECLARATIONS

Authors' contributions

Selection of material and writing the text: Archakova T

Design and correction of the review: Nedosugova L

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Consultant Doctor. Russian endocrinology clinical recommendations. Available from: <http://www.rosmedlib.ru/book/ISBN9785970436837.html>. [Last accessed on 24 Oct 2018] (in Russian)
2. Foley RN, Culleton BF, Parfrey PS, Harnett JD, Kent GM, et al. Cardiac disease in diabetic end-stage renal disease. *Diabetologia* 1997;40:1307-12.
3. Tsujimoto T, Kajio H, Takahashi Y, Kishimoto M, Noto H, et al. Asymptomatic coronary heart disease in patients with type 2 diabetes with vascular complications: a cross-sectional study. *BMJ Open* 2011; doi: 10.1136/bmjopen-2011-000139.
4. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes care* 1993;16:434-44.
5. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. *Circulation* 2003;108:2154-69.
6. Dedov II, Shestakova MV. Results of the implementation of the subprogram "diabetes mellitus of the federal target program" prevention and control of socially significant diseases 2007-2012. Available from: <https://cyberleninka.ru/article/n/rezultaty-realizatsii-podprogrammy-saharnyy-diabet-federalnoy-tselevoy-programmy-preduprezhdenie-i-borba-s-sotsialno-znachimymi>. [Last accessed on 24 Oct 2018] (in Russian)
7. Suzuki C, Nakamura S, Ishibashi-Ueda H, Yoshihara F, Kawano Y. Evidence for severe atherosclerotic changes in chronic hemodialysis patients: comparative autopsy study against cardiovascular disease patients without chronic kidney disease. *Ther Apher Dial* 2011;15:51-7.
8. Xue JL, Frazier ET, Herzog CA, Collins AJ. Association of heart disease with diabetes and hypertension in patients with ESRD. *Am J Kidney Dis* 2005;45:316-23.
9. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004;351:1296-305.
10. Tanaka M, Yasaka M, Nagano K, Otsubo R, Oe H, et al. Moderate atheroma of the aortic arch and the risk of stroke. *Cerebrovasc Dis* 2006;21:26-31.
11. Sen S, Oppenheimer SM, Lima J, Cohen B. Risk factors for progression of aortic atheroma in stroke and transient ischemic attack patients. *Stroke* 2002;33:930-5.
12. Ku E, Campese V. Is lipid management effective for all stages of CKD? *Blood Purif* 2013;35:26-30.
13. Bhowmik D, Tiwari SC. Metabolic syndrome and chronic kidney disease. *Indian J Nephrol* 2008;18:1-4.
14. Kato A, Takita T, Maruyama Y, Kumagai H, Hishida A. Impact of carotid atherosclerosis on long-term mortality in chronic hemodialysis patients. *Kidney Int* 2003;64:1472-9.
15. Gluba A, Olechnowicz-Tietz S, Paradowska A, Banach M, Rysz J. The risk of atherosclerosis in patients with chronic kidney disease. *Int Urol Nephrol* 2013;45:1605-12.
16. Vaziri ND. Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences. *Am J Physiol Renal Physiol* 2006;290:F262-72.
17. Mutluay R, Degertekin CK, Poyraz F, Yılmaz MI, Yücel C, et al. Dialysis type may predict carotid intima media thickness and plaque presence in end-stage renal disease patients. *Adv Ther* 2012;29:370-82.
18. Ragino YI, Nikitin YP. Oxidized and structurally-modified low-density lipoproteins in atherosclerosis. *Atherosclerosis* 2006;2:3-32. (in Russian)
19. Stocker R, Kearney JF Jr. Role of oxidative modifications in atherosclerosis. *Physiol Rev* 2004;84:1381-478.
20. Medical Theses. Mechanisms and role of atherogenic modification of lipoproteins in atherogenesis. Available from: <http://medical-diss.com/medicina/mechanizmy-i-rol-aterogennoy-modifikatsii-lipoproteidov-v-aterogeneze>. [Last accessed on 24 Oct 2018] (in Russian)
21. Ylä-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest* 1989;84:1086-95.
22. Belova LA, Oglobina OG, Belov AA, Kukharchuk VV. Processes of modification of lipoproteins. Physiological and pathogenetical role of modified lipoproteins. *Vopr Med Khim* 2000;46:8-21. (in Russian)
23. Sukhorukov VN, Karagodin VP, Orekhov AN. Atherogenic modifications of low-density lipoproteins. *Biomed Khim* 2016;62:391-402. (in Russian)
24. Lankin VZ, Konovalova GG, Tikhaze AK, Nedosugova LV. The influence of glucose on the free radical peroxidation of low density lipoproteins in vitro and in vivo. *Biomed Khim* 2012;58:339-52. (in Russian)
25. Borodachev EN, Sobenin IA, Karagodin VP, Bobryshev Yu.V, Orekhov A. Multiple modification of low density lipoproteins in diabetes. *Pathogenesis* 2013;11:16-21. (in Russian)
26. Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, et al. Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc Natl Acad Sci U S A* 1994;91:9441-5.
27. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 2000;342:1478-83.
28. Giachelli CM. Vascular calcification mechanisms. *J Am Soc Nephrol* 2004;15:2959-64.
29. Volgina G, Seleznev D, Balkarova O, Lovchinsky E. Extrinsic calcification in patients with CKD. *Vrach* 2012;7:2-8. (in Russian)

30. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. *Circulation* 1995;92:2157-62.
31. Ribeiro S, Ramos A, Brandão A, Rebelo JR, Guerra A, et al. Cardiac valve calcification in haemodialysis patients: role of calcium-phosphate metabolism. *Nephrol Dial Transplant* 1998;13:2037-40.
32. Kalpakian MA, Mehrotra R. Vascular calcification and disordered mineral metabolism in dialysis patients. *Semin Dial* 2007;20:139-43.
33. Raggi P, Boulay A, Chasan-Taber S, Amin N, Dillon M, et al. Cardiac calcification in adult hemodialysis patients. A link between end-stage renal disease and cardiovascular disease? *J Am Coll Cardiol* 2002;39:695-701.
34. Bhan I, Thadhani R. Vascular calcification and ESRD: a hard target. *Clin J Am Soc Nephrol* 2009; doi: 10.2215/CJN.04800709.
35. Braun J, Oldendorf M, Moshage W, Heidler R, Zeitler E, et al. Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. *Am J Kidney Dis* 1996;27:394-401.
36. Komaba H, Igaki N, Takashima M, Goto S, Yokota K, et al. Calcium, phosphorus, cardiovascular events and all-cause mortality in hemodialysis patients: a single-center retrospective cohort study to reassess the validity of the Japanese Society for Dialysis Therapy guidelines. *Ther Apher Dial* 2008;12:42-8.
37. Bellasi A, Ferramosca E, Ratti C, Block G, Raggi P. Cardiac valve calcification is a marker of vascular disease in prevalent hemodialysis patients. *J Nephrol* 2012;25:211-8.
38. Lee CT, Chua S, Hsu CY, Tsai YC, Ng HY, et al. Biomarkers associated with vascular and valvular calcification in chronic hemodialysis patients. *Dis Markers* 2013;34:229-35.
39. Volkov MM, Degtereva OA, Shevyakova EV. Factors associated with calcification of valvular heart apparatus in patients on chronic hemodialysis. Available from: <https://cyberleninka.ru/article/n/factory-svyazannye-s-kaltsinatsiey-klapannogo-apparata-serdtsa-u-patsientov-na-hronicheskom-gemodialize>. [Last accessed on 24 Oct 2018] (in Russian)
40. Cheng CY, Kuro-o M, Razzaque MS. Molecular regulation of phosphate metabolism by fibroblast growth factor-23- klotho system. *Adv Chronic Kidney Dis* 2011;18:91-7.
41. Riminucci M, Collins MT, Fedarko NS, Cherman N, Corsi A, et al. FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting. *J Clin Invest* 2003;112:683-92.
42. Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* 2004;19:429-35.
43. Volgina G, Shtandel V, Balkarova O, Lovchinsky E. Hyperphosphatemia in chronic kidney disease: modern correction strategy. *Vrach* 2012;7:19-23. (in Russian)
44. Milovanova LY, Milovanov YS, Kozlovskaya LV, Mukhin NA. New markers of cardiorenal interrelations in chronic kidney disease. *Therapeutic Archives* 2013;6:17-24. (in Russian)
45. Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, et al. High levels of serum fibroblast growth factor (FGF)-23 are associated with increased mortality in long haemodialysis patients. *Nephrol Dial Transplant* 2009;24:2792-6.
46. Grabner A, Schramm K, Silswal N, Hendrix M, Yanucil C, et al. FGF23/FGFR4-mediated left ventricular hypertrophy is reversible. *Sci Rep* 2017;7:1993.
47. Inaba M, Okuno S, Imanishi Y, Yamada S, Shioi A, et al. Role of fibroblast growth factor-23 in peripheral vascular calcification in non-diabetic and diabetic hemodialysis patients. *Osteoporos Int* 2006;17:1506-13.
48. Chan GC, Divers J, Russell GB, Langefeld CD, Wagenknecht LE, et al. FGF23 concentration and APOL1 genotype are novel predictors of mortality in African Americans with type 2 diabetes. *Diabetes Care* 2018;41:178-86.
49. Gutiérrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med* 2008;359:584-92.
50. Suliman ME, Stenvinkel P, Bárány P, Heimbürger O, Anderstam B, et al. Hyperhomocysteinemia and its relationship to cardiovascular disease in ESRD: influence of hypoalbuminemia, malnutrition, inflammation, and diabetes mellitus. *Am J Kidney Dis* 2003;41:S89-95.
51. Kardami E, Jiang ZS, Jimenez SK, Hirst CJ, Sheikh F, et al. Fibroblast growth factor 2 isoforms and cardiac hypertrophy. *Cardiovasc Res* 2004;63:458-66.
52. Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, et al. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* 2005;16:520-8.
53. Munoz Mendoza J, Isakova T, Cai X, Bayes LY, Faul C, et al. Inflammation and elevated levels of fibroblast growth factor 23 are independent risk factors for death in chronic kidney disease. *Kidney Int* 2017;91:711-9.
54. Rahman A, Gibney L, Person SD, Williams OD, Kiefe C, et al. Validity of self-reports of reasons for hospitalization by young adults and risk factors for discordance with medical records: the coronary artery risk development in young adults (CARDIA) study. *Am J Epidemiol* 2005;162:491-8.
55. Coen G, Manni M, Mantella D, Pierantozzi A, Balducci A, et al. Are PTH serum levels predictive of coronary calcifications in haemodialysis patients? *Nephrol Dial Transplant* 2007;22:3262-7.
56. Giachelli CM. Vascular calcification mechanisms. *J Am Soc Nephrol* 2004;15:2959-64.
57. Freedman BI, Divers J, Russell GB, Palmer ND, Bowden DW, et al. Plasma FGF23 and calcified atherosclerotic plaque in african americans with type 2 diabetes mellitus. *Am J Nephrol* 2015;42:391-401.
58. Lanzer P, Boehm M, Sorribas V, Thiriet M, Janzen J, et al. Medial vascular calcification revisited: review and perspectives. *Eur Heart J* 2014;35:1515-25.
59. Ishimura E, Okuno S, Kitatani K, Kim M, Shoji T, et al. Different risk factors for peripheral vascular calcification between diabetic and

- non-diabetic haemodialysis patients--importance of glycaemic control. *Diabetologia* 2002;45:1446-8.
60. Combe C, McCullough KP, Asano Y, Ginsberg N, Maroni BJ, et al. Kidney disease outcomes quality initiative (K/DOQI) and the dialysis outcomes and practice patterns study (DOPPS): nutrition guidelines, indicators, and practices. *Am J Kidney Dis* 2004;44:39-46.
 61. Titan SM, Zatz R, Gracioli FG, dos Reis LM, Barros RT, et al. FGF-23 as a predictor of renal outcome in diabetic nephropathy. *Clin J Am Soc Nephrol*. 2011;6:241-7.
 62. Palmer SC, Hayen A, Macaskill P, Pellegrini F, Craig JC, et al. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. *JAMA* 2011;305:1119-27.
 63. Qu W, Le TT, Azen SP, Xiang M, Wong ND, et al. Value of coronary artery calcium scanning by computed tomography for predicting coronary heart disease in diabetic subjects. *Diabetes Care* 2003;26:905-10.

Original Article

Open Access



Revascularization method for patients with infrainguinal arterial disease

Glushkov Nikolay, Ivanov Michael, Artemova Anastasia, Puzdriak Petr, Uryupina Anastasia, Bondarenko Pavel, Ivan Tigrov

Department of General Surgery, North-West State Medical University, Saint Petersburg 195067, Russia.

Correspondence to: Dr. Puzdriak Petr, Department of General Surgery, North-West State Medical University, Saint Petersburg 195067, Russia. E-mail: hirurg495@yandex.ru

How to cite this article: Nikolay G, Michael I, Anastasia A, Petr P, Anastasia U, Pavel B, Tigrov I. Revascularization method for patients with infrainguinal arterial disease. *Vessel Plus* 2018;2:35. <http://dx.doi.org/10.20517/2574-1209.2018.45>

Received: 9 Jun 2018 **First Decision:** 28 Aug 2018 **Revised:** 13 Sep 2018 **Accepted:** 18 Sep 2018 **Published:** 29 Oct 2018

Science Editors: Mario F. L. Gaudino, Igor A. Sobenin **Copy Editor:** Cui Yu **Production Editor:** Zhong-Yu Guo

Abstract

Aim: The aim of the study was to perform a comparative evaluation of the use of various methods of reconstructive assistance in the repair of the femoral-tibial segment in patients with peripheral arterial disease.

Methods: Two hundred and fifty-three patients with atherosclerotic lesions of arteries below the inguinal ligament were examined and revascularized. According to the type of reconstruction performed, the patients were divided into 3 groups: 98 patients underwent open operative interventions; 116 patients underwent endovascular interventions; 39 had hybrid reconstructions performed.

Results: Minor blood loss, and stability of hemodynamics in the perioperative context positively characterize hybrid effects. The time spent in the resuscitation department and the shorter hospitalization of patients after hybrid revascularization methods were revealed in comparison with open methods. The absence of dangerous complications and the primary patency of the operated segment in the early postoperative period, approaching 100%, characterize hybrid techniques as an effective method of treating patients with infrainguinal arterial disease.

Conclusion: Hybrid technologies are characterized by a shorter duration of surgical intervention, a low amount of blood loss and a lower incidence of complications in the early periods. The primary patency of the operated segment after hybrid techniques was higher than after open and endovascular surgical interventions.

Keywords: Peripheral arterial disease, hybrid intervention, claudication



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



INTRODUCTION

The comparative evaluation of the outcome after infrainguinal arterial disease (IAD) revascularization demonstrates controversial results^[1].

Bypass operations are the method of choice in the treatment of patients with IAD on the background of critical ischemia of the lower extremities^[2,3]. However, open interventions for distal segment are not always effective; there is a great deal of blood loss and the risk of infectious complications in comparison with minimally invasive interventions (balloon angioplasty and stenting)^[4,5]. The latter are now becoming increasingly common^[6]. Often endovascular interventions are used in the tibial artery reconstructions due to the high risk of poor wound healing after open operations^[7]. But the use of endovascular technologies at the level of the femoro-tibial segment is difficult with extensive stenotic and occlusive lesions, as well as arteries with wall calcification^[8].

The ascending spread of hybrid techniques has allowed the improvement of the results of surgical interventions at both proximal and distal segments, including patients with multilevel lesions, since the combined use of open and endovascular methods of revascularization allows to summarize the positive sides of each technique and to reduce the number of possible complications due to minimal trauma^[9].

Aim: to make a comparative assessment of the use of various reconstructive methods in the atherosclerotic lesions of the femoro-tibial segment.

METHODS

Two hundred and fifty-three patients with atherosclerotic lesions of arteries below the inguinal ligament were examined. According to the type of performed reconstruction, the patients were divided into 3 groups: the first group consisted of 98 patients who underwent open operative (OO) interventions; the second group consisted of 116 people who underwent endovascular procedures (EP): balloon angioplasty of femoral, popliteal or tibial arteries; the third group consisted of 39 patients, who had undergone hybrid surgery techniques (HS).

The primary endpoint was development of thrombosis of the operated segment, bleeding and surgical site infection, the need for re-interventions and amputations within 30 days after primary revascularization.

All patients underwent carbohydrate and lipid metabolism analysis: fasting glucose level, a day glucose fluctuations; for assessing of lipid metabolism the level of total cholesterol and its fractions were analyzed, as well as the atherogenicity coefficient value. The measurement of blood pressure in the perioperative period was carried out by direct and indirect methods.

Diabetes was diagnosed in accordance with the WHO recommendations. The evaluation of hypertension was carried out according to the WHO and the International Society for Hypertension classifications. The analysis of chronic heart failure (CHF) was carried out according to the New York Heart Association classification. All patients underwent a measurement of the ankle-brachial index before and after surgery; duplex scanning, CT angiography to determine the features of atherosclerotic lesion of the lower extremities arteries.

The frequency of patient concomitant disease is presented in [Table 1](#).

Variants of the performed operations are presented in [Table 2](#).

The evaluation of the operation time duration, blood loss volume, length of stay in the intensive care unit, in-hospital stay days, fluctuations in blood pressure and blood glucose level. In the postoperative period the

Table 1. Patient characteristics and concomitant disease

Criteria	OO	EP	HS	P
Sex, female, <i>n</i> (%)	26 (26.53%)	50 (43.1%)	12 (30.77%)	0.03
Age, year	70.47 ± 9.1	65.87 ± 10.3	65.45 ± 11.9	0.001
Diabetes, <i>n</i> (%)	37 (37.76%)	13 (11.21%)	10 (25.64%)	0.001
Hypertonic disease (HD), <i>n</i> (%)	85 (86.73%)	101 (87.07%)	28 (71.79%)	0.06
2 stage of HD, <i>n</i> (%)	59 (60.20%)	74 (75.51%)	23 (58.97%)	0.43
3 stage of HD, <i>n</i> (%)	23 (23.47%)	20 (17.24%)	5 (12.82%)	
CHF*, <i>n</i> (%)	37 (37.76%)	89 (76.72%)	17 (43.59%)	0.001
CHF, 2st by NYHA, <i>n</i> (%)	23 (23.47%)	72 (62.07%)	10 (25.64%)	0.08
CHF, 3st by NYHA, <i>n</i> (%)	11 (11.23%)	17 (14.65%)	7 (17.95%)	
CAD*, <i>n</i> (%)	81 (82.65%)	104 (89.66%)	28 (71.79%)	0.10
CAD, F/Class 1-2, <i>n</i> (%)	60 (61.23%)	61 (52.59%)	14 (35.89%)	0.075
CAD, F/Class 3-4, <i>n</i> (%)	21 (21.43%)	43 (37.07%)	10 (25.64%)	
Myocardial infarction, <i>n</i> (%)	8 (8.16%)	9 (7.76%)	12 (30.77%)	0.001
Chronic kidney disease, <i>n</i> (%)	21 (21.43%)	23 (19.83%)	6 (15.38%)	0.72
Smoking, <i>n</i> (%)	69 (70.41%)	58 (50%)	10 (25.64%)	0.001
CVD*, <i>n</i> (%)	27 (27.55%)	45 (38.79%)	7 (17.95%)	0.009

*CVD: cerebrovascular disease; CHF: chronic heart failure; NYHA: New York Heart Association; CAD: coronal arterial disease; OO: open operative; EP: endovascular procedures; HS: hybrid surgery techniques

Table 2. Surgical interventions

Type of surgery	OO	EP	HS
Superficial femoral artery loop endarterectomy	27	-	2
Femoro-popliteal bypass above knee	17	-	30
Femoro-popliteal bypass under knee	49	-	7
Femoro-tibial bypass	5	-	-
Balloon angioplasty of the superficial femoral artery, popliteal or tibial arteries	-	116	39

OO: open operative; EP: endovascular procedures; HS: hybrid surgery techniques

following criteria were assessed: the primary patency of the operated segment, the incidence of complications (thrombosis of the surgical site, bleeding, infectious complications, myocardial infarction, stroke, acute renal failure), the need for re-intervention on the operated segment and the number of amputations in the first 30 days after primary intervention.

Statistical processing of the obtained results was carried out using the Stata Statistica 10 data analysis package. We used the Kolmogorov-Smirnov criterion for determining the normality of the quantitative data distribution. The quantitative characteristics were presented in the following form: mean ± standard deviation. The identification of differences between groups was detected using the nonparametric Mann-Whitney *U* test (*t*-test). The description of qualitative features was carried out in the form of relative frequencies and expressed as a percentage. The reliability of the distribution of qualitative characteristics was determined using the χ^2 criterion. Differences between groups are considered reliable at $P < 0.05$.

RESULTS

Among the patients from the EP group, the greatest number of people with CHF and cerebrovascular disease were present. The group with hybrid operations contained the greatest proportion of people with postinfarction cardiosclerosis, that indicating a minimal invasive of this intervention. Among patients with open reconstruction were persons of the oldest age, and they were associated with a significant degree of calcification and prolonged occlusive-stenotic lesion [Table 1].

The degree of ischemia and features of atherosclerotic lesions were differentiated based on Transatlantic Intersociety Classification II (TASC II) in Figures 1 and 2.

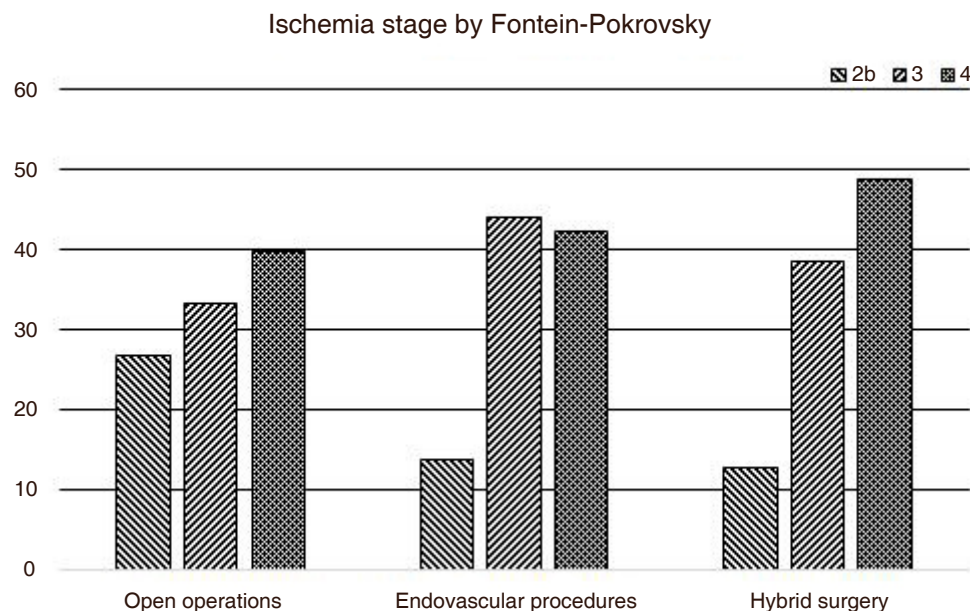


Figure 1. Patient distribution with different ischemia level in clinical groups, y-axis - %

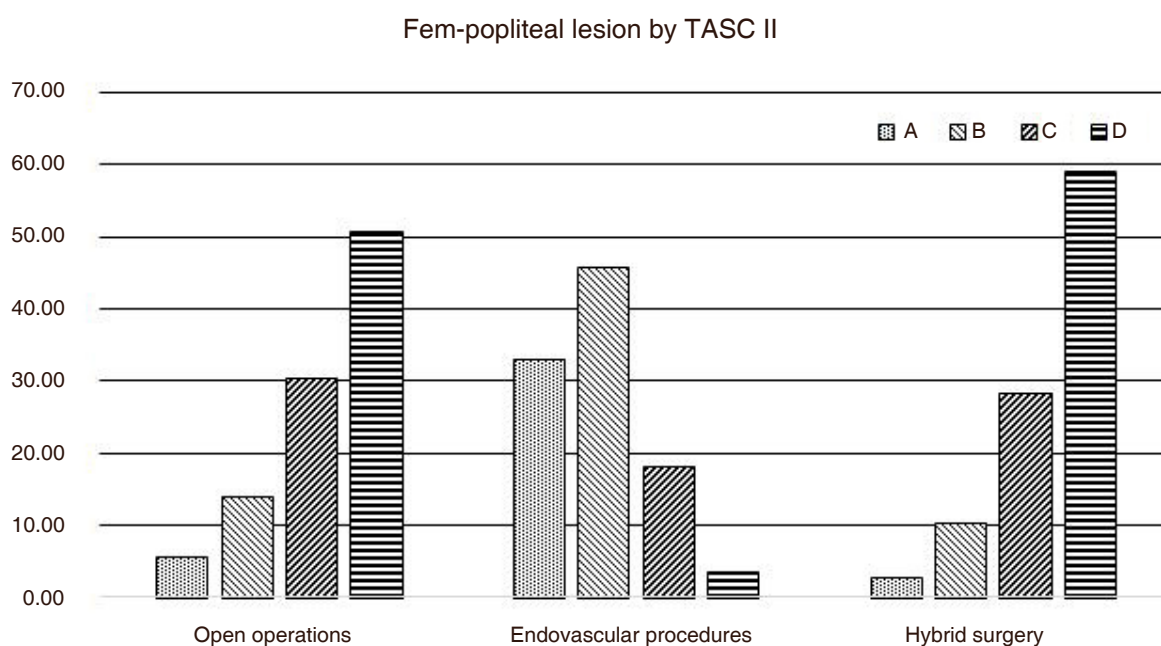


Figure 2. Distribution of atherosclerotic lesions A/B and C/D according to TASC II between clinical groups, y-axis - %

The greatest number of patients with critical ischemia was recorded in the HS group, the lowest in the OO group, which is due to the restriction on performing open reconstructions against the background of trophic changes (according to Rutherford).

The maximum number of persons with C/D lesions by TASC II were noted in the HS group; the minimum is in the EP group.

Analyzed metabolic changes in patient groups are shown in [Table 3](#).

Table 3. Patients metabolic disorders in clinical groups

Metabolic changes	OO	EP	HS	P
Fasting glucose level, mmol/L	7.13 ± 1.9	7.32 ± 1.9	6.34 ± 2.5	0.02
Cholesterol, mmol/L	6.79 ± 1.1	7.73 ± 1.6	6.66 ± 1.7	0.001
Triglycerides, mmol/L	1.77 ± 0.8	2.08 ± 1.4	3.33 ± 1.4	0.001
Low-density lipoproteins, mmol/L	4.08 ± 1.1	4.49 ± 1.2	3.77 ± 1.5	0.02
High-density lipoproteins, mmol/L	0.92 ± 0.3	1.08 ± 0.4	1.05 ± 0.3	0.06
Atherogenicity coefficient	7.23 ± 2.7	6.67 ± 2.6	5.38 ± 2.8	0.01
Body mass index	27.88 ± 3.6	28.48 ± 3.6	28.17 ± 2.7	0.43
sBP, mmHg	148.45 ± 18.1	153.47 ± 20.5	156.00 ± 20.3	0.03
dBp, mmHg	86.29 ± 8.3	79.64 ± 13.2	85.88 ± 7.1	0.001
ABI	0.40 ± 0.1	0.43 ± 0.1	0.39 ± 0.1	0.28

sBP: systolic blood pressure; dBp: diastolic blood pressure; ABI: ankle-brachial index; OO: open operative; EP: endovascular procedures; HS: hybrid surgery techniques

It should be noted that significant metabolic changes were registered in all analyzed groups, including significant disturbances of carbohydrate and lipid metabolism. But with a severe lipid balance violation, the method of choice for intervention was conventional.

The blood loss during hybrid interventions was significantly less than in the case of open reconstruction operations: this circumstance is especially significant considering the comorbid characteristics of patients from the OO group.

The time of patients' stay in the intensive care unit was shortest after endovascular interventions and longest after open methods of reconstruction. Hemodynamic instability was often noted after open operations.

As a result of hybrid surgery, there was no need for re-intervention and amputations. The primary patency of the operated segment in this group in the early postoperative period was 100%.

The postoperative period after open reconstructions was characterized by a relatively high incidence of complications: thrombosis of the operated segment and surgical site infection. Among patients who underwent hybrid interventions such negative outcomes were not recorded.

The number of perioperative myocardial infarctions were not different in groups of OO and EP with significant invasiveness of open surgery.

Features of the perioperative period are indicated in [Table 4](#).

When analyzing the results of revascularization, depending on the degree of ischemia according to Fontein-Pokrovsky classification, the complicated course of the postoperative period prevailed in cases of critical ischemia [\[Table 5\]](#).

DISCUSSION

Data on the effectiveness of various revascularization techniques for lesions of the femoral-popliteal segment are ambiguous: some authors state the best primary patency of shunting surgeries and the worst results of endovascular techniques: low values of primary patency (58%), high percentage of limb loss one year after surgery^[10]. Other authors consider endovascular interventions as the method of choice in the distal type of lesion^[11,12]. Data from several studies did not reveal a difference in the immediate outcomes of open and endovascular surgical interventions^[13].

Table 4. Characteristics of the perioperative period

Perioperative period	OO	EP	HS	P
Durations of the OO, min	206.17 ± 84.73	-	175.00 ± 92.95	0.19
Durations of the EP, min	-	90.63 ± 53.36	77.50 ± 66.22	0.22
BP fluctuation during the operation, mmHg	40.62 ± 23.33	30.55 ± 14.15	35.83 ± 14.48	0.001
Blood loss, mL	790.93 ± 244.32	-	473.91 ± 177.01	0.001
ICU* staying, h	29.76 ± 18.79	15.63 ± 7.03	25.88 ± 9.73	0.001
Myocardial infarction, <i>n</i> (%)	2 (2.04%)	2 (1.72%)	-	0.68
Stroke, <i>n</i> (%)	1 (1.02%)	-	-	0.45
Operated segment thrombosis, <i>n</i> (%)	8 (8.16%)	2 (1.72%)	-	0.02
Bleeding, <i>n</i> (%)	4 (4.08%)	3 (2.59%)	-	0.30
Surgical site infection, <i>n</i> (%)	10 (10.2%)	4 (3.45%)	-	0.02
Primary patency, <i>n</i> (%)	83 (84.69%)	113 (88.79%)	39 (100%)	0.01
Re-intervention, <i>n</i> (%)	15 (15.31%)	13 (11.21%)	-	0.04
Amputations, <i>n</i> (%)	8 (8.163%)	6 (5.17%)	-	0.16

*ICU: intensive care unit; OO: open operative; EP: endovascular procedures; HS: hybrid surgery techniques; BP: blood pressure

Table 5. Features of the operating period depending on the degree of ischemia

Operation	Degree of ischemia	OO	EP	P
Operated segment thrombosis, <i>n</i> (%)	Claudication	2 (3.6%)	-	0.04
	CLI	6 (11.6%)	2 (2.02%)	0.006
Bleeding, <i>n</i> (%)	Claudication	1 (1.8%)	-	0.156
	CLI	3 (5.8%)	3 (3.03%)	0.307
Surgical site infection complication, <i>n</i> (%)	Claudication	4 (8.7%)	1 (6.25%)	0.421
	CLI	6 (14.0%)	3 (3.03%)	0.006
Re-intervention in 30 days, <i>n</i> (%)	Claudication	3 (6.5%)	1 (6.25%)	0.775
	CLI	11 (21.2%)	12 (12.12%)	0.087
Amputations, <i>n</i> (%)	Claudication	1 (1.8%)	-	0.156
	CLI	7 (13.5%)	6 (6.06%)	0.06

CLI: critical limb ischemia; OO: open operative; EP: endovascular procedures

The advantages of endovascular techniques include minimal invasiveness and, accordingly, good tolerability of the operation, which is especially important for patients with combined lesions of the lower extremity arteries and arteries of the coronary or carotid pool^[14]. Among the shortcomings of EP, there is the risk of restenosis and thrombosis at the level of the operated segment, as well as limitations due to anatomical features^[15]. According to the results of this study, endovascular techniques are characterized by a lower primary patency compared to open and hybrid interventions within 30 days after the intervention.

The main disadvantages of open methods are: a significant probability of infectious complications, a longer surgical time and a significant surgical risk^[13]. In this study, a significant frequency of so-called “large” complications in the group of open operations was noted in the early period after the intervention.

Hybrid surgical interventions were characterized by positive outcomes at an early stage, combining the advantages of open and endovascular techniques^[16]. As a result, hybrid operations are characterized by a lower incidence of limb loss^[17]. According to the present study there were no amputations recorded in the early period after hybrid interventions.

Advantages of hybrid techniques in comparison with open reconstructions are reduced duration of hospitalization and blood loss^[18]. It is also interesting to note that the difference between hybrid and endovascular reconstructions was in the smaller amount of contrast used and the lesser occurrence of bleeding in the puncture zone^[19]. All this contributes to reducing the risk of contrast-induced nephropathy, as well as other complications, reducing the cost of hybrid surgical interventions compared with open

methods due to shorter hospitalization times and insignificant time spent in the intensive care unit^[20]. According to the present study, the length of hospitalization and the amount of blood loss were less in HS than in the group of open operations.

Quantitative differences between the analyzed groups should be considered as the limitation of this study, as well as the lack of information on the long-term results.

Conclusion: hybrid technologies are characterized by a shorter duration of surgical intervention, a low amount of blood loss and a lower incidence of complications in the early periods. These findings open new prospects for revascularization in persons with severe comorbid pathology.

DECLARATIONS

Authors' contributions

Data collection, data analysis, manuscript writing: Michael I, Petr P
Statistical analysis, manuscript review: Michael I, Anastasia A, Pavel B
Manuscript editing and reviewing: Petr P
Study supervisor, manuscript reviewing: Nikolay G
Data collection: Tigrov I, Anastasia U

Availability of data and materials

At request. Kindly email corresponding author.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This report is not considered research by NHS, as defined by the UK Policy Framework for Health and Social Care Research. An informed consent of participation in the study was obtained from participants and approved from NHS Research Ethics Committee (REC). There was no children under 16 participate in this study.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Krotovsky GS, Zudin AM. Tactics of treatment of patients with critical ischemia of the lower extremities. Available from: <http://textarchive.ru/c-1061963.html>. [Last accessed on 26 Oct 2018] (in Russian)
2. Nicoloff AD, Taylor LM Jr, McLafferty RB, Moneta GL, Porter JM. Patient recovery after infrainguinal bypass grafting for limb salvage. *J Vasc Surg* 1998;27:256-63.
3. Rutherford RB, Baker JD, Ernst C, Johnston KW, Porter JM, et al. Recommended standards for reports dealing with lower extremity ischemia: revised version. *J Vasc Surg* 1997;26:517-38.
4. Dosluoglu HH, Lall P, Cherr GS, Harris LM, Dryjski ML. Role of simple and complex hybrid revascularization procedures for symptomatic lower extremity occlusive disease. *J Vasc Surg* 2010;51:1425-35.

5. Glushkov NI, Ivanov MA, Artemova AS, Petrov DA, Maksimkina ES. The infection in vascular surgery and metabolic syndrome: a coincidence or correlation? *Kardiologiya I Serdechno-Sosudistaya Khirurgiya* 2017;10:56-61. (in Russian)
6. Russian Society of Surgeons. National recommendations for the management of patients with diseases of arteries of lower extremities. Available from: http://xn---9sdbex7bdduahu3a5d.xn--p1ai/upload/disease_arteries.pdf. [Last accessed on 26 Oct 2018] (in Russian)
7. Matsagkas M, Kouvelos G, Arnaoutoglou E, Papa N, Labropoulos N, et al. Hybrid procedures for patients with critical limb ischemia and severe common femoral artery atherosclerosis. *Ann Vasc Surg* 2011;25:1063-9.
8. Uchkin IG, Shugushev ZKh, Talov NA, Bagdasaryan AG, Gonsales AK, et al. Experience with hybrid techniques of surgical treatment of patients with lower limb critical ischaemia. *Angiol Sosud Khir* 2013;19:48-57. (in Russian)
9. Koidan AA, Batalin IV, Vavilov VN, Kaputin Y, Atmadzas AV, et al. Comparative results of various methods of arterial reconstruction in the defeat of the femoral-popliteal segment in patients with chronic critical ischemia of the lower limbs. *Reg Blood Circ Microcir* 2017;16:41-8. (in Russian)
10. Gordon IL, Conroy RM, Arefi M, Tobis JM, Stemmer EA, et al. Three-year outcome of endovascular treatment of superficial femoral artery occlusion. *Arch Surg* 2001;136:221-8.
11. Clark TW, Groffsky JL, Soulen MC. Predictors of long-term patency after femoropopliteal angioplasty: results form the STAR registry. *J Vasc Interv Radiol* 2001;12:923-33.
12. Cvetanovski MV, Jovev S, Cvetanovska M, Blazevski B, Colanceski R, et al. Femoropopliteal bypass vs percutaneous transluminal angioplasty and stenting in treatment of peripheral artery diseases of infrainguinal segment - short-term results. *Priloz* 2009;30:105-18.
13. Glushkov NI, Ivanov MA, Artemova AS. Results of various revascularization methods in patients with critical ischemia of the lower limbs in the background of peripheral atherosclerosis. *Kardiologiya I Serdechno-Sosudistaya Khirurgiya* 2017;10:50-6. (in Russian)
14. Zatevakhin I, Shipovsky V, Zolkin V. Balloon angioplasty in ischemia of lower limbs. Moscow: Medicine Pub; 2004. (in Russian)
15. Eroshkin IA, Eroshenko AV, Vasilev UG, Kokov LS. Results of endovascular methods for the treatment of critical ischemia of the lower limbs in patients with diabetes mellitus. *Int J Interv Cardioangiol* 2008;14:36-36a. (in Russian)
16. Antoniou GA, Sfyroeras GS, Karathanos C, Achouhan H, Koutsias S, et al. Hybrid endovascular and open treatment of severe multilevel lower extremity arterial disease. *Eur J Vasc Endovasc Surg* 2009;38:616-22.
17. Schillinger M, Diehm N, Baumgartner I, Minar E. TASC II section F on revascularization: commentary from an intervencionist's point of view. *J Endovasc Ther* 2007;14:734-42.
18. Troitskiĭ AV, Bekhtev AG, Khabazov RI, Beliakov GA, Lysenko ER, et al. Outcomes of hybrid operations in multi storeyed lesions of arteries of the aortoiliac and femoropopliteal segments. *Angiol Sosud Khir* 2012;6:67-77. (in Russian)
19. Nishibe T, Kondo Y, Dardik A, Muto A, Koizumi J, et al. Hybrid surgical and endovascular therapy in multifocal peripheral TASC D lesions: up to three-year follow-up. *J Cardiovasc Surg (Torino)* 2009;50:493-9.
20. Thomas M. Treatment of chronic lower limb ischemia. In: Jonathan DB, editor. *Vascular and endovascular surgery*. USA: Elsevier; 2006. pp. 35-6.

Review

Open Access



Vascular smooth muscle cell contractile function and mechanotransduction

Sultan Ahmed, Derek T. Warren

School of Pharmacy, University of East Anglia, Norwich, NR4 7TJ, UK.

Correspondence to: Dr. Derek T. Warren, School of Pharmacy, University of East Anglia, Norwich, NR4 7TJ, UK.
E-mail: derek.warren@uea.ac.uk

How to cite this article: Ahmed S, Warren DT. Vascular smooth muscle cell contractile function and mechanotransduction. *Vessel Plus* 2018;2:36. <http://dx.doi.org/10.20517/2574-1209.2018.51>

Received: 5 Jul 2018 **First Decision:** 19 Sep 2018 **Revised:** 15 Oct 2018 **Accepted:** 17 Oct 2018 **Published:** 5 Nov 2018

Science Editor: Alexander D. Verin **Copy Editor:** Cui Yu **Production Editor:** Zhong-Yu Guo

Abstract

Vascular smooth muscle cells (VSMCs) are the predominant cell type in the arterial wall and normally adopt a quiescent, contractile phenotype to regulate vascular tone. In the arterial wall, VSMCs are exposed to multiple mechanical cues, including stretch and matrix stiffness, which regulate VSMC contraction. However, during ageing and in vascular disease, such as atherosclerosis, hypertension and vascular calcification, the arterial wall stiffens and VSMC contraction contributes to this process. VSMCs display remarkable plasticity and changes in their mechanical environment promote VSMCs to adopt a proliferative, synthetic phenotype. VSMC phenotypic modulation is associated with altered expression of contractile proteins that generate actomyosin-based force. However, our understanding of precise mechanisms whereby altered mechanical landscape and mechanotransduction influence VSMC contraction remains limited. In this review, we discuss the present literature describing how VSMCs sense and respond to changes in their mechanical environment and how these changes influence VSMC contraction.

Keywords: Matrix stiffness, mechanotransduction, vascular smooth muscle cell, contraction

INTRODUCTION

Cardiovascular disease (CVD) remains one of most prevalent risk factors to health worldwide, and is the second highest cause of mortality within the UK^[1]. The aberration of health caused from CVD places a heavy burden on the health-care of developing countries as well as representing a major cause of death and morbidity in industrialised countries^[2,3]. CVD is an umbrella term, which holds host to multiple related diseases, including peripheral arterial disease, coronary heart disease and stroke^[4]. The risk factors of each vary depending on the specificity of the disease, however many present common symptoms, providing a crucial



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



link in treatment methods. Common physiological risk factors are hypertension, obesity, a rise in cholesterol and diabetes with treatment of each providing a larger scale preventative therapy of CVD^[5]. Despite this, the largest risk factor associated with CVD is ageing, an inevitable process that all individuals undergo.

Ageing causes an increase in CVD incidence and prevalence due to wide array of changes that occurs as a person becomes older. The cause of ageing requires a diverse and intricate investigation into the crosstalk between multiple genetic and environmental (i.e., diet, exercise and smoking) factors^[6]. A key modification observed is the structural and functional alterations within the vasculature. This includes the stiffening of the arteries, in particular the aorta. The function of the aorta is crucial in converting the large output of oxygenated blood from the left ventricle into a more controlled flow within the smaller arterioles and capillaries^[7]. Stiffening ultimately reduces aortic compliance and increases systolic arterial pressure that augments the overall vascular resistance^[8]. To compensate, the left ventricle adopts a compensatory mechanism creating a change in the end-systolic volume as well as prolonging systolic contraction. A direct consequence of this is the thickening of the left ventricle, which causes an aberrant hypertrophic physiology^[9]. Coupled to this, ageing also causes defects in the repair mechanisms of the vasculature which further drives the diseased-phenotypic changes^[10]. This, in turn, diminishes the capability of the vascular system to overcome the increased workload that is generated as a repercussion. Thus, ageing presents as the most predicative cause of CVD.

ARTERIAL STIFFNESS: CAUSE AND RELEVANCE IN CVD

Arterial stiffness is a predicative biomarker in ageing and CVD, including atherosclerosis, hypertension and obese populations^[11-13]. Normally, pulse pressure expands the elastic arteries, transferring energy from the blood to the arterial wall and slowing pulse velocity. In conditions of enhanced arterial stiffness, pulse pressure is no longer able to expand the artery, increasing pulse velocity, and pulse pressure is transmitted to the microcirculation of organs such as the heart and lungs^[14]. Vessels of the microcirculation are more fragile, resulting in damage to the microcirculation. The current gold standard method in assessing arterial stiffness is pulse wave velocimetry (PWV)^[15]. A higher PWV is linked to individuals who have greater risk of CVD^[16]. As a result, this method can be utilised to provide a predictive analysis of CVD independent of standard blood pressure measurements of the brachial artery^[17].

ARTERIAL STRUCTURE

Elastic arteries, including the aorta, are structurally composed of three layers; the tunica intima is the innermost layer [Figure 1]. It is comprised of a sheet of endothelial cells along with a basal membrane and collagen fibrils. The tunica media neighbours the tunica intima as the middle layer and is primarily composed of vascular smooth muscle cells (VSMCs) reinforced with elastin and collagen fibrils. The final and outermost layer is the tunica adventitia, containing largely connective tissue as a means to provide reinforcement to the structure of the aorta^[18]. This allows the aorta to act as an “elastic buffering chamber” in order to store and transmit blood to the peripheral circulation during systole and diastole, respectively^[19].

This function becomes aberrant when aortic stiffness increases. Stiffness augmentation of the aorta is ultimately driven by changes in extracellular matrix (ECM) composition, in particular enhanced elastic degradation as well as augmented collagen deposition^[20,21]. All three arterial layers are mechanoresponsive and remodel during vascular disease progression^[22-24]. In this review, we focus on the response of the VSMC layer to enhanced matrix stiffness. Elastin in small arteries and the aorta allows for vascular distensibility and in normal conditions, is found in high abundance within these vessels^[21]. However, this dogmatic view on arterial stiffness was recently found to be misleading, as an in depth analysis of the published data on hypertension found collagen levels to be inconsistent^[25]. Due to this, several studies have now found multiple contributing factors towards aortic stiffness, ranging from, but not limited to, mechanical stimuli, inflammatory cytokines and compositional changes in the ECM^[25]. The existing treatment therapies of aortic

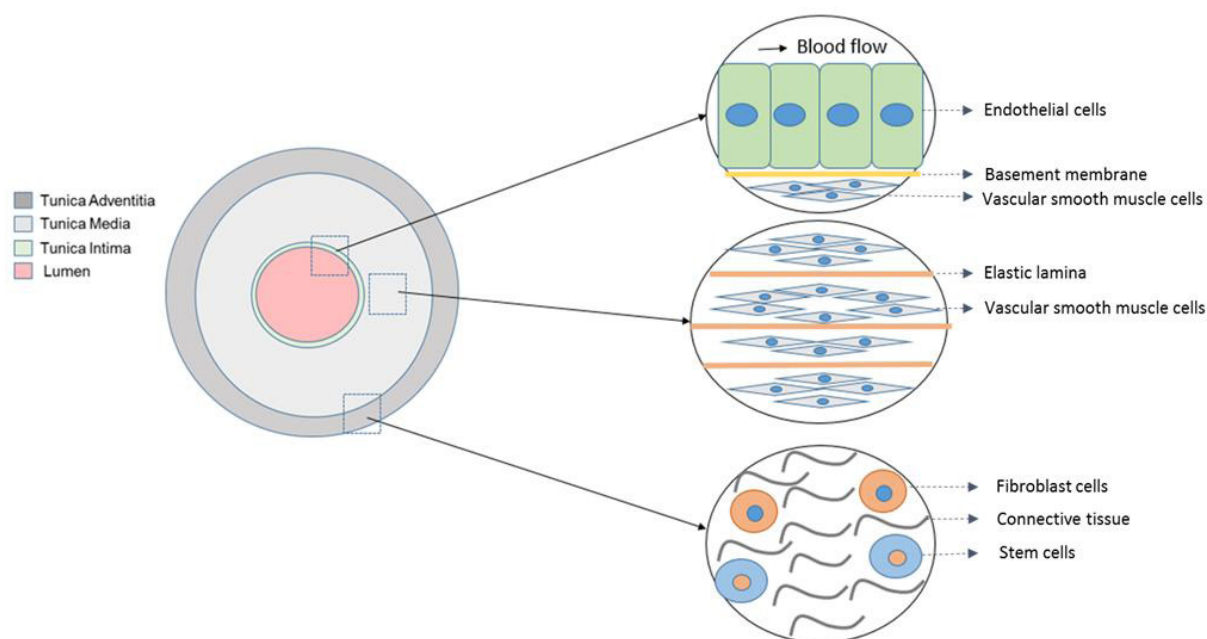


Figure 1. Schematic representation of arterial structural organisation

stiffness involve different anti-hypertensive drugs, which have an indirect effect on arterial stiffness^[25]. These drugs block calcium-channels and angiotensin II receptors, both of which modulate VSMC contraction and vascular tone, as opposed to ECM composition. Therefore, VSMCs have a fundamental role in the aortic stiffness and much of the focus has now shifted onto VSMC structure and function^[26].

VSMC PHENOTYPIC MODULATION AND THE CYTOSKELETON

VSMC are the predominant cell type within the arterial wall. They are arranged in a fibrous helix and regulate vessel diameter and vascular tone^[27]. Within a mature artery, VSMCs exist in a quiescent, contractile state and regulate vascular tone via vessel constriction^[28]. However, VSMCs retain phenotypic plasticity and can dedifferentiate into a proliferative, synthetic state^[29,30]. VSMC phenotypic modulation is associated with developmental and disease associated vessel remodeling, where VSMCs exhibit higher rates of proliferation, migration and altered ECM deposition^[29,30].

The key filamentous components of the VSMC cytoskeleton are the intermediate filaments, microtubules and actin. Intermediate filaments, including vimentin and desmin, maintain VSMCs 3D structure^[31]. In contrast, the properties of microtubules are not as clearly defined due to variable tissue types and staining methods. Actin filaments transmit mechanical signals to dense plaques which act as signalling hubs and are found dispersed within the cytoplasm^[24]. Three different isoforms of actin exist, alpha, beta and gamma actin, with alpha actin being the abundant isoform typical within contractile VSMCs^[24,32]. Changes in both extracellular and intracellular tension, alter actin cytoskeletal organisation and regulate cell contraction, migration and survival^[33].

VSMC phenotypic modulation is commonly associated with altered contractile marker expression^[34]. Contractile VSMCs possess smooth muscle myosin II (SM-myosin II), smoothelin and smooth muscle-actin and these are downregulated in models where arterial injury^[35]. SM-myosin II is the dominant myosin isoform found within contractile VSMC and is composed of both two heavy and light chains. There are two different types of light chains identified as myosin light chain-20 (MLC-20) and MLC-17, with the phosphorylation of the former regulating VSMC contraction^[36]. In contrast, synthetic VSMCs contain non-

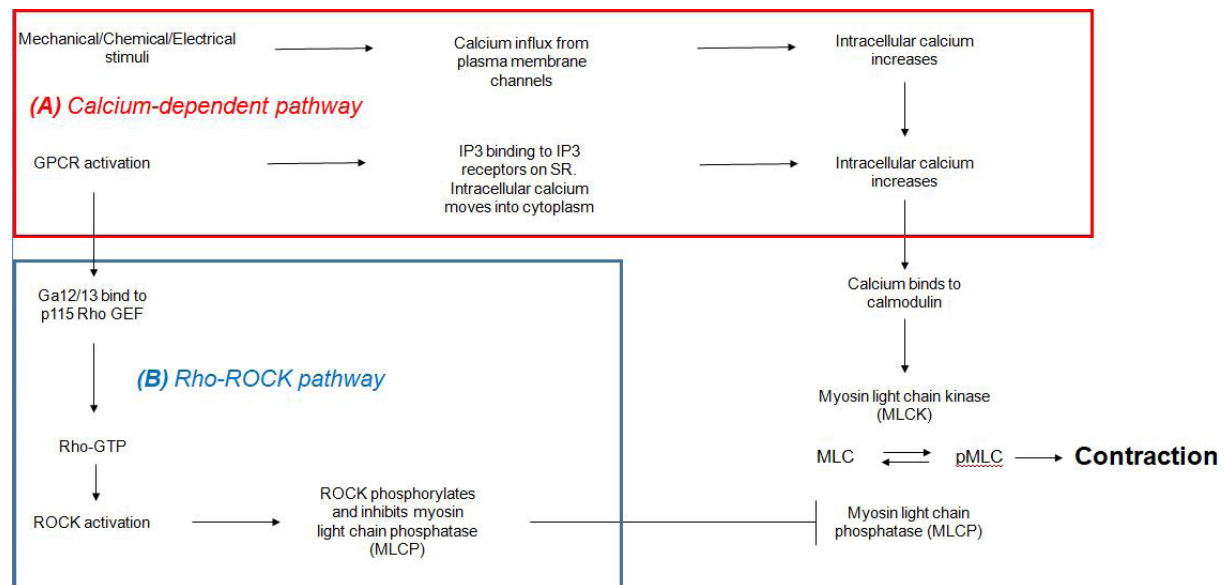


Figure 2. Calcium dependent and independent regulation of VSMC contraction. The two pathways work synergistically. Calcium dependent regulation is associated with transient phasic contraction whereas RhoA/ROCK regulation is associated with the prolonged tonic contraction of VSMCs. GPCR: G-protein coupled receptor; IP3: inositol triphosphate; SR: sarcoplasmic reticulum; Rho GEF: RhoGTPase guanine nucleotide exchange factors; ROCK: Rho-associated protein kinase; MLC: myosin light chain; VSMC: vascular smooth muscle cell

muscle myosin II (NM-myosin II), which is upregulated in the proliferative state^[35]. NM-myosin II is also expressed in the differentiated state, albeit at lower levels. Once phosphorylated, myosin associates with actin filaments to form the actomyosin complex. The ATPase activity of the myosin then results in rapid cycling of the cross-bridges formed between actin and myosin, thus causing a pulling of the actin thin filaments^[36]. The physiological function of the two myosin isoforms vary and the SM-myosin II has a higher immune-reactivity in tissue areas where faster phasic VSMC contractions were occurring^[37]. This is in contrast to NM-myosin II, which primarily regulates the slower tonic phase of VSMC contraction^[37]. The tonic contraction induced from NM-myosin II generates less force than phasic contraction produced from SM-myosin II^[37,38].

VSMC CONTRACTION

Calcium-dependent pathway

VSMC contraction occurs via two interlinked pathways that contribute synergistically to the contractile properties of VSMCs [Figure 2]. The first pathway, more commonly known as the calcium-dependent pathway, primarily involves augmenting cytoplasmic calcium levels to induce phasic contraction. Increased intracellular calcium can be triggered by mechanical, electrical and chemical stimuli, either by calcium influx from channels located on the plasma membrane or by release of calcium from the sarcoplasmic reticulum (SR)^[39]. Calcium entry from the extracellular space usually occurs via voltage gated calcium channels (VGCCs) or non-selective cation channels. Sub-populations of L-type, P/Q-type and T-type VGCCs are all found within VSMCs and are activated via depolarization^[40]. In addition, non-selective cation channels, found to predominantly be members of the transient receptor potential canonical family, allow for Na^+ and Ca^{2+} influx following receptor occupancy or capacitative calcium entry^[40].

Release of calcium from the SR is predominantly mediated by the activation of G-protein coupled receptors (GPCRs) (i.e., the AT_1 receptor) coupled to the G_{aq} G-protein. The G_{aq} protein, when in its GTP-bound state, causes activation of phospholipase C which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol triphosphate (IP₃) and diacylglycerol^[41,42]. IP₃ binds to the IP₃ receptors present on

the sarcolemma, which causes opening of the calcium channels and subsequent depletion of calcium intracellular store.

After an increase of cytoplasmic calcium concentration has been established, calmodulin becomes bound by 4 calcium ions. The resulting calcium-calmodulin complex interacts with and activates myosin light chain kinase (MLCK)^[43]. Next, MLCK phosphorylates MLC-20 (also known as the regulatory light chain) on the serine-19 and threonine-18 residues. The phosphorylation of serine-19 causes a resulting increase in the activity of the Mg^{2+} -ATPase and this effect is further enhanced by the phosphorylation of the latter residue. From this, the cross-bridge cycling is initiated and the myosin head can actively pull on the thin filament of the actin to induce contraction of the muscle^[44].

Rho/Rho-associated protein kinase pathway

In the absence of external contractile stimuli, the MLC-20 light chain remains phosphorylated at a low level. This low level leads to a slower tonic form of contraction, which regulates the vascular tone^[36]. A calcium-independent pathway that involves Rho/Rho-associated protein kinase (ROCK) signalling regulates VSMC tonic contractions [Figure 2]. This pathway not only caters to contractile function, but also extends to smooth muscle cell migration, proliferation and apoptosis^[45]. RhoA, part of the Ras superfamily, is a GTPase which can act as a molecular switch between a GTP/GDP bound state^[46]. In resting conditions, the Rho GDP dissociation inhibitor targets GDP-Rho for binding, as a means to localise the GTPase from the membrane to the cytosol. However, activation of GPCR receptors, in particular $G_{12/13}$ subtypes, can catalyse GTP for GDP exchange in RhoA by binding to p115 RhoGTPase guanine nucleotide exchange factors^[47]. In its GTP bound form, RhoA can interact with target proteins by utilising its C-terminal geranyl-geranylated tail to anchor itself to the plasma membrane^[47].

One of the target proteins activated by RhoA is ROCK^[48]. ROCK is a member of the protein kinase A, G and C family of protein kinases, and is characterised as a serine/threonine kinase. There are two isoforms of this kinase, referred to as ROCK1 and ROCK2, with expression of both present in VSMCs^[49]. Its structure is composed of an N-terminal kinase domain, a central coiled-coil domain and a C-terminal pleckstrin homology domain that associates with the Rho GTPase^[47]. ROCK has many effects within VSMCs and influences actomyosin activity by two main pathways. Firstly, ROCK actively regulates cytoskeletal organisation by preventing actin filament depolymerisation^[48]. Secondly, ROCK inhibits myosin light chain phosphatase (MLCP). MLCP has a structure that is composed of three subunits; a 37 kDa catalytic subunit, a variable subunit and a myosin-binding subunit^[36]. The myosin-binding site is crucial for its regulation and is subject to phosphorylation, specifically at residues. Threonine-695/697 (major site), serine-849/854 and threonine-850/855^[47,50]. Phosphorylation prevents MLCP from regulating the MLC phosphorylation state and increases the basal phosphorylated MLC level, stimulating VSMC contraction and augmenting vascular tone^[50].

MEMBRANE ANCHORS TO THE ACTIN CYTOSKELETON

VSMCs make connective junctions to their surrounding environment, which includes the ECM and neighboring cells within the vasculature. These adhesions play a vital role in determining morphology and VSMC function. The adhesion molecules that are utilised by VSMCs can be separated, despite their structural and functional similarities^[51].

Cell-cell adhesions

Cadherins are the primary molecules in cell-cell adhesion formation. The most abundant isoforms are E (epithelial)-, P (placental)- and N (neuronal)-cadherins, all of which belong to the type I classical cadherin family^[52,53]. N-cadherin is the predominant cadherin in VSMCs and mediates cell-cell adhesion formation with neighbouring endothelial cells as well as other VSMCs^[53]. The N-cadherin adhesion plays important roles

in regulating VSMC function. N-cadherin adhesions suppress both VSMC proliferation and apoptosis^[54,55]. N-cadherin is also involved in VSMC migration; however, its exact role currently remains unclarified^[56].

N-cadherin is composed of a large extracellular N-terminal domain, flanked by a single trans-membrane anchoring domain and a small cytoplasmic tail^[52]. The large extracellular domain consists of five cadherin (EC) repeat regions that are important in coupling N-cadherin into a parallel homodimer via linkage between adjacent EC repeats^[52,53]. EC repeats require calcium binding which stabilises the interaction between the parallel cadherin molecules^[56,57]. The cadherin homodimer of one cell interacts with the homodimer of an adjacent cell by interchanging a specific beta strand, referred to as the A* strand, found in the EC1 domain^[58]. This interaction, referred to as trans-binding, is necessary for the formation of cell-cell adhesions^[53]. The cytoplasmic tail of N-cadherin is linked to the actin cytoskeleton via a number of cytoskeleton-associated proteins, including β -catenin, p120-catenin and α -catenin. α -catenin is recruited to the adhesion structure via β -catenin and plays a crucial role in providing a link between the N-cadherin-based junction and the actin cytoskeleton^[59].

Cadherin-based mechanotransduction is observed in multiple cell types and induces adhesion and cytoskeletal remodelling, altered adhesion strength and changes in actomyosin activity. Evidence suggests that N-cadherin-based adhesions are important for VSMC contraction and mechanotransduction; β -catenin recruitment to N-cadherin-based adhesions is necessary for VSMC contraction, and N-cadherin is essential for VSMC myogenic response to changes in pressure. Despite this evidence, our understanding of the role of N-cadherin-based mechanotransduction in VSMC function remains poorly defined.

Cell-matrix adhesions

Cell-matrix adhesions possess integrin receptors at their core^[60]. Integrins span the plasma membrane and physically associate with different ECM components^[61]. Integrin receptors perform both structural and mechanosensing signalling functions within cell-matrix adhesions. Integrins form heterodimers, consisting of an alpha and beta subunit^[62] and are structurally comprised of an extracellular ligand-binding domain, which binds the ECM, and a cytosolic domain, which is anchored to the actin cytoskeleton^[63]. Before transducing intracellular tension to the ECM, the integrin receptors must mature via the recruitment of further integrin receptors as well as other cytoskeletal components^[64]. Talin and alpha-actinin bind directly to the cytosolic domain of integrin and talin binding promotes the recruitment of additional components to cell matrix adhesions, including vinculin, paxillin and focal adhesion kinase^[62,65]. Vinculin consists of 3-structural regions known as the head, neck and tail domain^[66]. The vinculin binding site is auto-inhibited by interactions between its head and tail domain^[66]. This interaction is disrupted by talin/alpha actinin, via individual binding or cooperatively with PIP2^[67]. Once disrupted, the activated form can then associate with cell-matrix adhesions via talin^[67]. Physical stress induces exposure of vinculin binding sites on talin's rod domain allowing vinculin binding. In addition, the vinculin molecule also associates with actin filaments, thereby crosslinking the actin cytoskeleton to the integrin receptors. This allows force transduction from the contractile machinery of VSMCs to be transmitted to the ECM^[66].

The linker of nucleoskeleton and cytoskeleton complex

The nuclear envelope (NE) is a double lipid bilayer that consists of an outer nuclear membrane (ONM) and an inner nuclear membrane (INM), separated by a perinuclear space [Figure 3]. The ONM and INM are continuous and join at nuclear pores^[68]. A meshwork of A-type and B-type lamin intermediate proteins and lamina associated proteins, collectively known as the nuclear lamina, underlies the INM and provides structural support to the NE^[68]. The linker of nucleoskeleton and cytoskeleton (LINC) complex, comprised of nesprin-family members and SUN-domain containing proteins, spans the NE. Giant nesprin-1/2 isoforms reside on the ONM and associate with filamentous actin via N-terminal calponin homology (CH) domains^[69]. Within the perinuclear space, binding of the nesprin Klarsicht, Anc-1, Syne-1 homology-

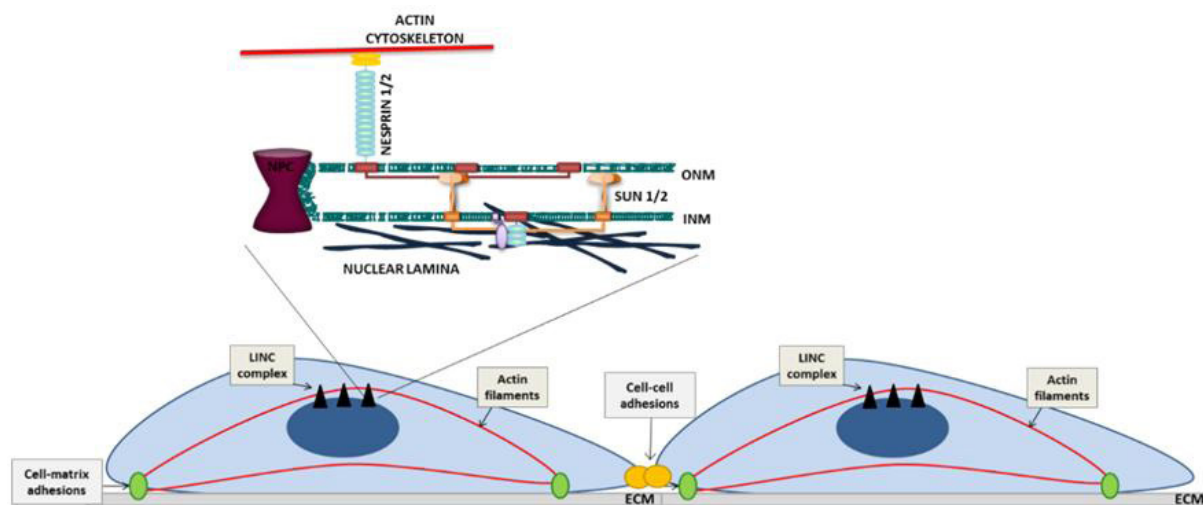


Figure 3. Schematic representation of adhesion organisation and the LINC complex in VSMCs. ONM: outer nuclear membrane; INM: inner nuclear membrane; ECM: extracellular matrix; LINC: linker of nucleoskeleton and cytoskeleton; VSMC: vascular smooth muscle cell

domain to the SUN-domain of SUN1/2 maintains LINC complex stability^[70,71]. At the INM, SUN1/2 interact with lamins A/C^[71], allowing the plasma membrane and nucleus to function as a mechanically coupled system [Figure 3].

The LINC complex is a regulator of cytoskeletal organisation and directly transmits biophysical signals into the nucleus. The LINC complex is subjected to mechanical tension and actomyosin-generated forces are directly transduced across the NE to the nuclear lamina^[72,73]. However, the LINC complex exists in mechanical balance with mechanotransduction and LINC disruption triggers cell-matrix adhesion, cell-cell adhesion and cytoskeletal reorganisation^[74,75]. LINC complex disruption also alters actomyosin activity, nesprin disruption enhances actomyosin activity in skeletal muscle progenitor and endothelial cells, whereas lamin A disruption in skeletal muscle progenitor cells enhanced actomyosin activity and reduced actomyosin activity in fibroblast cells^[76,77]. This suggests that the LINC complex plays cell-specific roles in regulating actomyosin activity and disruption of the nuclear lamin A during ageing alters VSMC morphology and cell-matrix organisation^[78]. However, the role of the LINC complex in VSMC actomyosin regulation remains unknown. Although the mechanism of this LINC complex/actomyosin feedback remains unknown, lamin A/C and SUN2 regulate Rac1 and RhoA activity, respectively^[78,79].

MECHANOTRANSDUCTION AND THE ECM

Sensing the extracellular environment

Extracellular mechanical cues directly regulate VSMC function, including actomyosin activity, adhesion, differentiation and migration^[66]. To achieve this, VSMCs must convert mechanical signals into biochemical response via a process known as mechanotransduction. Mechanosensors range from stretch-sensitive channels, cytoskeletal filaments, cytosolic proteins and nuclear proteins, all of which undergo conformational changes when encountering intra/extracellular tension^[80].

Conformational changes induced by tension alters mechanosensors modification, interactions and localisation within the cell^[81]. Vinculin, in particular, acts as a regulator of mechanical stress in addition to its role as a mechano-coupler. As a result, cells regulate their function by actively exerting and resisting forces both to and from the ECM as a means to adjust their mechanical properties^[82]. Force is transmitted via cell-matrix adhesions, which serve as bidirectional signalling conduits, enabling “inside-out” and “outside-in” signalling^[82]. This is crucial for the maintenance of normal physiology as well as injury-repair,

by offsetting signalling pathways that induce appropriate gene expression. Due to this, the cell can enable structural remodelling of the cytoskeleton, which allows for correct adjustment of the vascular tone and in turn the blood flow^[24].

Mechanotransduction: role of stretch

The cyclical process of the cardiac pumping creates numerous mechanical stimuli, all of which the vascular smooth muscle is exposed to. Examples are transmural pressure, circumferential wall tension and vascular shear strain^[24]. Shear stress predominantly acts on the tunica intima, where endothelial cells reside. In the tunica media, VSMCs are subjected to cyclic stretch that originates from the pulsatile blood pressure^[27]. Pulsatile stretch is cyclical in nature due to a rise in blood flow during the systolic phase and then a gradual decrease when in the diastolic phase^[83]. This form of pressure induces rapid cell-matrix and actin filament reorganisation^[24]. Stretch also physically opens mechanically gated cation channels, promoting Ca^{2+} ion entry and VSMC contraction^[84,85]. Stretch signals also regulate activity of several important signalling molecules, including protein kinase C and Akt. Therefore stretch signals regulate a range of VSMC functions, including proliferation, migration and apoptosis^[27].

CVD and the role of matrix stiffness

VSMCs can sense the stiffness of their surrounding matrix and respond by exerting actomyosin-generated tension on the ECM. As the stiffness of a material increases, its elasticity decreases^[86]. Therefore, as matrix stiffness is augmented, vessel stretch signals decrease and there is a switch from transient stretch signals to sustained stiffness signalling. Decreased arterial compliance is commonly found in the early stages of numerous CVDs such as atherosclerosis, restenosis and aneurism. Atherosclerosis is an inflammatory CVD that acts as the underlying cause of heart attack, stroke and cardiac death^[87]. The development of this disease primarily involves the endothelial intimal layer and the vascular smooth muscle medial layer^[88] and results in the formation of an atherosclerotic plaque, containing a lipid core surrounded by a fibrous cap. Atherosclerosis decreases aortic compliance and the stiffness of the individual components of an atherosclerotic plaque has been measured by atomic force microscopy, which displays a range of stiffness, from the soft lipid core (~5 kPa), to the relatively healthy cellular regions (~10 kPa), to the stiff fibrous cap (60-250 kPa)^[89].

VSMC phenotypic modulation, proliferation and migration is prevalent in atherosclerosis and there is a clear correlation between changes in arterial compliance and atherosclerotic disease progression^[11,90]. Yet our understanding of the influence of matrix stiffness on VSMC function remains limited. VSMCs exposed to enhanced matrix stiffness display increased intracellular tension and form larger cell-matrix adhesions^[25,91]. VSMC stiffness is also augmented in hypertension and diabetes^[25,26]. Importantly, treatment with actin inhibitors reduce VSMCs stiffness, suggesting that the stiffer aortic environments increase actomyosin force generation in VSMCs^[26].

In addition to altered actomyosin activity, matrix stiffness also potentially influences VSMC differentiation. VSMC phenotype is regulated by numerous environmental cues, including the ECM^[92]. Atherosclerotic plaques display reduced levels of elastin and collagen-I accumulation^[93]. Fetal aortic VSMCs display enhanced traction forces and actomyosin activity when matrix stiffness is increased from 10 kPa to 25 kPa^[93]. However, they fail to produce sufficient force to displace a 135 kPa matrix^[93], suggesting that VSMCs lose the ability to contract and deform their surrounding ECM under enhanced matrix stiffness. In agreement with this notion, VSMCs lose the ability to contract and deform the ECM when surrounded by stiff collagen-I fibrils in 3D models^[94]. In stiff environments, VSMCs exert higher levels of mechanical tension, which directs a proliferative change to the synthetic phenotype^[94]. Expression levels of key contractile marker proteins, such as alpha smooth muscle actin, are reduced when collagen-I gel concentration is increased^[92]. Furthermore, VSMCs transition to the synthetic state when stiffness and nanotopography of the substrate is increased^[95]. Therefore, ECM stiffness influences VSMC phenotypic modulation.

As described above, matrix stiffness influences both the expression of VSMC contractile proteins and the ability of VSMCs to physically contract and deform their surrounding ECM. Therefore, stiffened ECM is no longer remodeled by the intrinsic actomyosin activity of the VSMCs^[96]. However, this does not explain whether the absence of substrate deformation is due to a decline in VSMC actomyosin response or because the ECM has become too stiff to be manipulated. For example, a recent study investigated the *ex-vivo* vasoconstrictor response of young and old soleus muscle feed arteries. Despite an upregulation of ROCK activity, there was a decreased constrictor response in the aged vessels^[96]. Aged VSMCs were incapable of generating sufficient force to induce matrix remodeling^[96]. However, ECM deformation was used to assess the vasoconstrictor response and given that aged arteries are stiffer than their younger counterparts, VSMC actomyosin activity may remain intact^[97]. In agreement with this, cell-matrix adhesions were subjected to increased mechanical load in aged arteries, compared to their younger counterparts^[96]. Further investigation is now required to assess whether the mechanisms of VSMC actomyosin signaling is influenced by matrix stiffness.

Finally, directional cellular migration is also induced by gradients of ECM stiffness, known as durotaxis. Durotaxis contrasts from chemotaxis and haptotaxis due to an absence of soluble chemical signals or adhesive ligand density, respectively^[98]. Both healthy and diseased tissues possess heterogeneity in their mechanical stiffness indicating the presence of gradients^[89,99-102]. VSMC directional migration is prevalent in atherosclerosis and VSMCs are exposed to low (~5 kPa) and high matrix stiffness (> 200 kPa) within the atherosclerotic plaque. VSMCs orientate in the direction of an ECM stiffness gradient^[98] and show a directed migration towards a mechanical gradient when plated on gels coated with fibronectin as opposed to laminin^[103]. Fibronectin is found in high concentrations in atherosclerotic lesions, suggesting that ECM composition is a key component of VSMC durotaxis. It remains unknown whether durotaxis, chemotaxis and haptotaxis work cooperatively to regulate VSMC migration in atherosclerosis, however, matrix stiffness gradients may participate in the enhanced VSMC migration observed in vascular diseases such as atherosclerosis.

CONCLUSION

Evidence clearly dictates that matrix stiffness plays a crucial role in CVD. However, its effects on VSMCs, the predominant cell type within the aorta, remains poorly defined. The majority of VSMC research has been performed on tissue culture plastic or glass, which are over 1000 times stiffer than an arterial wall. There is a pressing need to utilise materials that more closely replicate both physiological and pathological stiffness in VSMC research. Several important questions remained unanswered: (1) the signalling pathways regulating VSMC function in response to matrix stiffness remain to be fully elucidated; (2) do VSMCs from different vascular beds possess unique force generating capabilities in response to matrix stiffness; and (3) how do other cell types, including endothelial cells, tune VSMC actomyosin activity in response to matrix stiffness? Answering these questions will facilitate an understanding of the aetiology of arterial stiffness on VSMC function that will potentially allow development of new therapeutic avenues for the treatment of a wide range of CVDs.

DECLARATIONS

Authors' contributions

Responsible for the design, literature review, writing and editing of this manuscript: Ahmed S, Warren DT

Availability of data and materials

Not applicable.

Financial support and sponsorship

A British Heart Foundation (BHF) Non-Clinical PhD Studentship (FS/17/32/32916) funded this work.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Bhatnagar P, Wickramasinghe K, Wilkins E, Townsend N. Trends in the epidemiology of cardiovascular disease in the UK. *Heart* 2016;102:1945-52.
2. Islam AM, Mohibullah A, Paul T. Cardiovascular disease in Bangladesh: a review. *Bangladesh Heart J* 2016;31:80-99.
3. Ness AR, Powles JW. Fruit and vegetables, and cardiovascular disease: a review. *Int J Epidemiol* 1997;26:1-13.
4. Stewart J, Manmathan G, Wilkinson P. Primary prevention of cardiovascular disease: a review of contemporary guidance and literature. *JRSM Cardiovasc Dis* 2017;6:2048004016687211.
5. Sun LY, Lee EW, Zahra A, Park JH. Risk factors of cardiovascular disease and their related socio-economical, environmental and health behavioral factors: focused on low-middle income countries-a narrative review article. *Iran J Public Health* 2015;44:435-44.
6. Södergren M. Lifestyle predictors of healthy ageing in men. *Maturitas* 2013;75:113-7.
7. Jani B, Rajkumar C. Ageing and vascular ageing. *Postgrad Med J* 2006;82:357-62.
8. Cecelja M, Chowienczyk P. Role of arterial stiffness in cardiovascular disease. *JRSM Cardiovasc Dis* 2012; doi: 10.1258/cvd.2012.012016.
9. Strait JB, Lakatta EG. Aging-associated cardiovascular changes and their relationship to heart failure. *Heart Fail Clin* 2012;8:143-64.
10. Ungvari Z, Kaley G, de Cabo R, Sonntag WE, Csizsar A. Mechanisms of vascular aging: new perspectives. *J Gerontol A Biol Sci Med Sci* 2010;65:1028-41.
11. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: pathophysiologic mechanisms and emerging clinical indications. *Vascuol Pharmacol* 2016;77:1-7.
12. Mitchell GF. Arterial stiffness and hypertension. *Hypertension* 2014;64:13-8.
13. Safar ME, Czernichow S, Blacher J. Obesity, arterial stiffness, and cardiovascular risk. *J Am Soc Nephrol* 2006;17:S109-11.
14. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, et al. Arterial stiffness and cardiovascular events: the Framingham heart study. *Circulation* 2010;121:505-11.
15. Sethi S, Rivera O, Oliveros R, Chilton R. Aortic stiffness: pathophysiology, clinical implications, and approach to treatment. *Integr Blood Press Control* 2014;7:29-34.
16. Shirwany NA, Zou MH. Arterial stiffness: a brief review. *Acta Pharmacol Sin* 2010;31:1267-76.
17. DeLoach SS, Townsend RR. Vascular stiffness: its measurement and significance for epidemiologic and outcome studies. *Clin J Am Soc Nephrol* 2008;3:184-92.
18. Tzamis A, Krawiec JT, Vorp DA. Elastin and collagen fibre microstructure of the human aorta in ageing and disease: a review. *J R Soc Interface* 2013;10:20121004.
19. Belz GG. Elastic properties and windkessel function of the human aorta. *Cardiovasc Drugs Ther* 1995;9:73-83.
20. Steed MM, Tyagi N, Sen U, Schuschke DA, Joshua IG, et al. Functional consequences of the collagen/elastin switch in vascular remodeling in hyperhomocysteinemic wild-type, eNOS^{-/-}, and iNOS^{-/-} mice. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L301-11.
21. Wagenseil JE, Mecham RP. Vascular extracellular matrix and arterial mechanics. *Physiol Rev* 2009;89:957-89.
22. Jufri NF, Mohamedali A, Avolio A, Baker MS. Mechanical stretch: physiological and pathological implications for human vascular endothelial cells. *Vasc Cell* 2015;7:8.
23. Humphrey JD. Mechanisms of arterial remodeling in hypertension: coupled roles of wall shear and intramural stress. *Hypertension* 2008;52:195-200.
24. Ye GJ, Nesmith AP, Parker KK. The role of mechanotransduction on vascular smooth muscle myocytes cytoskeleton and contractile function. *Anat Rec (Hoboken)* 2014;297:1758-69.
25. Sehgel NL, Vatner SF, Meininger GA. "Smooth muscle cell stiffness syndrome"-revisiting the structural basis of arterial stiffness. *Front Physiol* 2015;6:335.
26. Qiu H, Zhu Y, Sun Z, Trzeciakowski JP, Gansner M, et al. Short communication: vascular smooth muscle cell stiffness as a mechanism for increased aortic stiffness with aging. *Circ Res* 2010;107:615-9.

27. Haga JH, Li YS, Chien S. Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells. *J Biomech* 2007;40:947-60.
28. Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, et al. Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. *Pharmacol Rev* 2016;68:476-532.
29. Alexander MR, Owens GK. Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. *Annu Rev Physiol* 2012;74:13-40.
30. Owens GK. Molecular control of vascular smooth muscle cell differentiation and phenotypic plasticity. *Novartis Found Symp* 2007;283:174-91.
31. Yamin R, Morgan KG. Deciphering actin cytoskeletal function in the contractile vascular smooth muscle cell. *J Physiol* 2012;590:4145-54.
32. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillesen D, et al. A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. *J Cell Biol* 1986;103:2787-96.
33. Papakonstanti EA, Stournaras C. Cell responses regulated by early reorganization of actin cytoskeleton. *FEBS Lett* 2008;582:2120-7.
34. Rzuclido EM, Martin KA, Powell RJ. Regulation of vascular smooth muscle cell differentiation. *J Vasc Surg* 2007;45:A25-32.
35. Rensen SS, Doevendans PA, van Eys GJ. Regulation and characteristics of vascular smooth muscle cell phenotypic diversity. *Neth Heart J* 2007;15:100-8.
36. Webb RC. Smooth muscle contraction and relaxation. *Adv Physiol Educ* 2003;27:201-6.
37. Eddinger TJ, Meer DP. Myosin II isoforms in smooth muscle: heterogeneity and function. *Am J Physiol Cell Physiol* 2007;293:C493-508.
38. Löfgren M, Ekblad E, Morano I, Arner A. Nonmuscle myosin motor of smooth muscle. *J Gen Physiol* 2003;121:301-10.
39. Woodrum DA, Brophy CM. The paradox of smooth muscle physiology. *Mol Cell Endocrinol* 2001;177:135-43.
40. Martinsen A, Dessy C, Morel N. Regulation of calcium channels in smooth muscle: new insights into the role of myosin light chain kinase. *Channels (Austin)* 2014;8:402-13.
41. Fridlyand LE, Philipson LH. Pancreatic beta cell G-protein coupled receptors and second messenger interactions: a systems biology computational analysis. *PloS One* 2016;11:e0152869.
42. Inagami T, Eguchi S, Tsuzuki S, Ichiki T. Angiotensin II receptors AT1 and AT2: new mechanisms of signaling and antagonistic effects of AT1 and AT2. In: Dhalla NS, Zahradka P, Dixon IMC, Beamish RE, editors. *Angiotensin II receptor blockade physiological and clinical implications*. Boston: Springer; 1998. pp. 129-39.
43. Walsh MP. Calmodulin and its roles in skeletal muscle function. *Can Anaesth Soc J* 1983;30:390-8.
44. Lee S, Kumar S. Actomyosin stress fiber mechanosensing in 2D and 3D. *F1000Res* 2016; doi: 10.12688/f1000research.8800.1.
45. Schwartz MA. Integrins and extracellular matrix in mechanotransduction. *Cold Spring Harb Perspect Biol* 2010;2:a005066.
46. Bar-Sagi D, Hall A. Ras and Rho GTPases: a family reunion. *Cell* 2000;103:227-38.
47. Fukata Y, Amano M, Kaibuchi K. Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. *Trends Pharmacol Sci* 2001;22:32-9.
48. Amano M, Nakayama M, Kaibuchi K. Rho-kinase/ROCK: a key regulator of the cytoskeleton and cell polarity. *Cytoskeleton (Hoboken)* 2010;67:545-54.
49. Liao JK, Seto M, Noma K. Rho kinase (ROCK) inhibitors. *J Cardiovasc Pharmacol* 2007;50:17-24.
50. Murányi A, Derkach D, Erdodi F, Kiss A, Ito M, et al. Phosphorylation of Thr695 and Thr850 on the myosin phosphatase target subunit: inhibitory effects and occurrence in A7r5 cells. *FEBS Lett* 2005;579:6611-5.
51. Burute M, Thery M. Spatial segregation between cell-cell and cell-matrix adhesions. *Curr Opin Cell Biol* 2012;24:628-36.
52. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, et al. *Cell-cell adhesion and communication*. Molecular cell biology. New York: W. H. Freeman; 2000.
53. Sun Z, Parrish AR, Hill MA, Meininger GA. N-cadherin, a vascular smooth muscle cell-cell adhesion molecule: function and signaling for vasomotor control. *Microcirculation* 2014;21:208-18.
54. Lyon CA, Johnson JL, White S, Sala-Newby GB, George SJ. EC4, a truncation of soluble N-cadherin, reduces vascular smooth muscle cell apoptosis and markers of atherosclerotic plaque instability. *Mol Ther Methods Clin Dev* 2014;1:14004.
55. Lyon CA, Wadey KS, George SJ. Soluble N-cadherin: a novel inhibitor of VSMC proliferation and intimal thickening. *Vascul Pharmacol* 2016;78:53-62.
56. Lyon CA, Koutsouki E, Aguilera CM, Blaschuk OW, George SJ. Inhibition of N-cadherin retards smooth muscle cell migration and intimal thickening via induction of apoptosis. *J Vasc Surg* 2010;52:1301-9.
57. Perez TD, Nelson WJ. Cadherin adhesion: mechanisms and molecular interactions. *Handb Exp Pharmacol* 2004; doi: 10.1007/978-3-540-68170-0_1.
58. Shapiro L, Weis WI. Structure and biochemistry of cadherins and catenins. *Cold Spring Harb Perspect Biol* 2009;1:a003053.
59. Weis WI, Nelson WJ. Re-solving the cadherin-catenin-actin conundrum. *J Biol Chem* 2006;281:35593-7.
60. Wozniak MA, Madzelewska K, Kwong L, Keely PJ. Focal adhesion regulation of cell behavior. *Biochim Biophys Acta* 2004;1692:103-19.
61. Berrier AL, Yamada KM. Cell-matrix adhesion. *J Cell Physiol* 2007;213:565-73.
62. Suki B, Parameswaran H, Imsirovic J, Bartolák-Suki E. Regulatory roles of fluctuation-driven mechanotransduction in cell function. *Physiology (Bethesda)* 2016;31:346-58.
63. Bachir AI, Zareno J, Moissoglu K, Plow EF, Gratton E, et al. Integrin-associated complexes form hierarchically with variable stoichiometry in nascent adhesions. *Curr Biol* 2014;24:1845-53.

64. Romer LH, Birukov KG, Garcia JG. Focal adhesions: paradigm for a signaling nexus. *Circ Res* 2006;98:606-16.
65. Choev DS, Volberg T, Livne A, Eisenstein M, Martins B, et al. Conformational states during vinculin unlocking differentially regulate focal adhesion properties. *Sci Rep* 2018;8:2693.
66. Mierke CT. The role of vinculin in the regulation of the mechanical properties of cells. *Cell Biochem Biophys* 2009;53:115-26.
67. Carisey A, Ballestrem C. Vinculin, an adapter protein in control of cell adhesion signalling. *Eur J Cell Biol* 2011;90:157-63.
68. Burke B, Ellenberg J. Remodelling the walls of the nucleus. *Nat Rev Mol Cell Biol* 2002;3:487-97.
69. Warren DT, Zhang Q, Weissberg PL, Shanahan CM. Nesprins: intracellular scaffolds that maintain cell architecture and coordinate cell function? *Expert Rev Mol Med* 2005;7:1-15.
70. Haque F, Lloyd DJ, Smallwood DT, Dent CL, Shanahan CM, et al. SUN1 interacts with nuclear lamin A and cytoplasmic nesprins to provide a physical connection between the nuclear lamina and the cytoskeleton. *Mol Cell Biol* 2006;26:3738-51.
71. Crisp M, Liu Q, Roux K, Rattner JB, Shanahan C, et al. Coupling of the nucleus and cytoplasm: role of the LINC complex. *J Cell Biol* 2006;172:41-53.
72. Guilluy C, Osborne LD, Van Landeghem L, Sharek L, Superfine R, et al. Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. *Nat Cell Biol* 2014;16:376-81.
73. Lombardi ML, Jaalouk DE, Shanahan CM, Burke B, Roux KJ, et al. The interaction between nesprins and sun proteins at the nuclear envelope is critical for force transmission between the nucleus and cytoskeleton. *J Biol Chem* 2011;286:26743-53.
74. Stewart RM, Zubek AE, Rosowski KA, Schreiner SM, Horsley V, et al. Nuclear-cytoskeletal linkages facilitate cross talk between the nucleus and intercellular adhesions. *J Cell Biol* 2015;209:403-18.
75. Chambliss AB, Khatau SB, Erdenberger N, Robinson DK, Hodzic D, et al. The LINC-anchored actin cap connects the extracellular milieu to the nucleus for ultrafast mechanotransduction. *Sci Rep* 2013;3:1087.
76. Schwartz C, Fischer M, Mamchaoui K, Bigot A, Lok T, et al. Lamins and nesprin-1 mediate inside-out mechanical coupling in muscle cell precursors through FHOD1. *Sci Rep* 2017;7:1253.
77. Chancellor TJ, Lee J, Thodeti CK, Lele T. Actomyosin tension exerted on the nucleus through nesprin-1 connections influences endothelial cell adhesion, migration, and cyclic strain-induced reorientation. *Biophys J* 2010;99:115-23.
78. Porter LJ, Holt MR, Soong D, Shanahan CM, Warren DT. Prelamin A accumulation attenuates rac1 activity and increases the intrinsic migrational persistence of aged vascular smooth muscle cells. *Cells* 2016; doi: 10.3390/cells5040041.
79. Thakar K, May CK, Rogers A, Carroll CW. Opposing roles for distinct LINC complexes in regulation of the small GTPase RhoA. *Mol Biol Cell* 2017;28:182-91.
80. Alonso JL, Goldmann WH. Cellular mechanotransduction. *AIMS Biophys* 2016;3:50-62.
81. Belaadi N, Aureille J, Guilluy C. Under pressure: mechanical stress management in the nucleus. *Cells* 2016; doi: 10.3390/cells5020027.
82. Wrighton KH. Cell adhesion: the 'ins' and 'outs' of integrin signalling. *Nat Rev Mol Cell Biol* 2013;14:752.
83. Anwar MA, Shalhoub J, Lim CS, Gohel MS, Davies AH. The effect of pressure-induced mechanical stretch on vascular wall differential gene expression. *J Vasc Res* 2012;49:463-78.
84. Ducret T, El Arrouchi J, Courtois A, Quignard JF, Marthan R, et al. Stretch-activated channels in pulmonary arterial smooth muscle cells from normoxic and chronically hypoxic rats. *Cell calcium* 2010;48:251-9.
85. Zou H, Lifshitz LM, Tuft RA, Fogarty KE, Singer JJ. Visualization of Ca²⁺ entry through single stretch-activated cation channels. *Proc Natl Acad Sci U S A* 2002;99:6404-9.
86. Humphrey JD, Harrison DG, Figueroa CA, Lacolley P, Laurent S. Central artery stiffness in hypertension and aging: a problem with cause and consequence. *Circ Res* 2016;118:379-81.
87. Tabas I, Garcia-Cardena G, Owens GK. Recent insights into the cellular biology of atherosclerosis. *J Cell Biol* 2015;209:13-22.
88. Kher N, Marsh JD. Pathobiology of atherosclerosis--a brief review. *Semin Thromb Hemost* 2004;30:665-72.
89. Tracqui P, Broisat A, Toczek J, Mesnier N, Ohayon J, et al. Mapping elasticity moduli of atherosclerotic plaque in situ via atomic force microscopy. *J Struct Biol* 2011;174:115-23.
90. Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. *Circ Res* 2016;118:692-702.
91. Hytönen VP, Wehrle-Haller B. Mechanosensing in cell-matrix adhesions - converting tension into chemical signals. *Exp Cell Res* 2016;343:35-41.
92. Timraz SBH, Rezgui R, Boularaoui SM, Teo JCM. Stiffness of extracellular matrix components modulates the phenotype of human smooth muscle cells in vitro and allows for the control of properties of engineered tissues. *Procedia Eng* 2015;110:29-36.
93. Sazonova OV, Isenberg BC, Herrmann J, Lee KL, Purwada A, et al. Extracellular matrix presentation modulates vascular smooth muscle cell mechanotransduction. *Matrix Biol* 2015;41:36-43.
94. McDaniel DP, Shaw GA, Elliott JT, Bhadriraju K, Meuse C, et al. The stiffness of collagen fibrils influences vascular smooth muscle cell phenotype. *Biophys J* 2007;92:1759-69.
95. Chaterji S, Kim P, Choe SH, Tsui JH, Lam CH, et al. Synergistic effects of matrix nanotopography and stiffness on vascular smooth muscle cell function. *Tissue Eng Part A* 2014;20:2115-26.
96. Seawright JW, Sreenivasappa H, Gibbs HC, Padgham S, Shin SY, et al. Vascular smooth muscle contractile function declines with age in skeletal muscle feed arteries. *Front Physiol* 2018;9:856.
97. Kohn JC, Lampi MC, Reinhart-King CA. Age-related vascular stiffening: causes and consequences. *Front Genet* 2015;6:112.
98. Isenberg BC, Dimilla PA, Walker M, Kim S, Wong JY. Vascular smooth muscle cell durotaxis depends on substrate stiffness gradient strength. *Biophys J* 2009;97:1313-22.

99. Berry MF, Engler AJ, Woo YJ, Pirolli TJ, Bish LT, et al. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. *Am J Physiol Heart Circ Physiol* 2006;290:H2196-203.
100. Liu F, Tschumperlin DJ. Micro-mechanical characterization of lung tissue using atomic force microscopy. *J Vis Exp* 2011; doi: 10.3791/2911.
101. Lopez JJ, Kang I, You WK, McDonald DM, Weaver VM. In situ force mapping of mammary gland transformation. *Integr Biol (Camb)* 2011;3:910-21.
102. Plodinec M, Loparic M, Monnier CA, Obermann EC, Zanetti-Dallenbach R, et al. The nanomechanical signature of breast cancer. *Nat Nanotechnol* 2012;7:757-65.
103. Hartman CD, Isenberg BC, Chua SG, Wong JY. Vascular smooth muscle cell durotaxis depends on extracellular matrix composition. *Proc Natl Acad Sci U S A* 2016;113:11190-5.

Original Article

Open Access



Blood pressure control and vascular protection with a fixed-dose combination of lisinopril + amlodipine + rosuvastatin in hypertensive patients

Sergey V. Nedogoda, Elena V. Chumachek, Alla A. Ledyeva, Vera V. Tsoma, Alla S. Salasyuk, Victoria O. Smirnova, Victoria Yu. Hripaeva, Roman V. Palashkin, Ekaterina A. Popova

Department of Therapy and Endocrinology, Volgograd State Medical University, Ministry of Healthcare of the Russian Federation, Volgograd 400066, Russian Federation.

Correspondence to: Dr. Sergey V. Nedogoda, Department of Therapy and Endocrinology, Tsiolkovskogo str, 1, Volgograd 400001, Russian Federation. E-mail: nedogodasv@rambler.ru

How to cite this article: Nedogoda SV, Chumachek EV, Ledyeva AA, Tsoma VV, Salasyuk AS, Smirnova VO, Hripaeva VY, Palashkin RV, Popova EA. Blood pressure control and vascular protection with a fixed-dose combination of lisinopril + amlodipine + rosuvastatin in hypertensive patients. *Vessel Plus* 2018;2:37. <http://dx.doi.org/10.20517/2574-1209.2018.36>

Received: 23 May 2018 **First Decision:** 27 Aug 2018 **Revised:** 26 Sep 2018 **Accepted:** 1 Oct 2018 **Published:** 6 Nov 2018

Science Editor: Alexander D. Verin **Copy Editor:** Cui Yu **Production Editor:** Zhong-Yu Guo

Abstract

Aim: Assessment of the possibility of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin (Equamer) to achieve further angioprotection in patients with arterial hypertension and high pulse wave velocity (PWV) despite the previous combination antihypertensive therapy (AHT).

Methods: The 24-week open-label multi-center observational study involved 60 patients who received dual combination AHT for 6 months. All patients underwent 24 h blood pressure (BP) monitoring, applanation tonometry (determination of the augmentation index and central BP), measurement of the pulse wave velocity and laboratory tests [blood lipids, fasting glucose test, homeostasis model assessment of insulin resistance (HOMA-IR), leptin, ultra-sensitive C-reactive protein (us-CRP)] before and after switching to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin (Equamer).

Results: According to the office BP measurements, switching the patients from the dual combinations to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin has resulted in a further decrease of 14.3% in systolic BP (SBP) and 18.5% in diastolic BP (DBP). According to the 24 h BP monitoring data, the SBP has decreased by 16.1% and the DBP by 21.8%. The combination of lisinopril + amlodipine + rosuvastatin has reduced the SBP by 14.4%, the augmentation index by 14.5% and the central SBP by 8.1% ($P < 0.01$ vs. baseline). The fixed-dose combination of lisinopril + amlodipine + rosuvastatin has provided a 44%-decrease in low-density lipoproteins, a 36.1%-decrease in triglycerids and a 10.3%-increase in high-density lipoproteins ($P < 0.01$ vs. baseline). The use of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin has provided a definite decrease in the insulin resistance, as well as levels of us-CRP and leptin.



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Conclusions: The fixed-dose combination of lisinopril + amlodipine + rosuvastatin provides improved BP control, better vessel elasticity indicators (augmentation index, PWV, central BP), boosts the lipid and carbohydrate metabolism and helps to reduce the inflammation and leptin resistance in patients who initially received a dual combination AHT.

Keywords: Arterial hypertension, pulse wave velocity, central blood pressure, augmentation index, leptin, inflammation, combination antihypertensive therapy, lisinopril, amlodipine, rosuvastatin

INTRODUCTION

Analysis of trends in the development of the modern concept of reduction in cardiovascular risk and mortality reveals several important directions.

First, there is a trend today to “tighten” the blood pressure (BP)^[1] and lipid^[2] targets, which should lead to a further reduction in total and cardiovascular mortality in patients with arterial hypertension (AH) and atherosclerosis. Second, the co-relation of the improved indicators of elasticity of different caliber vessels, survival rate, and reduced risk of cardiovascular complications of AH can now be considered proven^[3]. Principal differences have been found among the classes of antihypertensive drugs in the angioprotective effect (influence on the augmentation index, central systolic and pulse pressure) and, ultimately, in the influence on the typical end points (total and cardiovascular mortality, stroke, myocardial infarction)^[4,5]. The same refers to individual representatives of antihypertensive classes^[6-13] and various combinations of antihypertensive drugs^[14-16]. It has been shown that, upon achievement of the same BP level, lower mortality occurs in the hypertensive patients with a decreased pulse wave velocity (PWV)^[17]. Third, low-intensity non-infectious inflammation becomes an important and independent target of pharmacotherapy^[18-20], because its reduction [first of all, in ultra-sensitive C-reactive protein (us-CRP)] allows not only to reduce the risk of cardiovascular complications, but also to solve the problem of vascular comorbidity^[21]. Fourth, there is a growing awareness that polypill is not only an opportunity to increase the patient’s medication adherence by reducing the number of tablets taken, but also an effective multi-target pharmacotherapy option intended to achieve the BP and lipid targets^[22] and hence maximally reduce the risk of cardiovascular complications.

In this respect, it would be practically important to assess the possibilities of a fixed-dose combination of antihypertensive drugs with statins to provide a more pronounced angioprotection, to achieve the BP and lipid targets and to suppress the inflammation in those hypertensive patients who previously underwent a combination antihypertensive therapy (AHT).

The aim of this study was to assess the possibility of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin (Equamer, “Gedeon Richter”) to achieve further angioprotection in patients with AH and high PWV despite the previous combination AHT.

METHODS

The purpose of the study was to assess changes in the indicators characterizing the elasticity of different caliber vessels [PWV, wave reflection index (AI), central BP (CBP), intima-media thickness of the common carotid artery, flow-dependent vasodilation], insulin resistance and inflammation after switching the patients from the two-component antihypertensive combinations to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin. The target was considered to be BP < 140/90 mmHg.

The open-label study included patients who met all of the following criteria: age from 18 to 65 years, previous combined AHT conducted for at least 6 months, PWV above 10 m/s, age-standardized; signed informed consent of the patient to participate in the study.

Patients with at least one of the following criteria could not be involved in the study: increased sensitivity to angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARBs), calcium antagonists (CAs) and hydrochlorothiazide; instable angina, recent myocardial infarction less than 1-month-old, cardiogenic shock, clinically significant aortic stenosis; decompensated heart failure; severe AH (BP above 170/100 mmHg) that requires a 3-component combination AHT, severe comorbidities; alcohol abuse, pronounced impairments of the kidney function (level of creatinine in the blood 2 times higher than the upper normal limit), liver function (activity of alanine and aspartate aminotransferases in the blood 2 times higher than the upper normal limit); malignant neoplasms; pregnancy or lactation; inability to understand the essence of the programme and give informed consent to participate therein.

The study involved 60 patients (26 men and 34 women, mean age 55.2 ± 6.5 years and body mass index 28.8 ± 4.6 kg/m²).

The study included 4 patients visits to the physician: V1 - entry visit, V2, V3, V4 - follow-up visits 4, 12 and 24 weeks after the entry visit. At the first visit, the previous AHT was discontinued and the fixed-dose combination of lisinopril 10 mg + amlodipine 5 mg + rosuvastatin 10 mg was prescribed to every patient. In the course of the study, the physician had the opportunity to intensify the lipid-lowering therapy by prescribing the fixed-dose combination of lisinopril 10 mg + amlodipine 5 mg + rosuvastatin 20 mg in cases when the target low-density lipoproteins (LDL) of ≤ 2.5 mmol/L was not reached for the high-risk patients after 4 weeks of therapy (at Visit 2).

Initially and after the course therapy, all the patients underwent 24 h BP monitoring, examination of the vessel wall elasticity, echocardiography and laboratory testing.

The 24 h BP monitoring was performed using the Spacelabs 90207 device (USA). During the daytime hours (from 7 to 23 o'clock), measurements were made every 15 min, during the night (from 23 to 7 o'clock) - every 30 min. A special cuff was used to measure the BP in the patients with excessive body weight.

The PWV, AI and central aortic pressure were determined using the SphygmoCor device^[23,24].

In 2010, a group of Spanish researchers published modified SCORE scales for the calculation of vascular age for European countries with high and low cardiovascular disease levels^[25]. The method of vascular age calculation from these scales involves calculating the absolute cardiovascular risk (ACVR) from the standard SCORE scales and then comparing the percentage of ACVR with the age of the vessels from the modified SCORE scale. To perform these calculations, the following data are required: patient's gender, passport age, smoking status, levels of systolic BP and total cholesterol (TC).

Serum adipocytokines were determined by enzyme immunoassay using Mediagnost kits, leptin high sensitive (0.05-5 ng/mL) and BCM diagnostics adiponectin. Blood was drawn into a plastic test tube without a stabilizer. After centrifugation at 1000 RPM for 10 min, 1 mL of serum was collected. Before the determination of leptin and adiponectin levels, the samples were stored at -20 °C.

The insulin resistance was estimated by the homeostasis model assessment (HOMA) index. The study was conducted strictly on an empty stomach, after an 8-12 h period of night fasting. Plasma glucose and plasma insulin were studied. The glucose level was determined by the hexokinase method (La Roche reagents, La Roche automatic analyzer). Plasma insulin was determined by enzyme immunoassay [Insulin ELISA (Mercodia AB, Sweden)]. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using the following formula: $\text{HOMA-IR} = \text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{IU/mL)} / 22.5$.

Table 1. Clinical and demographic characteristics of the cohort involved in the study

Indicator	
Age, years	55.2 ± 6.5
BMI, kg/m ²	28.8 ± 4.6
WC, cm	86.9 ± 13.0
Body fat %	38.7 ± 7.6

BMI: body mass index; WC: waist circumference

Table 2. Changes in BP and HR after 6 months

Indicator	Initially	6 months
SBP, mmHg	156.2 ± 10.0	133.7 ± 9.3*
DBP, mmHg	97.6 ± 11.7	79.6 ± 4.5*
HR, bpm	74.6 ± 7.7	71.7 ± 8.8

* $P < 0.05$ vs. baseline; BP: blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate

CRP was determined by immunoturbidimetric analysis [hs-CRP ELISA (Biomerica, USA)].

The statistical processing of the data obtained was carried out using the bio-medical data package statistical software package. The continuous quantitative initial and demographic characteristics were tested using the independent samples *t*-test. When the characteristic values were not normally distributed, the Mann-Whitney test was used. For the qualitative characteristics, either Fisher's exact test or χ^2 test was applied depending on the number of observations in each cell of the contingency table. The data were presented in the form of $M \pm m$, where M is the mean and m is the standard error. To validate the changes before and after the treatment, Student's paired *t*-test was used.

The study design did not include randomization or calculation of the sample size since a limited number of patients with the characteristics required attend our outpatient clinic, considering this a limitation of the study, and thus an observer-dependent bias, carrying out the daily clinical practice according to clinical guidelines.

The study was conducted in accordance with the good clinical practice standards and the principles of the Helsinki Declaration. The study protocol was approved by the Regional Ethics Committee. Written informed consent was obtained from all the participants prior to their involvement in the study.

RESULTS

The clinical and demographic characteristics of the patients involved in the study are presented in [Table 1](#).

Initially, the patients were treated with the following dual antihypertensive combinations: (1) ACEI + diuretic - 58.9%; (2) ARB + diuretic - 23.1%; (3) β -blocker (BB) + ARB - 3.6%; (4) CA + ACEI - 3.6%; (5) BB + ACEI - 3.6%; (6) CA + diuretic - 1.8%; (7) BB + diuretic - 1.8%; (8) CA + ARB - 1.8%; and (9) imidazoline receptor agonist + ACEI - 1.8%.

At the time of entry into the study, statins were received by 21.4% (atorvastatin 40 mg by 25.1% and 20 mg by 41.6%; rosuvastatin 5 mg by 16.7% and 10 mg by 8.3%; simvastatin 20 mg by 8.3%). After 4 weeks of treatment, 23.2% of the patients needed doubling the dose of rosuvastatin.

According to the office BP measurements [[Table 2](#)], switching the patients from the dual antihypertensive combinations to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin has resulted in a

Table 3. Changes in the data of the 24 h BP monitoring after 6 months

Indicator	Initially	6 months
Day SBP, mmHg	153.1 ± 8.2	128.6 ± 17.1*
Day DBP, mmHg	94.5 ± 9.8	73.9 ± 6.0*
Day HR, bpm	80.6 ± 11.5	76.3 ± 13.2
Night SBP, mmHg	138.9 ± 26.3	118.8 ± 9.3*
Night DBP, mmHg	82.2 ± 11.8	66.5 ± 6.0*
Night HR, bpm	68.2 ± 12.9	67.2 ± 14.9
SBP time index - 24 h, %	35.5 ± 11.3	21.0 ± 8.3*
DBP time index - 24 h, %	23.2 ± 10.5	16.2 ± 6.8*

* $P < 0.05$ vs. baseline; BP: blood pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate

Table 4. Changes in the indicators of the vessel wall elasticity after 6 months

Indicator	Initially	6 months
IMT, mm	1.07 ± 0.22	1.03 ± 0.2
Carotid-femoral PWV	12.5 ± 1.3	10.7 ± 1.4*
Central SBP, mmHg	143.1 ± 15.2	131.4 ± 8.5*
Augmentation index	27.5 ± 9.1	23.5 ± 9.2*
Vascular age, years	60.8 ± 10.0	54.6 ± 8.9*

* $P < 0.05$ vs. baseline; IMT: intima-media thickness; PWV: pulse wave velocity; SBP: systolic blood pressure

decrease of 14.4% in systolic BP (SBP) and 18.4% in diastolic BP (DBP) ($P < 0.05$ vs. baseline) in the absence of changes in the heart rate.

The data of the 24 h BP monitoring have confirmed that switching to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin makes it possible to reduce the average daily SBP by 16.1% and DBP by 21.8%, the average nightly SBP by 14.5% and DBP by 19.1% ($P < 0.05$ vs. baseline). At the same time, in all the groups of the initial therapy, switching to the fixed-dose combination has been accompanied by a definite decrease in the BP variability [Table 3].

The rate of achieving the target BP of $< 140/90$ mmHg with the use of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin has been 73.2%, whereas 23.2% of these patients have achieved a BP level of $< 130/80$ mmHg.

Table 4 shows the trends in the indicators characterizing the state of the arterial wall of the vessels belonging to the elastic or muscular type, as well as amortizing vessels, after switching the patients to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin: there has been a decrease of 14.7% in the PWV ($P < 0.05$), 14.4% in the augmentation index ($P < 0.05$) and 8.1% in the CBP ($P < 0.05$), which has reduced the vascular age by 10.2% ($P < 0.05$).

The fixed-dose combination therapy of lisinopril + amlodipine + rosuvastatin provides favorable performance of the lipid metabolism indicators: there has been a decrease of 29.4% in the TC ($P < 0.05$), a decrease of 31.9% in the triglycerids ($P < 0.05$), a decrease of 38.1% in LDL ($P < 0.05$), and an increase of 10.5% in high-density lipoproteins ($P < 0.05$). The rate of achieving the LDL target of ≤ 2.5 mmol/L with the use of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin has been 71.4%. After switching to the fixed-dose combination, all the groups have shown positive trends in the studied biochemical indicators characterizing the functions of the liver and kidneys, although it has not been statistically significant [Table 5].

Switching the patients to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin has been

Table 5. Changes in the studied biochemical indicators after 6 months

Indicator	Initially	6 months
TC, mmol/L	6.4 ± 1.0	4.5 ± 1.0*
HDL, mmol/L	1.2 ± 0.3	1.3 ± 0.3*
TG, mmol/L	2.5 ± 0.7	1.7 ± 0.5*
LDL, mmol/L	4.1 ± 1.1	2.5 ± 1.1*
ALT, u/L	27.9 ± 10.8	24.8 ± 7.8
AST, u/L	29.3 ± 8.1	24.3 ± 6.8
Creatinine, μmol/L	81.6 ± 9.5	72.7 ± 8.3

* $P < 0.05$ vs. baseline; TC: total cholesterol; HDL: high-density lipoproteins; TG: triglycerids; LDL: low-density lipoproteins; ALT: alanine transaminase; AST: aspartate aminotransferase

Table 6. Changes in the indicators of carbohydrate metabolism after 6 months

Indicator	Initially	6 months
Fasting plasma glucose, mmol/L	7.0 ± 1.3	6.2 ± 1.0*
Insulin, μIU/mL	15.5 ± 5.9	13.8 ± 6.3*
HOMA index, μIU/mL	4.8 ± 2.1	3.7 ± 1.5*

* $P < 0.05$ vs. Baseline; HOMA: homeostasis model assessment

Table 7. Trends in the adipokine levels after 6 months

Indicator	Initially	12 weeks
Leptin, ng/mL	16.3 ± 7.7	14.6 ± 7.9*
Adiponectin, μg/mL	6.9 ± 2.6	7.6 ± 2.8*
us-CRP, mg/L	3.1 ± 1.6	2.7 ± 1.5*

* $P < 0.05$ vs. baseline; us-CRP: ultra-sensitive C-reactive protein

Table 8. Changes in the anthropometric indicators after 6 months

Indicator	Initially	6 months
Weight, kg	82.8 ± 9.2	81.6 ± 8.4
BMI, kg/m ²	28.8 ± 3.5	28.4 ± 3.4
WC, cm	86.9 ± 13.0	83.1 ± 11.5
HC, cm	104.1 ± 13.6	100.1 ± 13.5
Body fat %	38.7 ± 7.7	37.7 ± 8.2

BMI: body mass index; WC: waist circumference; HC: hip circumference

accompanied by an improvement in the carbohydrate metabolism indicators [Table 6]: a decrease of 11.1 % in the insulin ($P < 0.05$) and 22.9% in the HOMA index ($P < 0.05$).

Particular attention should be paid to the possibility of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin to have a positive effect on the level of key adipokines: the average leptin level has become lower by 10.7% ($P < 0.05$), us-CRP - by 11.8% ($P < 0.05$) and the adiponectin level has increased by 9.9% ($P < 0.05$) [Table 7].

There have been no statistically significant changes in the anthropometric indicators revealed during the study [Table 8].

DISCUSSION

The importance of assessing the possibility of further angioprotection with the use of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin in patients with AH undergoing a two-component

AHT is confirmed by the initiation of The LOW CBP study (Targeted LOWering of CBP in patients with hypertension: a randomised controlled trial) and the findings that the combinations of perindopril + amlodipine, valsartan + amlodipine, azelnidipine + olmesartan have a more pronounced positive effect on aortic elasticity than the combinations of atenolol + hydrochlorothiazide, atenolol + amlodipine, olmesartan + hydrochlorothiazide^[12-13]. We must also add the trend to a “tighter” control of BP, lipids and inflammation^[1,2,18-21]. Therefore, it is appropriate to try to solve these problems using the fixed-dose combination of lisinopril + amlodipine + rosuvastatin which is also able to perform the task of a multi-target pharmacotherapy.

First of all, it should be noted that switching patients from different two-component antihypertensive combinations (mainly the renin-angiotensin-aldosterone system blockers and diuretics) therapy which they underwent for at least 6 months to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin has provided the achievement of the target BP in 7 out of 10 patients, given the fact that the study did not involve the use of maximum doses of antihypertensive drugs in this combination. This is apparently explained by an enhancement of the hypotensive potential due to the statins^[26]. The decrease in BP has been confirmed by the data of the 24 h BP monitoring, and all the groups of the initial therapy have shown a positive effect of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin on the BP variability, which can be considered as an important component in the correction of systemic hemodynamic atherothrombotic syndrome^[27].

The improved control of BP after the use of the fixed-dose combination of lisinopril + amlodipine + rosuvastatin and the lipid target achievement in 7 out of 10 patients has been naturally accompanied by positive changes in the indicators of vessel wall elasticity (PWV, CBP, AI, vascular age). These favorable changes should be explained not only by the achievement of the BP and lipid targets, but also the positive effect on adipokines (leptin and adiponectin) and low-intensity non-infectious inflammation (us-CRP), which represents an additional factor of angioprotection^[28-30].

It is important to emphasize that the use of rosuvastatin in combination with lisinopril and amlodipine has been accompanied by a decrease in the insulin resistance and a positive effect on the carbohydrate metabolism, which removes all the questions about the diabetogenic potential of statins when used in this therapy option.

Thus, it can be said that switching patients from two-component antihypertensive combinations to the fixed-dose combination of lisinopril + amlodipine + rosuvastatin provides better control of BP, lipids, angioprotection and reduction of inflammation in combination with improved carbohydrate metabolism and balance of adipokines.

DECLARATIONS

Authors' contributions

Conceived of the study: Nedogoda SV

Recruitment and clinical assessment, statistical analysis: Chumachek EV, Ledyeva AA, Tsoma VV, Salasyuk AS, Smirnova VO, Hripaeva VY, Palashkin RV, Popova EA

Drafted the initial version of the report: Chumachek EV, Salasyuk AS

Revision and editing of the report: all authors

Availability of data and materials

Reader can ask or mail to corresponding author for materials.

Financial support and sponsorship

The authors state that there is no need to disclose financial support with respect to this publication.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study protocol was approved by the Local Ethics Committee of the Volgograd Medical University. Each patient was informed about the study and gave their consent.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on clinical practice guidelines. *J Am Coll Cardiol* 2018;71:e127-248.
2. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, et al. 2016 ESC/EAS guidelines for the management of dyslipidemias. *Eur Heart J* 2016;37:2999-3058.
3. Nemcsik J, Cseppekál O, Tislér A. Measurement of arterial stiffness: a novel tool of risk stratification in hypertension. *Adv Exp Med Biol* 2017;956:475-88.
4. Dahlöf B, Sever PS, Poulter NR, Wedel H, Beevers DG, et al. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-blood pressure lowering arm (ASCOT-BPLA): a multicentre randomised controlled trial. *Lancet* 2005;366:895-906.
5. Manisty CH, Zambanini A, Parker KH, Davies JE, Francis DP, et al. Differences in the magnitude of wave reflection account for differential effects of amlodipine- versus atenolol-based regimens on central blood pressure: an Anglo-Scandinavian Cardiac Outcome Trial substudy. *Hypertension* 2009;54:724-30.
6. Chen X, Huang B, Liu M, Li X. Effects of different types of antihypertensive agents on arterial stiffness: a systematic review and meta-analysis of randomized controlled trials. *J Thorac Dis* 2015;7:2339-47.
7. Morgan T, Lauri J, Bertram D, Anderson A. Effect of different antihypertensive drug classes on central aortic pressure. *Am J Hypertens* 2004;17:118-23.
8. Mackenzie IS, McEniery CM, Dhakam Z, Brown MJ, Cockcroft JR, et al. Comparison of the effects of antihypertensive agents on central blood pressure and arterial stiffness in isolated systolic hypertension. *Hypertension* 2009;54:409-13.
9. Elliott WJ, Childers WK. Should β blockers no longer be considered first-line therapy for the treatment of essential hypertension without comorbidities? *Curr Cardiol Rep* 2011;13:507-16.
10. Hirata K, Vlachopoulos C, Adji A, O'Rourke MF. Benefits from angiotensin-converting enzyme inhibitor 'beyond blood pressure lowering': beyond blood pressure or beyond the brachial artery? *J Hypertens* 2005;23:551-6.
11. London GM, Pannier B, Guerin AP, Marchais SJ, Safar ME, et al. Cardiac hypertrophy, aortic compliance, peripheral resistance, and wave reflection in end-stage renal disease. Comparative effects of ACE inhibition and calcium channel blockade. *Circulation* 1994;90:2786-96.
12. Dhakam Z, McEniery CM, Yasmin, Cockcroft JR, Brown MJ, et al. Atenolol and eprosartan: differential effects on central blood pressure and aortic pulse wave velocity. *Am J Hypertens* 2006;19:214-9.
13. Mahmud A, Feely J. Favourable effects on arterial wave reflection and pulse pressure amplification of adding angiotensin II receptor blockade in resistant hypertension. *J Hum Hypertens* 2000;14:541-6.
14. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 2006;113:1213-25.
15. Boutouyrie P, Achouba A, Trunet P, Laurent S; EXPLOR Trialist Group. Amlodipine-valsartan combination decreases central systolic blood pressure more effectively than the amlodipine-atenolol combination: the EXPLOR study. *Hypertension* 2010;55:1314-22.
16. Matsui Y, Eguchi K, O'Rourke MF, Ishikawa J, Shimada K, et al. Association between aldosterone induced by antihypertensive medication and arterial stiffness reduction: the J-CORE study. *Atherosclerosis* 2011;215:184-8.
17. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, et al. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation* 2001;103:987-92.
18. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 2003;107:363-9.

19. Yusuf S, Bosch J, Dagenais G, Zhu J, Xavier D, et al. Cholesterol lowering in intermediate-risk persons without cardiovascular disease. *N Engl J Med* 2016;374:2021-31.
20. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 2008;359:2195-207.
21. Roberts ER, Green D, Kadam UT. Chronic condition comorbidity and multidrug therapy in general practice populations: a cross-sectional linkage study. *BMJ Open* 2014;4:e005429.
22. American Diabetes Association. Standards of medical care in diabetes 2017: summary of revisions. *Diabetes Care* 2017;40:S4-5.
23. Doupis J, Papanas N, Cohen A, McFarlan L, Horton E. Pulse wave analysis by applanation tonometry for the measurement of arterial stiffness. *Open Cardiovasc Med J* 2016;31:188-95.
24. Van Bortel LM, De Backer T, Segers P. Standardization of arterial stiffness measurements make them ready for use in clinical practice. *Am J Hypertens* 2016;29:1234-6.
25. Cuende JJ, Cuende N, Calaveras-Lagartos J. How to calculate vascular age with the SCORE project scales: a new method of cardiovascular risk evaluation. *Eur Heart J* 2010;31:2351-8.
26. Briasoulis A, Agarwal V, Valachis A, Messerli FH. Antihypertensive effects of statins: a meta-analysis of prospective controlled studies. *J Clin Hypertens (Greenwich)* 2013;15:310-20.
27. Kario K. Orthostatic hypertension-a new haemodynamic cardiovascular risk factor. *Nat Rev Nephrol* 2013;9:726-38.
28. Ionescu DD; PREFER Investigators. Antihypertensive efficacy of perindopril 5-10 mg/day in primary health care: an open-label, prospective, observational study. *Clin Drug Investig* 2009;29:767-76.
29. Fennessy PA, Campbell JH, Mendelsohn FA, Campbell GR. Angiotensin-converting enzyme inhibitors and atherosclerosis: relevance of animal models to human disease. *Clin Exp Pharmacol Physiol* 1996;23:S30-2.
30. Koz C, Baysan O, Yokusoglu M, Uzun M, Yildirim M, et al. The effects of perindopril on aortic elasticity and inflammatory markers in hypertensive patients. *Med Sci Monit* 2009;15:PI41-5.

Review

Open Access



Endoscopic radial artery harvesting for coronary artery bypass grafting

Ajita Naik^{1*}, Mohamed Rahouma^{1*}, Cristiano Spadaccio^{2,3}, Kritika Mehta¹, Massimo Baudo¹, Mohamed Kamel¹, Faiza Khan¹, Irbaz Hameed¹, Matthew Wingo¹, Yongle Ruan¹, Ahmed Abouarab¹, Mohamed Hossny¹, Leonard N. Girardi¹, Mario Gaudino¹

¹Department of Cardiothoracic Surgery, Weill Cornell Medicine, NY 10065, USA.

²Department of Cardiothoracic Surgery, Golden Jubilee National Hospital, Clydebank, Glasgow G814DY, UK.

³Institute of Cardiovascular and Medical Sciences, Veterinary and Life Sciences, College of Medical, University of Glasgow, Glasgow G128QQ, UK.

*The two authors made equal contributions.

Correspondence to: Dr. Mohamed Rahouma, Department of Cardiothoracic Surgery, Weill Cornell Medicine, 525 East 68th Street, Box 110, NY 10065, USA. E-mail: mhmdrahouma@gmail.com

How to cite this article: Naik A, Rahouma M, Spadaccio C, Mehta K, Baudo M, Kamel M, Khan F, Hameed I, Wingo M, Ruan Y, Abouarab A, Hossny M, Girardi LN, Gaudino M. Endoscopic radial artery harvesting for coronary artery bypass grafting. *Vessel Plus* 2018;2:38. <http://dx.doi.org/10.20517/2574-1209.2018.62>

Received: 3 Aug 2018 **First Decision:** 11 Sep 2018 **Revised:** 29 Sep 2018 **Accepted:** 29 Sep 2018 **Published:** 7 Nov 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Cai-Hong Wang **Production Editor:** Zhong-Yu Guo

Abstract

This article summarizes the current research on endoscopic technique of radial artery harvesting as a graft for coronary artery bypass grafting (CABG) surgery. Based on the available data, we reviewed various grafts available for CABG. Radial artery as a graft in CABG surgery has recently gained popularity. We sought to investigate the impact of radial artery harvesting techniques on clinical outcomes. Endoscopic harvest approach was found to be feasible in all patients when performed by skillful surgeon while local arm complications were found to be infrequent. However, when compared to open approach for harvest, it takes longer but provides higher patient satisfaction and cosmetic result.

Keywords: Endoscopic radial artery harvesting, coronary artery bypass grafting, harvesting techniques, wound complication, endoscopic technique cost, patency rates

INTRODUCTION

Coronary artery bypass grafting (CABG) is considered one of the mainstays of myocardial revascularization particularly in patients with left main coronary artery disease, multi-vessel coronary artery disease, and diabetes^[1,2].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Conduits available in CABG can be broadly classified into arterial and venous grafts. Saphenous vein (SV), Internal Thoracic Artery (ITA), Radial Artery (RA), Ulnar Artery (UA), and right gastroepiploic artery can be used as grafts^[3,4]. Venous grafts have been found to be more prone to thrombosis, intimal hyperplasia, and atherosclerosis in comparison with arterial grafts^[5]. Arterial grafts are thus more widely preferred over venous grafts^[6].

The RA has been suggested as a suitable conduit for CABG in the 70's^[7] in virtue of advantages like uniform size and easy availability^[8]. Its use was initially abandoned due to concerns of vasospasm, but progressively reconsidered in light of the positive long-term results described in the last decade. At least 6 randomized clinical trials and several observational studies have positively compared patency rates and outcomes of RA vs. RITA and saphenous vein graft (SVG), and very recent evidence from a large patient-level meta-analysis has declared the actual "renaissance" of the RA^[9]. Bilateral internal thoracic artery (BITA) grafting is not widely used due to a higher incidence of sternal complications when compared to single internal thoracic artery grafting (SITA) (0.6% vs. 1.9%, 95% CI: 1.5-6.8) which necessitates additional measures to improve prognosis. Current recommendations are:

Use of ITA in left anterior descending (LAD) bypass in patients where benefits greatly outweigh risks, i.e., when the procedure has to be performed.

An alternative to LITA should be used in patients where benefits outweigh risks, i.e., when additional studies with broad objectives are required.

BITA should be considered in cases where risk of sternal complications is minimal and benefits outweigh risks, i.e., when additional studies with broad objectives are required.

Risk of infection with BITA, should be reduced by skeletonizing the grafts, encouraging smoking cessation, having tight glycemic control, and ensuring adequate sternal stabilization^[10].

Both endoscopic and open techniques are available options for RA harvesting and carry different advantages and complications.

This review will focus on RA and will examine the main features of this conduit along with the impact of the available harvesting techniques on clinical outcomes.

PREOPERATIVE CONSIDERATIONS

While there are no major absolute contraindications to the use of RA, some disadvantages include its increased tendency to spasm owing to a thick tunica media. It is also associated with higher risk of atherosclerosis and intimal hyperplasia compared to ITA^[3]. Despite initial reports of lower patency rate of RA grafts in diabetic patients when compared to SVG at 1 year (89.28% were patent in RA vs. 97.05% patent in SVG)^[11], the more recent literature and the results of a large patient-level meta-analysis confirm the suitability of RA in diabetics and its theoretical advantage in terms of sternal wound infections when compared to BITA^[9,12,13].

Pre-operative suitability of the radial artery is most commonly assessed by the modified Allen's test which is used to determine the patency of the vessels supplying the hand. If the patient's hand flushes within 5-15 s after the examiner releases the occlusive pressure applied on both the RA and the UA, it is considered a positive modified Allen's test. If the hand takes longer than 15 s the test is considered negative. A positive modified Allen's test translates into good blood circulation in the forearm^[14]. However, the reliability of this test has been repeatedly questioned. Jarvis *et al.*^[15] recommended a Doppler ultrasound to be the gold standard. They

determined the sensitivity and specificity of the modified Allen's test to be 54.5% and 91.7% respectively with a diagnostic accuracy of 81.7% at the conventional cut off value of 6 second. Doppler ultrasound, on the other hand had sensitivity and specificity of 100% and 27% respectively with a diagnostic accuracy of 52%^[15,16]. Starnes *et al.*^[17] raised concerns that the high false negative rate of the modified Allen's test could lead to unnecessary exclusion of some patients by placing some patients at an incorrectly high risk of digit ischemia. They recommended direct digit pressure measurement, which is simpler and more accurate to determine the adequacy of collateral circulation in the hand prior to CABG.

In 1998, Buxton *et al.*^[18] were the first to describe the successful use of the ulnar artery as a conduit in a series of 8 patients. The idea of collateral circulation to the hand allowing the use of either the radial or ulnar artery for harvest was reiterated in a larger, more recent study of 25 patients described by Newcomb *et al.*^[19] in 2006. Although, both studies conclude that routine use of the ulnar artery is not recommended due to its close proximity to the ulnar nerve with significant potential for resultant motor and sensory deficits in the hand along with the fact that the ulnar artery tends to be the dominant artery to the hand^[18,19].

Another factor to be considered before choosing the RA for graft is the previous use of the conduit for invasive diagnostic procedures, which exposes the artery to post trans-radial access (TRA) occlusion. Interventional cardiologists commonly use the radial artery as access during procedures like percutaneous cardiovascular intervention (PCI) and angiography^[20]. Our group previously reported significant endothelial damage in the vessel post-TRA leading to reduced vasodilatory function of the vessel with no clear evidence of return of baseline function with time. Along with the biological dysfunction, the risk of RA thrombotic occlusion has been estimated at 7.7% at 1 day and 5.5% at > 7 days after the procedure in a recent large meta-analysis with specific clinical factors (i.e., age, diabetes, reduced artery size, female gender, peripheral vascular disease, smoking, low body weight and lack of statin use) and procedural factors (i.e., use of non-hydrophilic catheters, prolonged postprocedural high pressure compression, *etc.*) being described as mainly responsible for occurrence of RA occlusion^[21]. Additionally, an association between TRA and graft occlusion has been described, leading to the recent recommendations on the use of non-punctured RA as graft for CABG and on the preservation of the RA during angiographic diagnostic procedures in surgical candidates^[20,22]. In situations with limited graft options, it is recommended that the surgeon should perform Doppler ultrasound preoperatively to positively determine the patency and the diameter of the vessel. The use of the distal end of the artery should also be avoided^[22].

It was found that the incidence of the RA graft not being suitable for use after findings of non-satisfactory collateral circulation pre-operatively was around 5%^[23-25]. This does not take away from the versatility of the RA graft which can be used as a single free graft, as a Y graft, or as a sequential graft^[21].

Apart from the previously mentioned reasons for graft failure amongst venous and arterial grafts, another very important aspect to address is the degree of native artery stenosis when considering the RA for use as conduit. Tatoulis *et al.*^[26] explained that arterial graft patency rate is proportional to native artery stenosis severity so the more severe the stenosis the higher the patency rate of the arterial conduit. Radial arteries are known to be more sensitive and show best results when the native artery has at least 80% stenosis^[26]. This phenomenon is termed competitive flow and results in graft failure if the flow through the graft matches flow through the native artery after bypass. Vein grafts do not experience this phenomenon since they have much less resistance with a larger diameter^[27].

INTRAOPERATIVE CONSIDERATIONS

Radial artery graft harvest could be performed open or endoscopically [open radial artery harvest (ORAH) or endoscopic radial artery harvest (ERAH)], preferably from the non-dominant arm^[28]. The vessel might be harvested in skeletonized or pedicled fashion. Despite some advocating an advantage in conduit length and

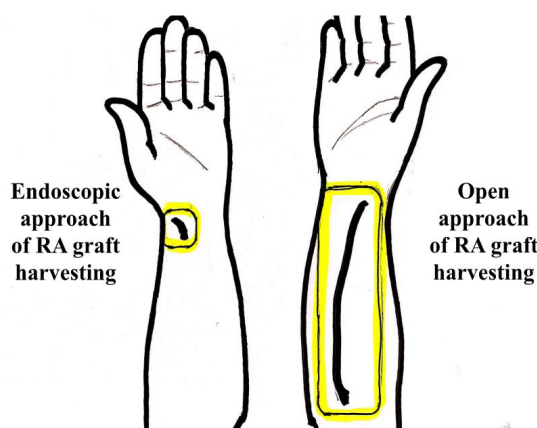


Figure 1. Endoscopic vs. open radial artery (RA) harvesting incision

diameter when harvested in skeletonized fashion ($P < 0.01$)^[29], others point out the disadvantage of longer harvesting time and the risk for endothelial damage especially when the Harmonic scalpel is used^[30]. The Harmonic scalpel uses ultrasonic energy for tissue dissection which is well known to cause increased release of nitric oxide leading to vasodilation and endothelial damage^[31]. Considering the lack of clear evidence pointing towards a significant improvement in patency rate using the skeletonizing technique, this approach should be discouraged^[16].

The open technique requires the forearm to be incised in its entirety. The artery is accessed via a curvilinear incision along the edge of the brachioradialis muscle. The incision is initiated 1 cm distal to the elbow and extends up to 1 cm proximal to the wrist. After retraction of the superficial veins, the fascia is incised to expose the radial artery in the mid arm under the belly of brachioradialis muscle [Figure 1]^[32].

Endoscopic approach of RA harvesting involves a longitudinal incision about 2-3 cm long proximal to the wrist crease. Dissection is done using bipolar scissors or bisector under direct endoscopic vision. Dissection is done as a pedicle with accompanying venae comitantes included. Carbon dioxide (CO_2) is insufflated at 10-12 mmHg to provide a working tunnel. Addition of CO_2 insufflation prevents spasms in the artery. This is followed by anterior dissection along the radial artery bilaterally up to the level of the antecubital fossa. Posterior dissection is then done up to the level of the radial plexus. Dissection is carried out laterally to achieve adequate branch length for sealing later. Fasciotomy enhances visualization and prevents the development of compartment syndrome. This is followed by branch division using a C-ring to stabilize the pedicle. The graft is then finally retrieved^[33].

Navia *et al.*^[34] found that ERAH requires more equipment in comparison with ORAH, hence it is more expensive than ORAH. Shapira *et al.*^[35] showed that the cost of an endoscopic kit, including the disposable Harmonic shears, is \$550. ERAH was also found to take a longer duration as compared to ORAH with an associated steep learning curve in inexperienced hands^[36]. The reported learning curve for endoscopic harvest ranges from 5 to 30 cases^[37]. Although, Kiaii *et al.*^[38] found that the endoscopic approach requires a significantly lower harvest time (36.5 ± 9.4 min) compared to the open approach (57.7 ± 9.4 min) when performed by a surgeon adequately experienced in endoscopic harvest.

POSTOPERATIVE OUTCOMES

Patency rates

Radial artery as a graft for CABG was first introduced by Carpentier *et al.*^[7], in 1973, however, its use was abandoned because of concerns of spasm and intimal hyperplasia. In 1975, Curtis *et al.*^[8] found that in the

late post-operative period 64.7% of grafts (22 out of 34 grafts in 29 patients) had occlusive intimal changes. On the other hand, later in the early 90's, Acar *et al.*^[39] demonstrated a 93.5% patency rate at 9 month follow-up concluding that the RA was still a reasonable alternative to complement IMA. Ikeda *et al.*^[40] found the patency rates of RA graft to be 91% (24 out 26 grafts), which was comparable to ITA graft patency rate of 97% (139 out 143 grafts) in the mid post-operative period (27 ± 10 months). Subsequently, Desai *et al.*^[41] also found that RA grafts were less likely to develop occlusion at one year when compared to saphenous venous grafts. RA grafts were found to have a higher patency rate at 5 year follow up when compared to SVG^[42]. Angiographic studies have demonstrated a patency rate of 80%-90% at 7-10 year follow-up^[43]. A more recent study reported an 84.4% patency rate at 20 years with a probability of graft failure at a similar time to LITA ($19.0\% \pm 0.2\%$ for LITA vs. $25.0\% \pm 0.2\%$ for the RA)^[44].

Moreover, a large patient-level meta-analysis, involving 1,036 patients from 6 trials, comparing RA with saphenous vein grafts showed a significant reduction in the incidence of adverse cardiac events like myocardial infarction, repeat revascularization, and death from cardiac causes with the use of the RA^[9].

Vasospasm was one of the major reasons which lead to a significant degree of reluctance in the use of the RA as a graft conduit in CABG procedures soon after its introduction. These concerns were related to the pronounced muscular profile of the RA wall as opposed to the more elastic wall of the ITA. Peri-operative arterial spasm is reported at 0.43% in all CABG surgeries, and He *et al.*^[45] have suggested this to be an underestimate owing to the chance of mild spasms going unreported. For this reason, a number of pharmacology prophylaxis protocols aimed at avoiding vasospasm have been developed^[46]. Treatment includes the use of calcium channel blockers with or without long-acting nitrates in the postoperative management of these patients^[46] or during surgery as per Reyes *et al.*^[47]. Chanda *et al.*^[48] recommend a combination of a calcium channel blocker like nifedipine, diltiazem, or verapamil with nitroglycerine for prevention of spasm. Organic nitrates like nitroglycerine have a short half-life but have a faster onset of action as compared to calcium channel blockers like nifedipine, verapamil, *etc.* These drugs are usually started immediately post operatively and continued for the first postoperative year. However, biological studies have demonstrated a progressive remodeling towards a more elastomuscular phenotype after implantation of the RA as a graft^[49], and clinical reports deny a benefit from antispastic pharmacological therapy^[50].

COMPARISON OF OUTCOMES BETWEEN ERAH AND ORAH

Harvesting technique may have an impact on postoperative outcomes and surgical complications. In early experiences in the late 90's, Royse *et al.*^[51] reported that post-operative numbness and paresthesia of the hand were a commonly observed complication although there was a 98.9% resolution within 3-6 months after surgery. Neurological complications such as sensory loss occurred in 1.6%-18.1%, while motor complications such as diminished thumb strength were observed in 5.5% of patients in other large series^[51-53].

After the introduction of endoscopic harvesting, the incidence of these complications significantly decreased. In an early prospective study from Patel *et al.*^[54] comparing ERAH with ORAH, major neurological complication restricting motor function post-operatively (8% vs. 1% patients at 1 month, $P < 0.05$), wound erythema, ecchymosis, mild numbness, or tingling were found to be significantly increased when using open approaches [Table 1].

More recently, the results of a propensity-matched study showed significantly lower hand ischemia (open 7.3% vs. endoscopic 0%, $P = 0.007$), by performing a modified Allen test prior to graft selection to confirm good collateral blood flow to the hand, and wound infection rate in the ERAH group, as well as better minor neurological outcomes (open 19.5% vs. endoscopic 3.6%, $P < 0.001$). Interestingly, freedom from cardiac-related mortality (open $96.3\% \pm 2.1\%$ vs. endoscopic $98.1\% \pm 1.8\%$, $P = 0.448$) as well as survival free from major cardiac and cerebrovascular adverse events (open $93.9\% \pm 2.6\%$ vs. endoscopic $93\% \pm 3.4\%$, $P = 0.996$)

Table 1. Comparison between endoscopic vs. open radial artery harvesting [6,11,14-17,33-35,38,45,51,53,54,60,63,64]

		ERAH	ORAH
Pre-operative considerations	Patient selection [11,60,63,64]	Comorbidities: - Diabetes - Hypertension	
	Graft selection [14-17,20]	Graft patency testing - Modified Allen's test - Doppler ultrasound - Direct digital pressure measurements Previous trans-radial artery approach	
Operative factors	Duration [38]	36.5 ± 9.4 min	57.7 ± 9.4 min
	Length of incision [33]	2-3 cm long	Full length of the forearm
	Cost [34,35]	\$550 for the endoscopic kit including the disposable harmonic shears	Less expensive due to fewer pieces of equipment required
	Expertise [36]	Steep learning curve	Easier to learn
Post-operative [36,51-54]	Hematoma (post operatively) [54]	5/100	0/100
	Wound infection (post operatively) [54]	7/100	1/100
	Neuralgia restricting motor function [54]	10/100	1/100
	Neuralgia restricting motor function [54]	8/100	1/100
	Neuralgia restricting motor function [54]	5/100	0/100
	Neuralgia restricting motor function [54]	1/100	0/100
	Ecchymosis (post operatively) [54]	21/100	2/100
	Wound erythema (post operatively) [54]	4/100	0/100
	Mild neuralgia [54]	31/100	18/100
	Mild neuralgia [54]	26/100	8/100
	Mild neuralgia [54]	14/100	4/100
	Mild neuralgia [54]	7/100	0/100
	Patency rate (mid-term follow up period)		91%
	Vasospasm (post operatively)		0.43%

ERAH: Endoscopic radial artery harvest; ORAH: open radial artery harvest

at 5 year follow-up were similar among the groups. This suggests that ERAH could provide additional short-term benefits in terms of improved cosmesis and reduced wound and neurologic complications without compromising the long-term clinical outcomes [55].

These results were confirmed by Burns *et al.* [56] by demonstrating non-inferiority of ERAH with regards to patency rates at 5 years when compared to ORAH (91.2% ERAH vs. 87.5% in ORAH, $P = 0.705$).

Finally, a recent meta-analysis of randomized controlled and propensity matched studies comparing the endoscopic approach of harvesting the RA graft with the open approach demonstrated a lower incidence of wound complications [odds ratio (OR) = 0.33, 95% CI: 0.14-0.77, $P = 0.01$] with similar patency rates and early mortality rates (OR = 1.32, 95% CI: 0.76-2.27, $P = 0.32$ and OR = 0.78, 95% CI: 0.10-6.11, $P = 0.81$) [Figure 2] [57].

Lastly, ERAH has a steep learning curve considering the need to master manipulation of the conduit along with the endoscope which requires an advanced hand-eye coordination. Initial experiences do indeed describe harvest times longer than one hour [58-60] [Table 1], but other reports from the neurosurgical arena, in which the RA is also widely used as a conduit, show that the learning curve associated with the endoscope can be overcome by practice on cadavers [61].

CONCLUSION

It is suggested that standard treatment for patients with multivessel disease is use of single or bilateral ITA along with additional arterial conduit [62]. When appropriate, the use of RA is recommended over SV graft since it is associated with better 5 year patency rates and improved patient longevity. The RA is preferred in patients at risk for sternal wound complications, such as diabetics who cannot tolerate BITA grafting [18,59].

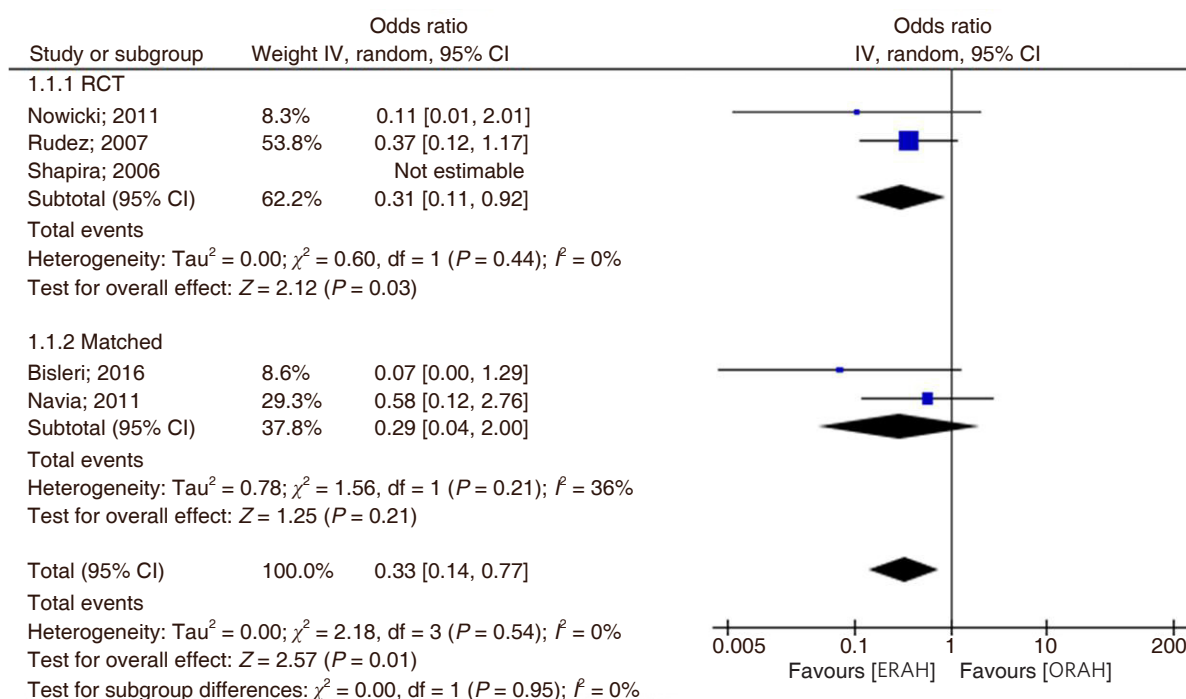


Figure 2. Forest plot of wound infection in endoscopic radial artery harvest (ERAH) vs. open radial artery harvest (ORAH)

The RA offers a decreased risk of atherosclerosis and post-operative complications compared to other graft options like venous grafts. ORAH has a higher risk of wound infection with a larger scar but a shorter harvest time and is easier to learn. ERAH is feasible in all patients when performed by skillful personnel, has fewer arm complications, and provides a higher patient satisfaction with better cosmetic results but takes a longer time to perform when compared to ORAH^[58,59]. The post-operative cardiac complications and outcomes remain comparable in the two approaches.

DECLARATIONS

Authors' contributions

Wrote the paper, created figures for paper: Naik A

Idea generation, helped in writing: Rahouma M

Author for different parts of paper: Rahouma M, Spadaccio C, Khan F

Reviewed and edited of paper: Spadaccio C, Mehta K, Baudo M, Kamel M, Khan F, Hameed I, Wingo M, Ruan Y, Abouarab A, Hossny M, Girardi LN, Gaudino M

Helped with review of figures and tables: Mehta K

Made table for paper: Baudo M

Availability of data and materials

Data and materials used to help write this article are available for public access.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Kolh P, Windecker S, Alfonso F, Collet JP, Cremer J, et al. 2014 ESC/EACTS guidelines on myocardial revascularization: the task force on myocardial revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). *Eur J Cardiothorac Surg* 2014;46:517-92.
2. Arisha MJ, Ibrahim DA, Abouarab AA, Ahmed MMR, Hussein MKK, et al. Percutaneous coronary intervention in the elderly: current updates and trends. Available from: https://www.researchgate.net/publication/326272491_Percutaneous_coronary_intervention_in_the_elderly_current_updates_and_trends. [Last accessed on 11 Oct 2018]
3. Martínez-González B, Reyes-Hernández CG, Quiroga-Garza A, Rodríguez-Rodríguez VE, Esparza-Hernández CN, et al. Conduits used in coronary artery bypass grafting: a review of morphological studies. *Ann Thorac Cardiovasc Surg* 2017;23:55-65.
4. Desai M, Seifalian AM, Hamilton G. Role of prosthetic conduits in coronary artery bypass grafting. *Eur J Cardiothorac Surg* 2011;40:394-8.
5. Parang P, Arora R. Coronary vein graft disease: pathogenesis and prevention. *Can J Cardiol* 2009;25:e57-62.
6. Gaudino M, Cellini C, Pragliola C, Trani C, Burzotta F, et al. Arterial versus venous bypass grafts in patients with in-stent restenosis. *Circulation* 2005;112:1265-9.
7. Carpentier A, Guermontprez JL, Deloche A, Frechette C, DuBost C. The aorta-to-coronary radial artery bypass graft. A technique avoiding pathological changes in grafts. *Ann Thorac Surg* 1973;16:111-21.
8. Curtis JJ, Stoney WS, Alford WC Jr, Burrus GR, Thomas CS Jr. Intimal hyperplasia. A cause of radial artery aortocoronary bypass graft failure. *Ann Thorac Surg* 1975;20:628-35.
9. Gaudino M, Benedetto U, Fremes S, Biondi-Zoccai G, Sedrakyan A, et al. Radial-artery or saphenous-vein grafts in coronary-artery bypass surgery. *N Engl J Med* 2018;378:2069-77.
10. Aldea GS, Bakaeen FG, Pal J, Fremes S, Head SJ, et al. The society of thoracic surgeons clinical practice guidelines on arterial conduits for coronary artery bypass grafting. *Ann Thorac Surg* 2016;101:801-9.
11. Goldman S, Sethi GK, Holman W, Thai H, McFalls E, et al. Radial artery grafts vs saphenous vein grafts in coronary artery bypass surgery: a randomized trial. *JAMA* 2011;305:167-74.
12. Schwann TA, Sleiman AKMEH, Yamine MB, Tranbaugh RF, Engoren M, et al. Incremental value of increasing number of arterial grafts: the effect of diabetes mellitus. *Ann Thorac Surg* 2018;105:1737-44.
13. Hoffman DM, Dimitrova KR, Lucido DJ, Dincheva GR, Geller CM, et al. Optimal conduit for diabetic patients: propensity analysis of radial and right internal thoracic arteries. *Ann Thorac Surg* 2014;98:30-7.
14. WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy. Geneva: World Health Organization; 2010. Annex I, Modified Allen test. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK138652/>. [Last accessed on 15 Oct 2018]
15. Jarvis MA, Jarvis CL, Jones PRM, Spyt TJ. Reliability of Allen's test in selection of patients for radial artery harvest. *Ann Thorac Surg* 2000;70:1362-5.
16. Gaudino M, Crea F, Cammertoni F, Mazza A, Toesca A, et al. Technical issues in the use of the radial artery as a coronary artery bypass conduit. *Ann Thorac Surg* 2014;98:2247-54.
17. Starnes SL, Wolk SW, Lampman RM, Shanley CJ, Prager RL. Noninvasive evaluation of hand circulation before radial artery harvest for coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 1999;117:261-6.
18. Buxton BF, Chan AT, Dixit AS, Eizenberg N, Marshall RD, et al. Ulnar artery as a coronary bypass graft. *Ann Thorac Surg* 1998;65:1020-4.
19. Newcomb A, Oqueli E, Buxton BF. Ulnar artery as a coronary artery bypass graft: five-year experience. In: He GW, editor. *Arterial grafting for coronary artery bypass surgery*. Berlin: Springer; 2006. p. 227-32.
20. Gaudino M, Burzotta F, Bakaeen F, Bertrand O, Crea F, et al. The radial artery for percutaneous coronary procedures or surgery? *J Am Coll Cardiol* 2018;71:1167-75.
21. Rashid M, Kwok CS, Pancholy S, Chugh S, Kedev SA, et al. Radial artery occlusion after transradial interventions: a systematic review and meta-analysis. *J Am Heart Assoc* 2016;5:e002686.
22. Mounsey CA, Mawhinney JA, Werner RS, Taggart DP. Does previous transradial catheterization preclude use of the radial artery as a conduit in coronary artery bypass surgery? *Circulation* 2016;134:681-8.
23. Calafiore AM, Di Giammarco G, Teodori G, D'Annunzio E, Vitolla G, et al. Radial artery and inferior epigastric artery in composite grafts: improved midterm angiographic results. *Ann Thorac Surg* 1995;60:517-23; discussion 523-4.
24. Brodman RF, Frame R, Camacho M, Hu E, Chen A, et al. Routine use of unilateral and bilateral radial arteries for coronary artery bypass graft surgery. *J Am Coll Cardiol* 1996;28:959-63.

25. Risteski PS, Akbulut B, Moritz A, Aybek T. The radial artery as a conduit for coronary artery bypass grafting: review of current knowledge. *Anadolu Kardiyol Derg* 2006;6:153-62.
26. Tatoulis J, Buxton BF, Fuller JA. Patencies of 2127 arterial to coronary conduits over 15 years. *Ann Thorac Surg* 2004;77:93-101.
27. Glineur D, Hanet C. Competitive flow and arterial graft a word of caution. *Eur J Cardiothorac Surg* 2012;41:768-9.
28. Baikoussis NG, Papakonstantinou NA, Apostolakis E. Radial artery as graft for coronary artery bypass surgery: advantages and disadvantages for its usage focused on structural and biological characteristics. *J Cardiol* 2014;63:321-8.
29. Amano A, Takahashi A, Hirose H. Skeletonized radial artery grafting: improved angiographic results. *Ann Thorac Surg* 2002;73:1880-7.
30. Rukosujew A, Reichelt R, Fabricius AM, Drees G, Tjan TD, et al. Skeletonization versus pedicle preparation of the radial artery with and without the ultrasonic scalpel. *Ann Thorac Surg* 2004;77:120-5.
31. Discigil B, King RM, Pearson PJ, Capellini VK, Rodrigues AJ, et al. High-frequency ultrasonic waves cause endothelial dysfunction on canine epicardial coronary arteries. *Braz J Cardiovasc Surg* 2008;23:190-6.
32. Blitz A, Osterday RM, Brodman RF. Harvesting the radial artery. *Ann Cardiothorac Surg* 2013;2:533-42.
33. Navia JL, Olivares G, Ehasz P, Gillinov AM, Svensson LG, et al. Endoscopic radial artery harvesting procedure for coronary artery bypass grafting. *Ann Cardiothorac Surg* 2013;2:557-64.
34. Navia JL, Brozzi N, Chiu J, Blackstone EH, Atik FA, et al. Endoscopic versus open radial artery harvesting for coronary artery bypass grafting. *J Cardiovasc Surg (Torino)* 2012;53:257-63.
35. Shapira OM, Eskenazi BR, Anter E, Joseph L, Christensen TG, et al. Endoscopic versus conventional radial artery harvest for coronary artery bypass grafting: functional and histologic assessment of the conduit. *J Thorac Cardiovasc Surg* 2006;131:388-94.
36. Rahouma M, Kamel M, Benedetto U, Ohmes LB, Di Franco A, et al. Endoscopic versus open radial artery harvesting: a meta-analysis of randomized controlled and propensity matched studies. *J Card Surg* 2017;32:334-41.
37. Krishnamoorthy B, Critchley WR, Venkateswaran RV, Barnard J, Caress A, et al. A comprehensive review on learning curve associated problems in endoscopic vein harvesting and the requirement for a standardised training programme. *J Cardiothorac Surg* 2016;11:45.
38. Kiaii BB, Swinamer SA, Fox SA, Stitt L, Quantz MA. A prospective randomized study of endoscopic versus conventional harvesting of the radial artery. *Innovations (Phila)* 2017;12:231-8.
39. Acar C, Jebara VA, Portoghese M, Beyssen B, Pagny JY, et al. Revival of the radial artery for coronary artery bypass grafting. *Ann Thorac Surg* 1992;54:652-60.
40. Ikeda M, Ohashi H, Tsutsumi Y, Hige K, Kawai T, et al. Angiographic evaluation of the luminal changes in the radial artery graft in coronary artery bypass surgery: a concern over the long-term patency. *Eur J Cardiothorac Surg* 2002;21:800-3.
41. Desai ND, Cohen EA, Naylor CD, Fremes SE. A randomized comparison of radial-artery and saphenous-vein coronary bypass grafts. *N Engl J Med* 2004;351:2302-9.
42. Al-Sabti HA, Kindi AA, Al-Rasadi K, Banerjee Y, Al-Hashmi K, et al. Saphenous vein graft vs. radial artery graft searching for the best second coronary artery bypass graft. *J Saudi Heart Assoc* 2013;25:247-54.
43. Tatoulis J, Buxton BF, Fuller JA, Meswani M, Theodore S, et al. Long-term patency of 1108 radial arterial-coronary angiograms over 10 years. *Ann Thorac Surg* 2009;88:23-9; discussion 29-30.
44. Gaudino M, Tondi P, Benedetto U, Milazzo V, Flore R, et al. Radial artery as a coronary artery bypass conduit: 20-year results. *J Am Coll Cardiol* 2016;68:603-10.
45. He GW, Taggart DP. Spasm in arterial grafts in coronary artery bypass grafting surgery. *Ann Thorac Surg* 2016;101:1222-9.
46. Myers MG, Fremes SE. Prevention of radial artery graft spasm: a survey of Canadian surgical centres. *Can J Cardiol* 2003;19:677-81.
47. Reyes AT, Frame R, Brodman RF. Technique for harvesting the radial artery as a coronary artery bypass graft. *Ann Thorac Surg* 1995;59:118-26.
48. Chanda J, Brichkov I, Canver CC. Prevention of radial artery graft vasospasm after coronary bypass. *Ann Thorac Surg* 2000;70:2070-4.
49. Gaudino M, Prati F, Caradonna E, Trani C, Burzotta F, et al. Implantation in coronary circulation induces morphofunctional transformation of radial grafts from muscular to elastomuscular. *Circulation* 2005;112:1208-11.
50. Patel A, Asopa S, Dunning J. Should patients receiving a radial artery conduit have post-operative calcium channel blockers? *Interact Cardiovasc Thorac Surg* 2006;5:251-7.
51. Royse AG, Royse CF, Shah P, Williams A, Kaushik S, et al. Radial artery harvest technique, use and functional outcome. *Eur J Cardiothorac Surg* 1999;15:186-93.
52. Meharwal ZS, Trehan N. Functional status of the hand after radial artery harvesting: results in 3,977 cases. *Ann Thorac Surg* 2001;72:1557-61.
53. Denton TA, Trento L, Cohen M, Kass RM, Blanche C, et al. Radial artery harvesting for coronary bypass operations: neurologic complications and their potential mechanisms. *J Thorac Cardiovasc Surg* 2001;121:951-6.
54. Patel AN, Henry AC, Hunnicutt C, Cockerham CA, Willey B, et al. Endoscopic radial artery harvesting is better than the open technique. *Ann Thorac Surg* 2004;78:149-53.
55. Bisleri G, Giroletti L, Hrapkiewicz T, Bertuletti M, Zembala M, et al. Five-year clinical outcome of endoscopic versus open radial artery harvesting: a propensity score analysis. *Ann Thorac Surg* 2016;102:1253-9.
56. Burns DJP, Swinamer SA, Fox SA, Romsa J, Vezina W, et al. Long-term patency of endoscopically harvested radial arteries: from a randomized controlled trial. *Innovations (Phila)* 2015;10:77-84.
57. Rahouma M, Kamel M, Benedetto U, Ohmes LB, Di Franco A, et al. Endoscopic versus open radial artery harvesting: a meta-analysis of randomized controlled and propensity matched studies. *J Card Surg* 2017;32:334-41.

58. Yoshizaki T, Arai H, Igari T, Tabuchi N, Tanaka H, et al. Endoscopic radial artery harvesting: our initial experience and results of the first 25 patients. *Ann Thorac Cardiovasc Surg* 2005;11:391.
59. Casselman FP, La Meir M, Cammu G, Wellens F, De Geest R, et al. Initial experience with an endoscopic radial artery harvesting technique. *J Thorac Cardiovasc Surg* 2004;128:463-6.
60. Karimi A, Ahmadi H, Davoodi S, Movahedi N, Marzban M, et al. Factors affecting postoperative morbidity and mortality in isolated coronary artery bypass graft surgery. *Surg Today* 2008;38:890-8.
61. Gonzalez LF, Patterson DL, Lekovic GP, Nakaji P, Spetzler RF. Endoscopic harvesting of the radial artery for neurovascular bypass. *Neurosurg Focus* 2008;24:E10.
62. Buxton BF, Hayward PAR, Newcomb AE, Moten S, Seevanayagam S, et al. Choice of conduits for coronary artery bypass grafting: craft or science? *Eur J Cardiothorac Surg* 2009;35:658-70.
63. Zacharias A, Schwann TA, Riordan CJ, Durham SJ, Shah AS, et al. Late results of conventional versus all-arterial revascularization based on internal thoracic and radial artery grafting. *Ann Thorac Surg* 2009;87:19-26.e2.
64. Aronson S, Boisvert D, Lapp W. Isolated systolic hypertension is associated with adverse outcomes from coronary artery bypass grafting surgery. *Anesth Analg* 2002;94:1079-84, table of contents.

Original Article

Open Access



Evaluation of different surgical modalities for coronary reconstruction of diffusely diseased left anterior descending artery

Mohamed H. Elsayed¹, Wael M. Hassanein¹, Samir A. Keshk¹, Mamdouh Zidan², Waheed G. Etman¹

¹Department of Cardiothoracic Surgery, University of Alexandria, Alexandria 21111, Egypt.

²Department of Radiology, University of Alexandria, Alexandria 21111, Egypt.

Correspondence to: Dr. Mohamed H. Elsayed, Department of Cardiothoracic Surgery, University of Alexandria, Champollion Street, Khartoum Square, Azarita, Alexandria 21111, Egypt. E-mail: moh.hassanein@gmail.com

How to cite this article: Elsayed MH, Hassanein WM, Keshk SA, Zidan M, Etman WG. Evaluation of different surgical modalities for coronary reconstruction of diffusely diseased left anterior descending artery. *Vessel Plus* 2018;2:39. <http://dx.doi.org/10.20517/2574-1209.2018.65>

Received: 13 Sep 2018 **First Decision:** 26 Oct 2018 **Revised:** 30 Oct 2018 **Accepted:** 1 Nov 2018 **Published:** 12 Nov 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Cui Yu **Production Editor:** Zhong-Yu Guo

Abstract

Aim: Endarterectomy has been shown to be an effective adjunct in treating diffusely diseased coronary arteries. Reconstruction of endarterectomized coronaries has been done by various techniques. We compare early results of left internal mammary artery (LIMA) patch to saphenous vein patch in left anterior descending artery (LAD) reconstruction.

Methods: We prospectively followed 30 patients with diffusely diseased LAD from January 2016 to January 2018. Patients were followed up clinically, by echocardiogram and CT coronary angiography.

Results: Twenty-seven patients were males (90%). The mean age was 59.23 ± 7.98 . Twenty-two patients (73.3%) had a LIMA onlay patch. The mean length of patch reconstruction was greater in the saphenous vein group than LIMA group (8.31 ± 1.16 cm vs. 5.64 ± 0.73 cm, $P < 0.001$). Postoperative myocardial infarction occurred in 1 patient from the LIMA group (4.5%) and 1 patient in the saphenous vein patch group (12.5%). Operative mortality occurred in 1 patient belonging to the LIMA group. Mean time of follow up was 17.59 ± 6.34 months. CT coronary angiography showed a patency rate of 93.1%.

Conclusion: Results of reconstruction by LIMA and saphenous vein patch are comparable in short-term follow up.

Keywords: Endarterectomy, reconstruction, left internal mammary artery patch



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



INTRODUCTION

The progressive application of percutaneous coronary interventions (PCI) to achieve myocardial revascularization has contributed to the referral of patients with distinctly less attractive anatomic substrates for surgery. Surgical candidates now are usually from an older age group, have more severe coronary lesions and suffer from multiple comorbidities. Of these perhaps diffuse coronary disease is one of the most troublesome situations the surgeon has to deal with.

A conventional anastomosis placed distally in a diffusely diseased vessel such as the left anterior descending artery (LAD) may leave a large area of myocardium supplied by large side branches unrevascularized, which defies the principle of coronary artery bypass grafting (CABG) aiming at complete revascularization^[1]. Hence endarterectomy has been revisited as an adjunct to conventional CABG in such cases.

Coronary endarterectomy is done by different surgical techniques, mainly open and closed. However in principle, they all entail removal of the atherosclerotic plaque or calcified core of the coronary vessel creating a neo-vascular bed which can be revascularized using one of the standard arterial or venous conduits.

Since Bailey's first coronary endarterectomy^[2], a lot has changed, namely the use of cardiopulmonary bypass, pharmacological support, and the growing experience of cardiac surgeons. In the current era results have changed significantly from earlier days where controversial debates were held about coronary endarterectomy due to its morbidity and mortality mainly perioperative myocardial infarction (MI)^[3]. It is therefore important to focus on the current results and proper indications for selecting this technique.

In this study we evaluate the outcome of different surgical modalities for coronary reconstruction in diffusely diseased LAD.

METHODS

Thirty patients with diffusely diseased left anterior descending coronary artery presenting to the Cardiothoracic Surgery Department in Alexandria Faculty of Medicine from January 2016 were included in this study and followed prospectively for at least 6 months. Informed consents were obtained from all patients prior to the procedure with explanation regarding the aim of the procedure and the possible side effects according to the guidelines of ethical committee at Alexandria Faculty of Medicine.

Inclusion criteria: primary elective CABG patients with diffuse LAD disease requiring endarterectomy with or without combined valvular procedure.

Exclusion criteria: patients needing endarterectomy in diffusely diseased vessels other than the LAD.

Indications for LAD endarterectomy: (1) chronic total occlusion of the LAD; (2) heavily calcific plaque impeding suturing of bypass graft to the coronary vessel; (3) multiple obstructions in the LAD; (4) diffusely diseased LAD with atherosclerosis extending into major side branches; and (5) soft atherosclerotic plaque for fear of sutures disrupting the plaque and causing distal embolization.

Surgical procedure: all procedures were done using median sternotomy and cardiopulmonary bypass. Cardioplegia was given in antegrade fashion and temperature was allowed to drift.

The most suitable soft spot was identified for LAD arteriotomy, and arteriotomy was done using super blade. Failure to pass 1 mm coronary probe through the arteriotomy confirmed the need for endarterectomy, which was often anticipated from the coronary angiography and other times not. The LAD arteriotomy was extended using the coronary scissors till a disease free area distal to the atherosclerotic plaque was identified

and the plaque was removed under direct vision using the fine dissecting spatula. This method allowed complete extraction of the plaque from the LAD as well as from septal and diagonal branches if present under vision. Passage of 1 mm probe was done distally to ensure the distal bed was free from plaques. The proximal end of the arteriotomy was not extended beyond the proximal occlusion for fear of competitive flow between the native coronary artery and the graft. The atherosclerotic plaque was sharply divided proximally. This was followed by reconstruction using the left internal mammary artery (LIMA) or on-lay saphenous patch. LIMA was always the first choice for reconstruction. Saphenous vein patch was used in very lengthy reconstructions needing a generous patch or when harvested LIMA appeared short to avoid having a LIMA-LAD anastomosis under tension.

In cases of reconstruction using the saphenous vein, a small part of the vein graft was completely opened longitudinally using scissors to convert it to a vein patch and fashioned to match the length of the arteriotomy. The vein was then sewn to the endarterectomized LAD using prolene 7-0. A small opening was then created in the proximal or mid part of the vein patch using a scalpel and LIMA was anastomosed to the vein graft using prolene 8-0.

In cases where LIMA was used to reconstruct the LAD the LIMA was incised to match the length of the arteriotomy and it was anastomosed directly to the LAD using prolene 7-0 or 8-0 in an onlay fashion. Sometimes the surgeons prefer to start this anastomosis at the toe of the anastomosis unlike the usual where anastomosis is started at the heel. This approach makes it easier to adjust the length of the LIMA patch in case of discrepancy between the length of LIMA patch and the arteriotomy. Bulldog was removed from LIMA to allow flow in the coronary anastomosis and check if there was any leak that can be repaired before removing the cross clamp. After completion of distal anastomoses, the cross clamp was removed. Vein grafts were cut to appropriate length and proximal anastomoses to the aorta were done with partial side biting clamp.

In our study, 2 different anticoagulation protocols were used. In both regimens intravenous unfractionated heparin was started in ICU on day 0 typically after 6 h or as soon as chest tube output dictated with target partial thromboplastin time of 60-90 s. In the dual antiplatelet therapy (DAPT) protocol, 75 mg clopidogrel and low dose aspirin were given on day 1. Clopidogrel was discontinued after 1 year and aspirin given for life. The second regimen was triple therapy with warfarin for 3 months with target international normalized ratio (INR) of 2 in addition to 75 mg clopidogrel (1 year) and low dose aspirin for life.

Echocardiography was done to all patients routinely prior to discharge. Following discharge, patients were followed up in outpatient clinic one week following discharge, then one month after discharge.

Patients enrolled in this study were contacted by phone following discharge to ask about their status regarding dyspnea and chest pain. They were asked to perform CT coronary angiography for assessment of LAD patency. CT coronary angiography was chosen for this study due to its sensitivity in assessment of bypass patency, as well as being less invasive than conventional coronary angiography.

Definitions: operative mortality was defined as death occurring during the hospitalization in which the surgery was performed or death occurring after hospital discharge, but within 30 days, unless clearly unrelated to the operation.

Stroke was defined as neurologic deficit of abrupt onset caused by disturbance in blood supply to the brain persisting > 24 h.

Renal failure: acute or worsening renal function resulting in one or both more of the following. Increase of serum creatinine to > 2 mg/dL and to 2 × the most recent preoperative creatinine or requirement for dialysis.

Table 1. Preoperative characteristics

Preoperative characteristics	No. (%), mean \pm SD, or median (range) (n = 30)
Age (years)	59.23 \pm 7.98
Males	27 (90)
Cardiac profile	
Unstable angina	26 (86.7)
Left main	5 (16.7)
Previous MI	18 (60)
Ejection fraction	51.23 \pm 9.02
Risk factors	
Hypertension	27 (90)
Smoking	16 (53.3)
Dyslipidemia	10 (33.3)
Diabetes mellitus	19 (63.3)
Insulin use	4 (14.3)
Comorbidities	
COPD	9 (30)
Renal impairment	0 (0)
Previous CVA	1 (3.3)
PVD	2 (6.7)
NYHA class	
II	15 (50)
III	15 (50)

MI: myocardial infarction; COPD: chronic obstructive pulmonary disease; CVA: cerebrovascular accident; PVD: peripheral vascular disease; NYHA: New York Heart Association

MI: elevation of biomarkers (creatine kinase-MB or troponin) to more than 5 times the 99th percentile of the normal reference range during the first 72 h after a CABG plus: (1) new pathologic Q waves or left bundle branch block; or (2) angiographically documented new graft or native coronary artery occlusion; or (3) imaging evidence of new loss of viable myocardium.

Statistical analyses were performed using IBM SPSS Statistics 22.0 software (IBM Corp, Armonk, NY). Normally distributed continuous variables are expressed as the mean \pm SD, and skewed continuous variables are expressed as the median with the range. For comparison of the 2 groups, *t* test, Wilcoxon signed rank test, or Fisher's exact test was used as deemed appropriate.

RESULTS

Baseline clinical characteristics and demographic data are summarized in [Table 1](#). The endarterectomized LAD was reconstructed using LIMA onlay patch in 22 patients (73.3%). Postoperative MI occurred in 2 patients, one of them only in the reconstructed LAD territory. There was one mortality occurring 5 days postoperative in the ICU due to MI and refractory ventricular arrhythmias resulting in operative mortality of 3.3%. Intraoperative and postoperative data are summarized in [Table 2](#).

At least 6 months after surgery all patients except for one case of mortality had a coronary CT angiogram done and showed 93.1 percent patency rate with 2 patients found to have stenotic LIMA-LAD anastomosis [[Table 3](#), [Figure 1](#)]. One patient belonged to the LIMA group and the other to the saphenous patch group. In addition to the CT angiography echocardiogram was done and the patients were followed up clinically regarding any chest pain and dyspnea. Angina occurred in 4 patients and they belonged to Canadian Cardiovascular Society (CCS) class I. [Tables 4 and 5](#) showed the improvement in New York Heart Association (NYHA) class and ejection fraction postoperatively.

[Tables 6 and 7](#) showed comparison between both methods of reconstruction using LIMA or LIMA and saphenous vein patch in relation to the baseline demographics, intraoperative and postoperative events.

Table 2. Intraoperative and postoperative data

Variables	No. (%), mean \pm SD, or median (range) (n = 30)	
Number of distal anastomosis	1	6 (20)
	2	6 (20)
	3	12 (40)
	4	6 (20)
Cross clamp time (min)		60.7 \pm 17.82
		60 (28.0-100.0)
Method of reconstruction	LIMA patch	22 (73.3)
	SVG patch + LIMA	8 (26.7)
Length of reconstruction (cm)		6.35 \pm 1.47
		6 (5-10)
Time of ventilation (h)		6 (3-110)
Inotropic support (days)		2.40 \pm 0.93
ICU stay (days)		4.93 \pm 1.41
Myocardial infarction		2 (6.7)
Need for dialysis		0 (0)
Stroke		0 (0)
Atrial fibrillation		7 (23.3)
Mediastinitis		1 (3.3)
Bleeding requiring exploration		2 (6.7)
Mortality		1 (3.3)

LIMA: left internal mammary artery; SVG: saphenous vein graft

Table 3. Follow up and CT angiographic patency

Variables	No. (%), mean \pm SD, or median (range) (n = 29)
Duration of follow up (months)	17.59 \pm 6.34
	20.0 (6.0-26.0)
Post operative ejection fraction	56.28 \pm 5.62
	58.0 (48.0-68.0)
Post op. NYHA class	
	I 20 (69.0)
LIMA LAD CT angio patency	II 9 (31.0)
	Patent 27 (93.1)
Postoperative angina	4 (13.8)
GI bleeding	1 (3.4)
Medications	
	Plavix aspirin 12 (41.4)
	Plavix aspirin + marevan 17 (58.6)

NYHA: New York Heart Association; LIMA: left internal mammary artery; LAD: left anterior descending artery; GI: gastrointestinal

DISCUSSION

Complete revascularization of coronary vessels is the main target of CABG and in particular the LIMA-LAD anastomosis, since a patent LIMA-LAD is the single most important determinant of the long-term and event-free survival^[4].

With advances in PCI, patients now referred to CABG are becoming more complex with multiple comorbidities as well as less attractive target vessels. Diffuse coronary artery disease is a problem that faces surgeons now with increasing frequency since in this subset of patients PCI produces less than optimal results^[5]. In up to 25% of patients with diffuse coronary disease conventional CABG as well would not optimally revascularize the ischemic territories^[6]. Therefore endarterectomy was revisited as an option to increase the surgical armamentarium in facing this complex lesion.

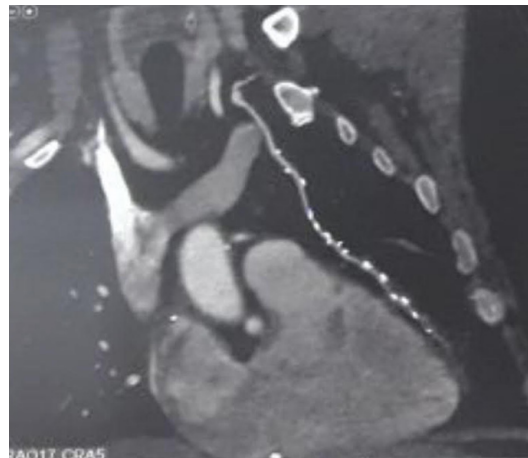


Figure 1. Representative image of CT coronary angiography showing left internal mammary artery (LIMA) patch with patent LIMA-left anterior descending artery anastomosis

Table 4. Comparison between pre and postoperative New York Heart Association class

NYHA class	Preoperative (<i>n</i> = 30)		Postoperative (<i>n</i> = 29)		<i>Z</i>	<i>P</i>
	No.	%	No.	%		
I	0	0.0	20	69.0	4.919*	< 0.001*
II	15	50.0	9	31.0		
III	15	50.0	0	0.0		

*Statistically significant at $P \leq 0.05$; *Z* and *P* values for Wilcoxon signed ranks test for comparing between pre and postoperative; NYHA: New York Heart Association

Table 5. Comparison between pre and postoperative ejection fraction

	Preoperative (<i>n</i> = 30)	Postoperative (<i>n</i> = 29)	<i>T</i>	<i>P</i>
Ejection fraction				
Min-Max	35.0-70.0	48.0-68.0	4.968*	< 0.001*
Mean \pm SD	51.23 \pm 9.02	56.28 \pm 5.62		
Median	49.0	58.0		

*Statistically significant at $P \leq 0.05$; *T* and *P* values for Paired *t*-test for comparing between pre and postoperative

Endarterectomy entails removal of the atherosclerotic core from the coronary vessel. In closed endarterectomy (also called pull out method) a small arteriotomy is used to dissect the atherosclerotic plaque out of the coronary vessel by using steady and gentle traction. It's a simpler technique but was criticized due to the possibility of occlusion of the distal LAD or its branches by insufficient endarterectomy the so called snowplow effect^[7]. In addition blind traction on the atherosclerotic core can lead to tears and iatrogenic intimal flaps that would lead to occlusion of the coronary vessel^[8].

In open endarterectomy a longitudinal arteriotomy is performed on the coronary vessel beyond the limits of the atheromatous plaque and the atherosclerotic plaque is dissected under vision from the coronary vessel and its side branches avoiding any residual obstruction and repairing any intimal flaps that might occur during the removal of the plaque. The coronary is then reconstructed using an onlay patch of saphenous vein or LIMA itself. Advocates of coronary reconstruction using the saphenous vein patch suggested that it is very easy to use, easy to harvest and enlarged the lumen of the reconstructed LAD^[6,8]. However others accused it of being more time consuming, as you convert one anastomosis into two anastomoses, first anastomosis of saphenous vein graft (SVG) to the endarterectomized LAD, then anastomosing the LIMA onto the saphenous vein patch. It was also suggested that this complex vascular bed formed of three

Table 6. Relation between method of reconstruction and preoperative characteristics

	Method of reconstruction		Test of Sig	P
	LIMA (n = 22) No. (%)	LIMA + vein (n = 8) No. (%)		
Gender				
Males	21 (95.5)	6 (75.0)	$\chi^2 = 2.727$	^{FE} P = 0.166
Age				
Mean \pm SD	59.4 \pm 7.69	58.75 \pm 9.27	T = 0.180	0.861
Median	60.0 (40.0-69.0)	62.0 (42.0-67.0)		
Cardiac profile				
Left main	4 (18.2)	1 (12.5)	$\chi^2 = 0.136$	^{FE} P = 1.000
Unstable angina	20 (90.9)	6 (75.0)	$\chi^2 = 1.285$	^{FE} P = 0.284
NYHA class				
I	0 (0.0)	0 (0.0)		
II	10 (45.5)	5 (62.5)	$\chi^2 = 0.682$	^{FE} P = 0.682
III	12 (54.5)	3 (37.5)		
Previous MI	14 (63.6)	4 (50.0)	$\chi^2 = 0.455$	^{FE} P = 0.678
Ejection fraction				
Mean \pm SD	50.27 \pm 8.59	53.88 \pm 10.23	T = 0.967	0.342
Median	48.0 (35.0-70.0)	54.0 (40.0-70.0)		
Coronary risk factors				
Hypertension	19 (86.4)	8 (100)	$\chi^2 = 1.212$	^{FE} P = 0.545
Smoking	13 (59.1)	3 (37.5)	$\chi^2 = 1.099$	^{FE} P = 0.417
Dyslipidemia	5 (22.7)	5 (62.5)	$\chi^2 = 4.176$	^{FE} P = 0.078
Diabetes mellitus	13 (59.1)	6 (75.0)	$\chi^2 = 0.639$	^{FE} P = 0.672
Insulin use	3 (13.6)	1 (12.5)	$\chi^2 = 0.007$	^{FE} P = 1.000
Comorbidities				
COPD	8 (36.4)	1 (12.5)	$\chi^2 = 1.591$	^{FE} P = 0.374
Renal impairment	0 (0.0)	0 (0.0)	-	-
Prev.CVA	1 (4.5)	0 (0.0)	$\chi^2 = 0.376$	^{FE} P = 1.000
PVD	1 (4.5)	1 (12.5)	$\chi^2 = 0.597$	^{FE} P = 0.469

χ^2 : Chi square test; FE: fisher exact; T: Student *t*-test; P: P value for comparing between the two groups; NYHA: New York Heart Association; COPD: chronic obstructive pulmonary disease; CVA: cerebrovascular accident; PVD: peripheral vascular disease

Table 7. Comparison between both groups of reconstruction

Variables	Method of reconstruction		Test of Sig	P
	LIMA (n = 22)	LIMA + vein (n = 8)		
Cross clamp time (min)				
Mean \pm SD	58.9 \pm 18.80	65.6 \pm 14.74	T = 0.910	0.371
Median (range)	60.0 (28.0-100.0)	70.0 (40.0-85.0)		
Length of reconstruction (cm)				
Mean \pm SD	5.64 \pm 0.73	8.31 \pm 1.16	T = 7.564	< 0.001*
Median (range)	5.50 (5.0-7.0)	8.0 (7.0-10.0)		
Time of ventilation (h)				
Mean \pm SD	15.82 \pm 15.82	8.0 \pm 5.50	U = 68.00	0.344
Median (range)	6.0 (3.0-110.0)	4.50 (4.0-17.0)		
Inotropic support (days)				
Mean \pm SD	2.41 \pm 1.01	2.38 \pm 0.74	U = 85.50	0.889
Median (range)	2.0 (1.0-5.0)	2.0 (2.0-4.0)		
Myocardial infarction	1 (4.5)	1 (12.5)	$\chi^2 = 0.597$	^{FE} P = 0.469
Atrial fibrillation	6 (27.3)	1 (12.5)	$\chi^2 = 0.716$	^{FE} P = 0.638
Bleeding requiring reexploration	1 (4.5)	1 (12.5)	$\chi^2 = 0.597$	^{FE} P = 0.469
Mortality	1 (4.5)	0 (0.0)	$\chi^2 = 0.376$	^{FE} P = 1.000
Patency rate*	20 (95.2)	7 (87.5)	$\chi^2 = 0.540$	^{FE} P = 0.483
Postoperative angina*	2 (9.5)	2 (25.0)	$\chi^2 = 1.167$	^{FE} P = 0.300

*One case of mortality (n = 29). χ^2 : Chi square test; FE: fisher exact; T: Student *t*-test; P: P value for comparing between the two groups; U: Mann Whitney test

components (LIMA, SVG, and native coronary) caused turbulence of flow due to difference in compliance of the the three components^[9]. Enlarging the lumen of the reconstructed LAD was accused of decreasing flow velocity^[9]. In addition long term patency was questioned due to the known predilection of veins to more rapid intimal hyperplasia.

As studies continued to support the use of arterial grafting, and showing arterial grafts to show better late patency rates than SVG, the use of onlay LIMA patching to endarterectomized LAD gained wider popularity^[10]. In this method the LIMA itself was used as a patch after adjusting its opening to the length of the LAD arteriotomy. Using the LIMA directly as a patch took advantage of the superior anti-atherosclerotic property of LIMA compared to SVG, besides the known vasomotor functions of the LIMA and its ability to adjust the flow rate to the distal runoff of the LAD by virtue of its release of endothelium derived relaxing factors^[10]. Nevertheless the verdict on the optimal method of LAD reconstruction is not yet clear.

Soylu *et al.*^[3] in a best evidence series published in 2014 included 150 articles in their search and stated in conclusion that open coronary endarterectomy appeared to be safer, carried a lower rate of mortality than closed endarterectomy, and that the use of LIMA may improve mortality.

With this large study in mind and with the theoretical advantages of open over closed endarterectomy stated previously the surgeons in our study were disinclined to use the closed traction method of endarterectomy in a vessel as precious as LAD and all patients operated on in this study had open endarterectomy of the LAD.

It is worth mentioning that Barra and his colleagues from France advocated a different method of using the LIMA in reconstruction of the LAD without endarterectomy^[11]. In this method LIMA onlay graft is sutured inside the coronary in such a fashion as to exclude atheromatous plaques from the lumen of the coronary artery. LIMA wall makes up 75% of the reconstructed vessel, and the newly reconstructed artery retains 25% of the native coronary artery. However this method was reserved mainly for non calcified plaques, since heavily calcific plaques can preclude suturing. They explained that by using this method they limit the use of endarterectomy and hence decrease the postoperative cascade of myofibrointimal hyperplasia and thrombosis since no area of the coronary is denuded of its covering endothelium.

Bridge or jump graft was also used for LAD reconstruction. This method was used in patients with multiple lesions in their LAD. Arteriotomies were performed proximal and distal to the site of coronary stenosis and a valveless saphenous segment was used as a bridge between the lesion to which LIMA was anastomosed^[12].

LIMA itself was used to perform jump grafts by performing a side to side anastomosis to the LAD proximal to the site of stenosis then another end to side anastomosis after jumping over the site of stenosis^[13]. Again the merits of both of these methods were mainly to avoid endareterectomy of the LAD. All of the above methods were considered by the surgeons in our study for the sake of avoiding endarterectomy and its histopathological consequences and endarterectomy was saved as the last option when other methods appeared to be futile.

In our study the use of the SVG (26.7%) was reserved for patients who needed a much lengthier reconstruction of the LAD and in cases where there was fear that the LIMA was too short or would be under tension if used in the reconstruction. This was reflected in our results showing a greater mean of reconstruction length in the saphenous vein group.

The cross clamp time was shorter in the LIMA patch group however did not reach statistical significance. Owais *et al.*^[14] and Myers *et al.*^[15] showed a statistically significant shorter cross clamp time in the LIMA patch group compared to the saphenous vein patch group.

Two of our patients suffered from postoperative MI, one of them in the LAD region (3.3 percent). Our results compared well to rates of perioperative MI mentioned in Schmitto *et al.*^[16] 3 percent, Byrne *et al.*^[17] 3 percent, Myers *et al.*^[15] 4 percent, Sundt *et al.*^[18] 2 percent and Nishi *et al.*^[19] 2.9 percent. There was no difference in our study between both groups LIMA and saphenous regarding MI and this was also the case in Myers *et al.*^[15] study.

There was one case of mortality in our study (3.3). Our explanation that the delay in starting anticoagulation for this patient who was bleeding led to a MI and started this vicious cascade of events leading to death.

Nishigawa *et al.*^[20] reported mortality of 1.1 percent, Schmitto *et al.*^[16] 5 percent, Takanashi *et al.*^[21] 2.7 percent, Byrne *et al.*^[17] 3 percent, Nishi *et al.*^[19] 2.9 percent. In comparing LIMA to saphenous group mortality was 4.5 percent in the LIMA group and none in the saphenous group and was not statistically significant. Myers *et al.*^[15] also had 4.1% mortality in the LIMA group, slightly higher than the saphenous patch group (3%) and was not statistically significant. Owais *et al.*^[14] had a 13 percent mortality in each of his groups, with no difference between both.

The CT coronary angiography done at 6 months postoperatively showed a patency rate of 93.1 percent, with 2 patients found to have stenotic LIMA-LAD anastomosis. One patient belonged to the LIMA group and the other to the saphenous patch group, with no statistical difference between both. Our explanation for the patient with occlusion in the LIMA and saphenous patch group was attributed to bleeding in the early postoperative hours which required reexploration in the operating room and delayed the start of the anticoagulation. The second patient had an uneventful postoperative course and this unfortunate event was not explained in his case.

In Nishigawa *et al.*^[20] study conventional angiography was done at a median of 7 days (range 0-85 days) and showed a patency rate of 91.6 percent. Takanashi *et al.*^[21] showed early patency of 94 percent.

Nishi *et al.*^[19] had early and late angiographic control of his patients. He had a 92.1 percent early patency rate and at midterm follow up at a mean of 21 months the patency rate was 89.1.

NYHA class significantly changed in our study following surgery, with 69 percent of patients falling in NYHA class 1 as opposed to none of the patients being in class 1 preoperatively. Schmitto *et al.*^[16] also noted an improvement in NYHA class after operation.

During follow up four of our patients reported recurrence of anginal pain (13.8 percent) and they belonged to CCS 1. There was no difference related to the technique of reconstruction. Sundt *et al.*^[18] reported recurrence of angina in 27 percent of their patients. Byrne *et al.*^[17] reported 90 percent freedom from anginal pain at 1 year. Sergeant *et al.*^[22] in their analysis of 9600 patients undergoing primary CABG reported recurrence of anginal pain in 8 percent of patients. Both authors attributed difference in recurrence of symptoms between patients undergoing endarterectomy and those undergoing primary grafting may be due to the diffuse and particularly severe nature of the coronary disease present among the population requiring endarterectomy and insufficient endarterectomy done in LAD or other territories.

Our anticoagulation protocol started with unfractionated heparin in the ICU. Some surgeons preferred DAPT and some triple therapy with DAPT in addition to warfarin. Takanashi *et al.*^[21] stated their anticoagulation protocol to be low-molecular-weight heparin, followed by triple therapy. Myers *et al.*^[15] used unfractionated heparin for 24 h and then switched to either warfarin (3 months) or clopidogrel (1 year) in addition to aspirin for life.

Nishi *et al.*^[19] administered low molecular weight heparin 6 h after arrival in the ICU followed by low-dose aspirin and warfarin (INR = 2.0), which were continued indefinitely.

Endarterectomy leaves behind a coronary vessel denuded of endothelium. This bare area acts as a nidus for thrombus formation and triggers a rapid coagulation cascade in the early postoperative period and can explain the high rate of postoperative MI. Therefore it seems obvious that thromboprophylaxis serves as a cornerstone in the outcome of those patients. Nevertheless anticoagulation regimens followed are based on surgeon's experience. This is due to lack of randomized controlled studies and the fact that there are no recommendations on anticoagulation and antiplatelet therapy in endarterectomy patients published in the guidelines yet.

In a recent review by Soylu *et al.*^[3] perioperative MI ranged from 0 to 19 percent, cerebrovascular accident ranged from 0 to 6 percent and the operative mortality ranged from 0 to 19 percent. Coronary endarterectomy was accompanied by acceptable patency rates, that ranged from 56% to 100% at a post-operative follow-up ranging from 6 months to 10 years. Our results match the results of large series in the literature.

In the current era, coronary endarterectomy appears as a valuable surgical option in diffusely diseased coronary vessels. With appropriate indications and in experienced hands, it can be done with acceptable morbidity, mortality, angiographic patency rates and lead to favorable outcomes in a high risk cohort.

In conclusion, results of reconstruction by LIMA and saphenous vein patch are comparable in short term follow up.

DECLARATIONS

Acknowledgments

Dr. Bassem Ramadan for sharing his expertise in coronary reconstruction.

Authors' contributions

Collection of data, analysis, follow up of patients, writing up the manuscript: Elsayed MH

Design of the study and revision, operated most of the cases: Hassanein WM

Revision of the manuscript: Keshk SA, Etman WG

Follow up of cases: Zidan M

Availability of data and materials

Alexandria University Cardiothoracic Surgery Department database.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Informed consents were obtained from all patients prior to the procedure with explanation regarding the aim of the procedure and the possible side effects according to the guidelines of ethical committee at Alexandria Faculty of Medicine.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

- Garcia S, Sandoval Y, Roukoz H, Adabag S, Canoniero M, et al. Outcomes after complete versus incomplete revascularization of patients with multivessel coronary artery disease: a meta-analysis of 89,883 patients enrolled in randomized clinical trials and observational studies. *J Am Coll Cardiol* 2013;62:1421-31.
- Bailey CP, May A, Lemmon WM. Survival after coronary endarterectomy in man. *J Am Med Assoc* 1957;164:641-6.
- Soylu E, Harling L, Ashrafian H, Athanasiou T. Does coronary endarterectomy technique affect surgical outcome when combined with coronary artery bypass grafting? *Interact Cardiovasc Thorac Surg* 2014;19:848-55.
- Lytle BW, Loop FD, Cosgrove DM, Ratliff NB, Easley K, et al. Longterm (5 to 12 years) serial studies of internal mammary artery and saphenous vein coronary bypass grafts. *J Thorac Cardiovasc Surg* 1985;89:248-58.
- Baranauskas A, Peace A, Kibarskis A, Shannon J, Abraitis V, et al. FFR result post PCI is suboptimal in long diffuse coronary artery disease. *Eurointervention* 2016;12:1473-80.
- Santini F, Casali G, Lusini M, D'Onofrio A, Barbieri E, et al. Mid-term results after extensive vein patch reconstruction and internal mammary grafting of the diffusely diseased left anterior descending coronary artery. *Eur J Cardiothorac Surg* 2002;21:1020-5.
- Shapira N, Lumia FJ, Gottdiener JS, Germon P, Lemole GM. Adjunct endarterectomy of the left anterior descending coronary artery. *Ann Thorac Surg* 1988;46:289-96.
- Fundarò P, Di Biasi P, Santoli C. Coronary endarterectomy combined with vein patch reconstruction and internal mammary artery grafting: experience with 18 patients. *Texas Heart Inst J* 1987;14:389-94.
- FitzGibbon GM, Leach AJ, Keon WJ, Burton JR, Kafka HP. Coronary bypass graft fate. Angiographic study of 1,179 vein grafts early, one year, and five years after operation. *J Thorac Cardiovasc Surg* 1986;91:773-8.
- Lüscher TF, Diederich D, Siebenmann R, Lehmann K, Stulz P, et al. Difference between endothelium-dependent relaxation in arterial and in venous coronary bypass grafts. *N Engl J Med* 1988;319:462-7.
- Barra JA, Bezon E, Mondine P, Resk A, Gilard M, et al. Coronary artery reconstruction for extensive coronary disease: 108 patients and two year follow-up. *Ann Thorac Surg* 2000;70:1541-5.
- Gucu A, Goncu T, Yavuz S, Ozluk OA, Eris C, et al. Alternative option in patients with multisegmental left anterior descending coronary artery disease for providing complete myocardial revascularization. *Int J Clin Exp Med* 2014;7:142-7.
- Nezic D, Knezevic A, Milojevic P, Jovic M, Sagic D, et al. Tandem pedicled internal thoracic artery conduit for sequential grafting of multiple left anterior descending coronary artery lesions. *Tex Heart Inst J* 2006;33:469-72.
- Owais T, Abdelfattah I, Osama A, Rasekh F, Girdauskas E, et al. Early term results of left internal mammary artery patch to left anterior descending artery and left internal mammary artery to on-lay saphenous vein patch in diffusely diseased left anterior descending artery: which is inferior and which is superior? *Int J Cardiovasc Res* 2016;5:3.
- Myers PO, Tabata M, Shekar PS, Couper GS, Khalpey ZI, et al. Extensive endarterectomy and reconstruction of the left anterior descending artery: early and late outcomes. *J Thorac Cardiovasc Surg* 2012;143:1336-40.
- Schmitt JD, Kolat P, Ortmann P, Popov AF, Coskun KO, et al. Early results of coronary artery bypass grafting with coronary endarterectomy for severe coronary artery disease. *J Cardiothorac Surg* 2009;4:52.
- Byrne JG, Karavas AN, Gudbjartson T, Leacche M, Rawn JD, et al. Left anterior descending coronary endarterectomy: early and late results in 196 consecutive patients. *Ann Thorac Surg* 2004;78:867-73.
- Sundt TM 3rd, Camillo CJ, Mendeloff EN, Barner HB, Gay WA Jr. Reappraisal of coronary endarterectomy for the treatment of diffuse coronary artery disease. *Ann Thorac Surg* 1999;68:1272-7.
- Nishi H, Miyamoto S, Takanashi S, Minamimura H, Ishikawa T, et al. Optimal method of coronary endarterectomy for diffusely diseased coronary arteries. *Ann Thorac Surg* 2005;79:846-53.
- Nishigawa K, Fukui T, Yamazaki M, Takanashi S. Ten-year experience of coronary endarterectomy for the diffusely diseased left anterior descending artery. *Ann Thorac Surg* 2017;103:710-6.
- Takanashi S, Fukui T, Miyamoto Y. Coronary endarterectomy in the left anterior descending artery. *J Cardiol* 2008;52:261-8.
- Sergeant P, Blackstone E, Meyns B. Is return of angina after coronary artery bypass grafting immutable, can it be delayed, and is it important? *J Thorac Cardiovasc Surg* 1998;116:440-53.

Case Report

Open Access



Ventricular septal defect and tricuspid and mitral valve insufficiency caused by penetrating trauma

Ana Lopez-Marco¹, Jennifer Williams¹, Christine Tan², Dheeraj Mehta¹

¹Department of Cardiothoracic Surgery, University Hospital of Wales, Cardiff CF14 4XW, UK.

²Department of Anaesthesia, University Hospital of Wales, Cardiff CF14 4XW, UK.

Correspondence to: Dr. Ana Lopez-Marco, Department of Cardiothoracic Surgery, University Hospital of Wales, Cardiff CF14 4XW, UK. E-mail: analopez1000@hotmail.com

How to cite this article: Lopez-Marco A, Williams J, Tan C, Mehta D. Ventricular septal defect and tricuspid and mitral valve insufficiency caused by penetrating trauma. *Vessel Plus* 2018;2:40. <http://dx.doi.org/10.20517/2574-1209.2018.67>

Received: 2 Oct 2018 **First Decision:** 16 Oct 2018 **Revised:** 17 Oct 2018 **Accepted:** 18 Oct 2018 **Published:** 5 Dec 2018

Science Editor: Mario F. L. Gaudino **Copy Editor:** Cui Yu **Production Editor:** Zhong-Yu Guo

Abstract

A 28-year old male sustaining a penetrating injury to the subxiphoid area presented to the emergency department fully conscious and haemodynamically stable. The CT scan revealed a localized infero-posterior pericardial collection. Emergency surgery was planned to evacuate the collection and assess the extent of injury. Intraoperative transoesophageal echocardiogram demonstrated severe tricuspid regurgitation due to transection of the papillary muscle, as well as a ventricular septal defect. Tricuspid repair with reconstruction of the papillary muscle, closure of the ventricular septal defect (VSD) and the right ventricular laceration was performed. Mitral regurgitation secondary to chordae rupture was identified following de-airing maneuvers, and subsequently underwent repair. Traumatic VSD and lesions of the mitral and tricuspid valves causing insufficiency have been reported before. They have been described in isolation or as combination of two lesions but never the combination of the three of them as described in this case.

Keywords: Cardiac trauma, penetrating wounds, emergency, ventricular septal defect, heart valve disease, transoesophageal echocardiography

INTRODUCTION

Penetrating cardiac injuries are usually secondary to stab or gunshot wounds. They represent a life threatening condition that often requires emergency surgery for evacuation of the commonly associated cardiac tamponade^[1-6]. The free ventricular walls, especially on the right side are more commonly affected^[1,5]. Injury of the cardiac valves and intraventricular septum is rare but it has been described, although more frequently associated with blunt cardiac trauma^[1-6].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Traumatic ventricular septal defect (VSD) has been reported in 1%-5% of penetrating cardiac injuries. The diagnosis is usually obscured by the priority of stabilization and treatment of other more obvious and lethal injuries. It is consequently sometimes missed and diagnosed after the patient is recovered from the initial trauma^[1-6].

Mitral and tricuspid insufficiency secondary to cardiac trauma has also been reported, more commonly after blunt trauma. The trauma can potentially injure the valves at three different levels; papillary muscle, chordae and leaflet. Their diagnosis is also difficult, requiring an expert echocardiographer available at the time of the secondary survey. Otherwise, they are commonly missed until a full echocardiographic study is requested once the patient is more stable. High level of suspicion is key for their diagnosis^[4-7].

The gold-standard diagnosis for penetrating cardiac trauma is the echocardiography, to confirm haemopericardium and assess intracardiac structures. The VSD, however, can be missed initially due to muscle spasm or clot sealing the defect, consequently being commonly diagnosed and treated as a delayed complication of penetrating injuries^[1-7].

CASE REPORT

A 28-year-old male presented to the accident and emergency department following a stab injury to the anterior chest. He sustained a 6 cm stab wound in the subxiphoid area. On arrival, he was fully conscious and haemodynamically stable, with a heart rate of 90 beats/min, blood pressure 130/80 mmHg and respiratory rate of 22 breaths/min. He was managed as per advanced trauma life support guidelines and being described as a “good responder” following commencement of fluid resuscitation.

Physical examination revealed a systolic murmur, multiple bruises to the forehead and sustained soft tissue swelling of the right orbit and face. Glasgow Comma Scale was 15/15 and his electrocardiogram revealed sinus tachycardia, with inversion of T wave in the inferior wall leads.

A thoracic and abdominal CT was done to assess the extent of the injuries. There was a mediastinal haematoma extending to the anterior cardiophrenic recess and moderate to large infero-posterior haemopericardium. The diaphragm remained intact [Figure 1].

He underwent a bedside transthoracic echocardiography (TTE) that reported preserved left ventricular function but with infero-posterior wall motion abnormality, causing tethering of the posterior leaflet of the mitral valve and therefore, moderate mitral regurgitation (MR). There was also moderate tricuspid regurgitation (TR) with pulmonary hypertension [pulmonary artery systolic pressure (PASP) 40 mmHg + central venous pressure, normal PASP values 15-25 mmHg]; as well as a 1 cm localized pericardial effusion in the infero-posterior wall, probably clotted. Right ventricular function was preserved.

He was therefore, prepared for emergency cardiac surgery to evacuate the haemopericardium and assess the extent of cardiac injuries. An intraoperative transoesophageal echocardiography (TOE) confirmed the infero-posterior pericardial collection as well as the inferior wall dyskinesia. There was severe TR due to rupture of the head of the posterior papillary muscle and a 2 cm VSD in the muscular interventricular septum causing a left-right shunt. The MR was mild-moderate and deemed to be secondary to the inferior wall dyskinesia. At this stage, there was no mitral prolapse [Figure 2A and B].

Surgery was performed through a median sternotomy, extending the stab wound superiorly. A clot was identified between the inferior wall and the diaphragm. As soon as the clot was dislodged, a profuse bleeding was identified from a 2 cm full thickness injury on the inferior wall of the right ventricle (RV). Control of the bleeding was achieved with manual pressure, whilst cardiopulmonary bypass (CPB) with bi-caval



Figure 1. Preoperative CT scan of thorax and abdomen. Coronal view showing a localized collection in the inferior/posterior pericardial walls

cannulation and mild hypothermia was established. Myocardial protection was achieved with intermittent antegrade cardioplegia.

Following a right atriotomy, visualisation of the septum was achieved through the tricuspid valve (TV); there was a 3 cm VSD just below the posterior leaflet of the TV, which was detached due to complete transection of the papillary muscle head.

The VSD was closed with interrupted non-absorbable sutures reinforced with teflon. The papillary muscle head was re-implanted with a goretex suture. The TV was tested with saline test confirming competency. Closure of the right atrium was performed with a continuous 3/0 prolene suture as well as the injury on the inferior wall of the RV using a continuous 3/0 prolene suture reinforced with 2 bands of teflon.

The patient was successfully removed from CPB. However the intraoperative TOE identified severe MR secondary to a new prolapse of A2 segment (or the central scallop of the anterior leaflet) with a flail chordae, which was probably partially ruptured and the complete transection occurred within the de-airing maneuvers [Figure 2C].

CPB was reinstituted and the left atrium (LA) was opened confirming the mitral aetiology; repair was performed with implantation of a goretex neo-chordae and a 30 mm physio II ring annuloplasty. The LA was closed and the CPB was weaned easily with good results of the repairs confirmed on TOE and with no residual interventricular shunt. After a long period to secure haemostasis, the chest was closed routinely after placing one mediastinal and one pericardial drains.

The postoperative period was satisfactory, being discharged by day 10 when the levels of oral anti-coagulation were satisfactory. Follow-up visit at 6 weeks confirmed that he was asymptomatic and in sinus rhythm. The echocardiography confirmed a good result of the repairs, with trace TR, no MR and no residual VSD. The left and right ventricular functions were preserved with residual inferior hypokinesis of the RV.

DISCUSSION

Penetrating chest trauma, usually due to stab or gunshot wounds, can produce a wide variety of cardiac injuries which are life threatening in most cases. The mortality at the scene has been reported as high as 80%^[2].

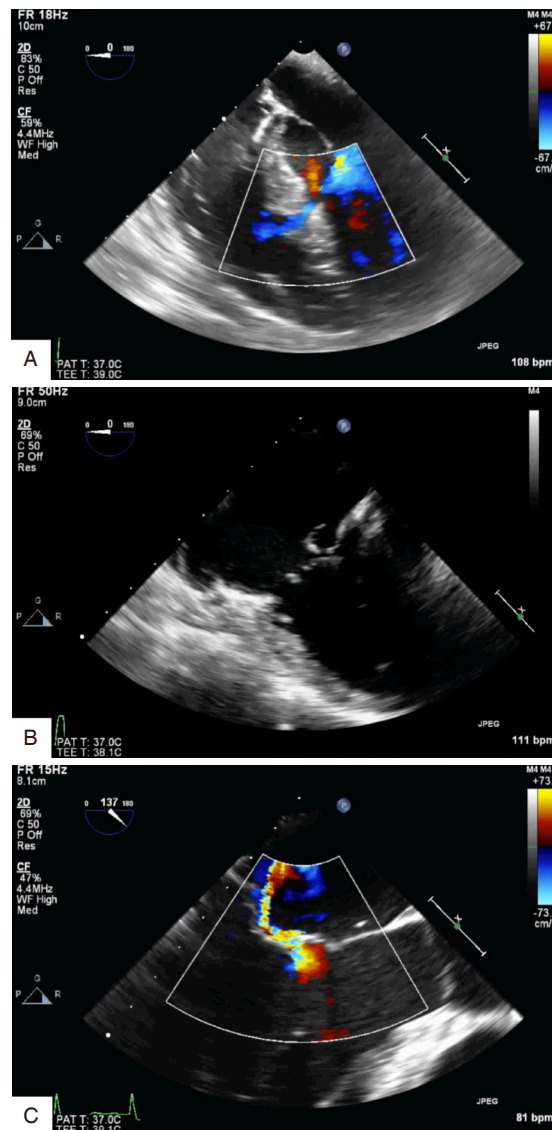


Figure 2. A: Intraoperative transoesophageal echocardiography (TOE) 4 chambers view with Doppler colour demonstrating the flow through the ventricular septal defect; B: intraoperative TOE, 4 chambers view, demonstrating the complete detachment of the tricuspid septal leaflet due to transection of the head of the papillary muscle; C: intraoperative TOE, apical long axis view with Doppler colour demonstrating the eccentric jet of mitral regurgitation caused by prolapse of A2 segment secondary to complete transection of the primary chordae

Of those who arrive in hospital, up to 70% survive to discharge, with rapid diagnosis and immediate treatment of injuries being the most important predictors of survival^[2]. In addition to the free wall of the heart or the great arteries, intracardiac lesions affecting the valves or the septum is also possible. Traumatic VSD and lesions of the mitral and TVs causing insufficiency have been reported before. They have been described in isolation or as combination of two lesions but never the combination of the three of them as described in this case^[1-7].

The surgical treatment of penetrating cardiac injuries in an emergency is evacuation of the commonly associated cardiac tamponade and simple cardiorrhaphy of the lacerations on the external surface of the heart^[1-7]. The subxiphoid approach for evacuation of the tamponade and lavage of the cavity has been reported as a safe alternative to full sternotomy for those cases who are haemodynamically stable and without active bleeding^[8].

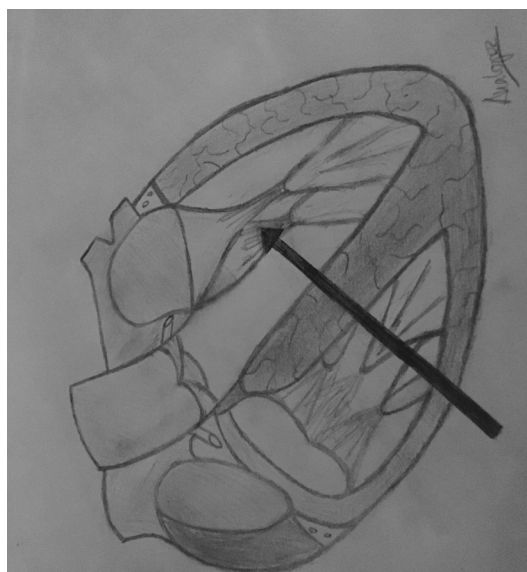


Figure 3. Drawing of a section of the heart showing the four chambers and postero-inferior walls. The thick arrow simulates the trajectory line of the stab injury to damage the inferior wall of the right ventricle, head of the papillary muscle supporting the posterior leaflet of the tricuspid valve, interventricular septum and chordae tendinae supporting A2 segment of the mitral valve

The gold-standard test for diagnosis in penetrating cardiac injuries is the echocardiography, which can confirm the presence of haemopericardium and assess the intra-cardiac structures. However, even in experienced hands, the diagnosis of intra-cardiac injuries, especially VSD can be obscured in this emergency setting. The VSD is often missed in the initial diagnosis due to focus on more threatening injuries or secondary to lack of intraoperative TOE^[1-5]. They are therefore commonly diagnosed as a delayed complication of penetrating cardiac injuries and treated according to the associated symptoms^[1-6].

The atrioventricular valves can be injured during cardiac trauma at different levels: chordal rupture, papillary muscle rupture or leaflet defects. They are more frequently caused by blunt trauma, and their diagnosis can also be obscured during the initial evaluation. The degree of the valve insufficiency and the associated symptoms will determine the indication and timing for surgical intervention. Surgery in the early stages after the trauma favors the feasibility of valve repair^[4-7].

In our case, the TOE was the key in the prompt diagnosis of the three simultaneous lesions [Figure 3], allowing them to be repaired in a single operation. The fact that the patient was haemodynamically stable and with only a localized collection in the pericardium (confirmed by the CT and TTE) directed us towards an emergency operation for evacuation of the collection. We were inclined to start with a subxiphoid approach to evacuate the collection and perform a pericardial lavage to evaluate active bleeding but considered the intraoperative TOE necessary to further scrutinize the valve regurgitations addressed by the TTE.

The subxiphoid approach would not have been enough in our case, independent of the associated lesions. Active bleeding was identified as soon as the clotted collection was dislodged with the sucker, prompting a median sternotomy. It was then when the lesion on the free wall of the RV was seen, but decided to establish CPB as the VSD and valvular problems were already identified by the TOE at this point.

To summarize, penetrating cardiac trauma can cause a variety of lesions. Thorough examination with an early echocardiographic assessment should be mandatory for the evaluation of intracardiac injuries. Intraoperative TOE should always be performed in these cases as the TTE can often miss some of the intracardiac injuries.

DECLARATIONS

Authors' contributions

Writing of the case report: Lopez-Marco A, Williams J

Consultant anaesthetist responsible for the case who also performed the intraoperative TOE and facilitated the images: Tan C

Operating consultant surgeon: Mehta D

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The patient whom this case report refers gave his written consent form for publication of clinical information as well as diagnostic images.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2018.

REFERENCES

1. Juneau D, Hermann D, Wells GL. Penetrating trauma resulting in ventricular septal defect. *World J Cardiovasc Surg* 2014;4:77-80.
2. Antoniadou L, Petrou PM, Eftychiou C, Nicolaides E. A penetrating heart injury resulting in ventricular septal defect. *Hellenic J Cardiol* 2011;52:71-4.
3. Sugiyama G, Lau C, Tak V, Lee DC, Burack J. Traumatic ventricular septal defect. *Ann Thorac Surg* 2011;91:908-10.
4. Topaloglu S, Aras D, Cagli K, Ergun K, Deveci B, et al. Penetrating trauma to the mitral valve and ventricular septum. *Tex Heart Inst J* 2006;33:392-5.
5. Doty JR, Cameron DE, Elmaci T, Salomon NW. Penetrating trauma to the tricuspid valve and ventricular septum: delayed repair. *Ann Thorac Surg* 1999;67:252-3.
6. Shiber J, Cardarelli M. Traumatic ventricular septal defect and tricuspid regurgitation. *J Emerg Med* 2012;43:e141-2.
7. Ma WG, Luo GH, Sun HS, Xu JP, Hu SS, et al. Surgical treatment of traumatic tricuspid insufficiency: experience in 13 cases. *Ann Thorac Surg* 2010;90:1934-8.
8. Nicol AJ, Navsaria PH, Hommes M, Ball CG, Edu S, et al. Sternotomy or drainage for a haemopericardium after penetrating trauma: a randomized controlled trial. *Ann Surg* 2014;259:438-42.

Review

Open Access



Volatile anesthetics in cardiac surgery: the impalpable benefit

Annalaura Di Pumpo, Chiara Candela, Fabrizio Cucciniello, Domenico Sarubbi, Felice Eugenio Agrò

Department of Anesthesia and Intensive Care Unit, Università Campus Bio-Medico di Roma, Rome 00128, Italy.

Correspondence to: Dr. Annalaura Di Pumpo, Department of Anesthesia and Intensive Care Unit, Università Campus Bio-Medico di Roma, Via Alvaro del Portillo 200, Rome 00128, Italy. E-mail: a.dipumpo@unicampus.it

How to cite this article: Di Pumpo A, Candela C, Cucciniello F, Sarubbi D, Agrò FE. Volatile anesthetics in cardiac surgery: the impalpable benefit. *Vessel Plus* 2018;2:41. <http://dx.doi.org/10.20517/2574-1209.2018.38>

Received: 29 May 2018 **First Decision:** 17 Oct 2018 **Revised:** 5 Nov 2018 **Accepted:** 5 Nov 2018 **Published:** 12 Dec 2018

Science Editors: Mario F. L. Gaudino, Cristiano Spadaccio **Copy Editor:** Cui Yu **Production Editor:** Huan-Liang Wu

Abstract

Nowadays, there are numerous studies demonstrating that volatile anesthetics reduce mortality and morbidity with a cardio-protective effect. The mechanisms involved in protecting perioperative cardiac ischemic damage provided by desflurane and sevoflurane are not fully known. Volatile anesthetics are commonly used in cardiac surgery. This mini-review aims to summarize the mechanism of action for cardio-protection of volatile anesthetics and discuss the potential therapeutic implications. Human studies have shown that volatile anesthetics can reduce mortality, but also the use of mechanical ventilation in cardiac patients, especially coronary artery bypass grafting. In contrast, total intravenous anesthesia has not shown any significant benefit compared to halogenated agents. Volatile anesthetics are among the few drugs that affect survival in the perioperative period. In addition, they can be administered in areas other than cardiac surgery due to their cardioprotective effects, which may add future perspectives in their use.

Keywords: Volatile anesthetics, preconditioning, cardiac surgery, cardio-protection

INTRODUCTION

Nowadays, there are numerous studies demonstrating that volatile anesthetics reduce mortality and morbidity with a cardio-protective effect^[1]. The mechanisms involved in protecting perioperative cardiac ischemic damage provided by desflurane and sevoflurane are not fully known.

In 1986, for the first time, this phenomenon is described as a response to short periods of sublethal ischemia leading to a protection against a subsequent lethal ischemia^[2].



© The Author(s) 2018. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



In 1988, Warltier *et al.*^[3] demonstrated that the use of halothane or isoflurane improved left ventricular systolic function. Subsequently, Cason *et al.*^[4] introduced the concept of cardiac pre-conditioning of the halogenates, demonstrating that their administration before ischemia protects the myocardium from a subsequent lesion.

Since then, numerous studies both *in vivo* and in animals have demonstrated this phenomenon^[5-9].

An international consensus conference included volatile agents among the few drugs that would decrease perioperative mortality in cardiac surgery^[10].

This mini-review aims to summarize the mechanism of action for cardioprotection of volatile anesthetics and discuss the potential therapeutic implications.

MECHANISM OF ACTION FOR CARDIAC PROTECTION

Thanks to the use of animal models, we have been able to relate the ischemia/reperfusion injury with the cardio-protection of volatile agents and establish a cause-effect relationship between the volatile anesthetic and cardio-diagnostic points such as the reduction of death cellular, improvement of contractile function and decrease in the incidence of arrhythmias^[11].

A dose-dependent signal appears to be based on anesthetic cardiac preconditioning: the degree of protection depends on the concentration of the drug administered and the duration of the administration^[12-15]. Lange *et al.*^[16] has shown that there is a threshold for the preconditioning of desflurane which is included between 0.5 and 1.0 minimum alveolar concentration. This threshold may decrease through the administration of desflurane by intervals. As soon as the level of cardioprotection from desflurane is reached, it cannot be further improved with desflurane increments.

All the volatile anesthetics that we use daily (desflurane, halothane, isoflurane and sevoflurane) produce cardiac preconditioning because they have the same mechanism of action but different power^[16].

Two types of preconditioning have been identified: one early, which lasts about 1 or 2 h, and one late, which occurs after 24 h and lasts up to 72 h. Early and late preconditioning have many characteristics, but probably involve different paths [Table 1]^[5].

Myocardial reperfusion has two effects: decreases cardiac damage but activates apoptosis of the cells producing myocardial dysfunction, which is linked to mitochondrial dysfunction, in particular, at the opening of mitochondrial permeability transition pores (mPTPs).

During reperfusion, volatile anesthetics avoid the opening of transitional pores of the mitochondrial permeability. In this way the different mechanisms of apoptosis are inhibited^[17,18]. In addition, anesthetics act on the signaling pathways linked to adrenergic receptors and adenosine bradykinin, both involved in cardioprotection^[19].

Myocardial post-conditioning by the halogenates is due to various cellular mechanisms^[16-18]: modulation of L-type calcium channels, inhibition of the release of reactive oxygen species from the mitochondria to the cytoplasm, mPTP closed, stimulation of G proteins coupled with β -adrenergic receptors.

Various pathways, including the activation of Gi (GiPCR) coupled Gi (inhibitors), phospholipase B and D receptors, diacylglycerol and protein kinase C, are involved, activating KATP channels^[5]. KATP channels are inhibited by intracellular ATP physiological concentrations and, when opened, produce a repolarizing internal potassium stream. MitKATP channel antagonists may inhibit the cardioprotective function of des-

Table 1. Difference between early and late preconditioning

Early preconditioning	Late preconditioning
It begins early	It begins 12-24 h after ischemia
Duration of 1-2 h	Duration of 72 h
It is due to the accumulation of adenosine	It is due to gene up-regulation

flurane and sevoflurane. The opening of the canal would lead to the reduction of the swelling of the inter-membrane space after ischemia, preserving the structure and mitochondrial function. Depolarization of the mitochondrial inner membrane also prevents the opening of the mPTP and inhibits the exchange of $\text{Na}^+ - \text{H}^+$, attenuating Ca^{2+} overload and cellular edema^[5]. Interferences have been described with the apoptotic cascade mediated by Bcl-2-associated death promoter and Bcl-2-associated X proteins and caspases 9, in addition to activation of nitric oxide endothelial synthase^[5,20,21] which may give a cardioprotective effect. Also reactive oxygen species (ROS) have an important task: the opening of the KATP channels causes an increase in the intracellular concentrations of ROS, at the same time, the production of ROS can also precede and cause the opening of the KATP channels. Activators of the KATP channel and sevoflurane may attenuate the overproduction of ROS during reperfusion. Therefore, halogenates, in order to achieve preconditioning, must cause ROS production. Preconditioning, in turn, allows a reduction of ROS excess, during reperfusion^[21,22]. Volatile anesthetics also reduce platelet adhesion to the vascular wall after ischemia^[23].

Regarding the late preconditioning, it is due to cardioprotective proteins that are expressed after the translation of the first genes induced by cardiac preconditioning. The most common genes expressed virtually by any type of stress conditioning include antioxidants such as superoxide dismutase, glutathione peroxidase and heme oxygenase, genes associated with cell defense [heat shock proteins(HSP) such as HSP70 and HSP10, aldose reductase, Bcl-xS] and cycloid-oxygenase 2^[24].

Ischemic preconditioning is the process implemented by the myocardial tissue at the cellular level that provides myocardial protection against the damage due to the ischemia/reperfusion phenomenon in the cardiac tissue^[25].

After administration of volatile agents, the systolic function improves because we have a reduction in myocardial oxygen consumption due to depression of myocardial contractility and improvement of blood flow in several capillary beds^[26].

Human studies have shown that volatile anesthetics can reduce mortality, but also the use of mechanical ventilation in cardiac patients, especially coronary artery bypass grafting (CABG). In contrast, total intravenous anesthesia (TIVA) (and more specifically propofol) has not shown any significant benefit compared to halogenated agents. The studies of Fortis *et al.*^[27] and Schilling *et al.*^[28] have shown that halogenates lead to a reduction of the inflammatory response in acute lung injury. In addition, the volatile agents cause neuroprotection after brain damage^[29], reduce hepatic damage^[30] and the incidence of acute renal injury after an ischemic insult^[31]. As a result, volatile anesthetics can also play an important role in preventing cardiac surgery complications.

CLINICAL IMPLICATIONS

In 2011, there was an international consensus conference that included volatile agents among the few drugs that would decrease perioperative mortality in cardiac surgery^[10]. Volatile anesthetics (desflurane, isoflurane and sevoflurane) have pharmacological characteristics that generate cardiac protection, unlike the drugs used for TIVA.

Indeed, the 2007 Landoni meta-analysis showed a reduction in perioperative cardiac troponin release and reduced mortality in patients receiving volatile anesthetics compared to patients receiving a TIVA^[32].

To endorse the hypothesis, that from these benefits we can obtain a reduced mortality rate in patients who receive volatile agents, there are numerous studies. These results led to an ordinary use of volatile anesthetics compared to TIVA, although propofol is a drug that has a much lower cost than halogenated drugs. For example, a bottle of sevoflurane costs 74 euro, while a bottle of 2% propofol costs 1.6 euro.

There are no studies that discourage the use of propofol^[33] but we must remember that when using TIVA in cardiac surgery we do not have remote ischemic preconditioning^[34], due to the inhibition of the organo-protective properties of this technique^[35]. Furthermore, there are at least 8 studies showing an increase in mortality with the use of TIVA, while there is no study that demonstrates an increase in survival with TIVA^[32,36,37].

DISCUSSION

In the meta-analysis of Zangrillo *et al.*^[38], it has been shown that halogenated agents can decrease mortality and have additional cardioprotective effects compared to TIVA. In this meta-analysis, it has been shown that in patients undergoing cardiac surgery, the use of volatile agents and/or the combination of volatile agents with remote preconditioning produces lower mortality compared to TIVA with longer follow-up. The meta-analysis of the Bayesian network compares different groups of patients both directly and indirectly, with a consolidated method in clinical research. This meta-analysis includes all randomized trials in adult cardiac systems that compare volatile agents, TIVA and remote ischemic preconditioning. This study is an invitation to use volatile anesthetics during general anesthesia because there is no evidence of beneficial properties of TIVA except when combined with volatile agents. This meta-analysis has some limitations: most of the studies included in the study are small, single centers and not double-blind. In fact, for example, some authors do not preside over whether patients taking drugs such as sulfonylurea, theophylline or allopurinol have been excluded, as these drugs appear to influence the preconditioning mechanism. Another limitation may be the use of intraoperative opioids. In fact, opioids reduce the cardiovascular effect and can hinder the cardioprotective effects of volatile agents^[38].

Furthermore, ischemic preconditioning can lower postoperative cardiac biomarker levels^[39,40] and even mortality during cardiac surgery^[41].

The first study^[32], published in 2007, which showed a greater survival of patients subjected to halogenated agents compared to TIVA, was a meta-analysis of small randomized clinical trials. The meta-analysis showed that sevoflurane and desflurane were associated with significant reductions in myocardial infarction (2.4% vs. 5.1%) and mortality (0.4% vs. 1.6%). In a large multi-center study, patients who received sevoflurane showed a 30-day lower postoperative mortality than those who received TIVA (2.2% vs. 3.1%)^[36]. Instead, De Hert *et al.*^[42] did not find any difference in the post-operative troponin T release between volatile anesthetics and TIVA but showed a significant difference in one-year mortality among the various groups (3.3% in the sevoflurane group, 6.7% in the desflurane group and 12.3% in the TIVA group ($P = 0.034$)^[42]. A large multi-center study analyzed the 30-day mortality rate in patients undergoing CABG where halogenated anesthetics or TIVA was used. Mortality was lower with halogenates and the mortality rate was lower than with the use of halogenated anesthetics^[43]. A recent meta-analysis by Landoni showed a 50% reduction in mortality compared to TIVA (desflurane 1.8% vs. 4.0%, isoflurane 0.7% vs. 2.0% and sevoflurane 1.2% vs. 3.0%)^[44].

In contrast, some authors have shown that patients with severe preoperative ischemic stress benefit from TIVA because of its antioxidant effects^[45]. However, no increase in survival with TIVA has been demonstrated, indeed some studies have shown worse outcomes if propofol is compared with halogenates^[36].

There is disagreement about the type of cardiac surgery that benefits most from halogenated cardiac protection. Most studies have shown that cardioprotection occurs mainly in CABG while the evidence of haloge-

nates in valve surgery is controversial^[46,47]. A 2006 study showed that the use of sevoflurane leads to better preservation of myocardial function and less postoperative release of troponin I in patients undergoing aortic valve replacement^[47]. In contrast, the study of Pinaud *et al.*^[48] in 2015, demonstrated that preconditioning does not have cardioprotective effects in patients undergoing valve surgery without CABG.

There are no clear advantages of the use of volatiles in valve surgery. A first reason is that myocardial infarction can simulate a preconditioned state by improving the effect of halogenated agents^[49]. In fact, as demonstrated by Murry *et al.*^[2], short periods of transient myocardial ischemia protect the heart from extensive damage in longer periods of ischemia. Furthermore, valvular surgery causes an increase in troponin due to higher surgical injuries and anatomical changes that modify the geometry and function of the left ventricle, with acute increase in the afterload, especially in mitral valve surgery^[50].

We must remember that when using TIVA in cardiac surgery we do not have remote ischemic preconditioning^[34]. This led to the demonstration that TIVA leads to an increase in mortality, while there is no study showing that it increases survival^[32,36,37].

However, TIVA remains widely used for rapid interventions. As demonstrated by Çaparlar *et al.*^[51], patients eligible for the preferential ward were older and the time for rapid suitability was shorter in the TIVA group compared to sevoflurane (82.1% *vs.* 57.5% and 8 min *vs.* 12 min, $P < 0.05$).

At the same time, it must be stated that volatile agents, such as desflurane, are proving to be an excellent alternative to propofol for fast-track interventions^[52,53].

Thus we obtain two results: the cardiac preconditioning given by the volatile agent and rapid extubation times.

CONCLUSION

Volatile anesthetics are among the few drugs that affect survival in the perioperative period^[54]. In addition, they can be administered in other areas other than cardiac surgery due to their cardioprotective effects, which may add future perspectives in their use.

Notwithstanding the numerous studies in favor of volatile anesthetics, it is necessary to give a definitive answer regarding the greater survival of patients with the simple use of volatile anesthetics, with randomized trials provided with a very large sample. The largest study currently underway is recruiting 10,600 participants and has the task of conclusively demonstrating how the simple use of a halogenated agent improves one-year survival in patients undergoing CABG.

DECLARATIONS

Authors' contributions

Data collection, manuscript revision: Di Pumpo A

Study design, literature analysis, manuscript drafting: Di Pumpo A, Candela C

Literature analysis, manuscript critical revision: Cucciniello F, Sarubbi D

Study design, manuscript critical revision: Agrò FE

Availability of data and materials

Literature search (Pubmed, Scopus).

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Approved by the institution where the work was carried out.

Copyright

© The Author(s) 2018.

REFERENCES

1. Landoni G, Fochi O, Torri G. Cardiac protection by volatile anaesthetics: a review. *Curr Vasc Pharmacol* 2008;6:108-11.
2. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124-36.
3. Warltier DC, al-Wathiqui MH, Kampine JP, Schmeling WT. Recovery of contractile function of stunned myocardium in chronically instrumented dogs is enhanced by halothane or isoflurane. *Anesthesiology* 1988;69:552-65.
4. Cason BA, Gamperl AK, Slocum RE, Hickey RF. Anesthetic-induced preconditioning: previous administration of isoflurane decreases myocardial infarct size in rabbits. *Anesthesiology* 1997;87:1182-90.
5. Chiari P, Bouvet F, Piriou V. Anaesthetic-induced myocardial preconditioning: fundamental basis and clinical implications. *Ann Fr Anesth Reanim* 2005;24:383-96. (in French)
6. Varadarajan SG, An J, Novalija E, Stowe DF. Sevoflurane before or after ischemia improves contractile and metabolic function while reducing myoplasmic Ca(2+) loading in intact hearts. *Anesthesiology* 2002;96:125-33.
7. Ross S, Foëx P. Protective effects of anaesthetics in reversible and irreversible ischaemia-reperfusion injury. *Br J Anaesth* 1999;82:622-32.
8. Toller WG, Kersten JR, Pagel PS, Hettrick DA, Warltier DC. Sevoflurane reduces myocardial infarct size and decreases the time threshold for ischemic preconditioning in dogs. *Anesthesiology* 1999;91:1437-46.
9. Preckel B, Schlack W, Comfère T, Obal D, Barthel H, et al. Effects of enflurane, isoflurane, sevoflurane and desflurane on reperfusion injury after regional myocardial ischaemia in the rabbit heart in vivo. *Br J Anaesth* 1998;81:905-12.
10. Landoni G, Augoustides JG, Guarracino F, Santini F, Ponschab M, et al. Mortality reduction in cardiac anesthesia and intensive care: results of the first International Consensus Conference. *Acta Anaesthesiol Scand* 2011;55:259-66.
11. Muntean DM, Ordodi V, Ferrera R, Angoulvant D. Volatile anaesthetics and cardioprotection: lessons from animal studies. *Fundam Clin Pharmacol* 2013;27:21-34.
12. Riess ML, Kevin LG, Camara AK, Heisner JS, Stowe DF. Dual exposure to sevoflurane improves anesthetic preconditioning in intact hearts. *Anesthesiology* 2004;100:569-74.
13. Ludwig LM, Patel HH, Gross GJ, Kersten JR, Pagel PS, et al. Morphine enhances pharmacological preconditioning by isoflurane: role of mitochondrial K(ATP) channels and opioid receptors. *Anesthesiology* 2003;98:705-11.
14. Kato R, Foëx P. Myocardial protection by anesthetic agents against ischemia-reperfusion injury: an update for anesthesiologists. *Can J Anesth* 2002;49:777-91.
15. Kehl F, Krolikowski JG, Mraovic B, Pagel PS, Warltier DC, et al. Is isoflurane-induced preconditioning dose related? *Anesthesiology* 2002;96:675-80.
16. Lange M, Redel A, Lotz C, Smul TM, Blomeyer C, et al. Desflurane-induced postconditioning is mediated by beta-adrenergic signaling: role of beta 1- and beta 2-adrenergic receptors, protein kinase A, and calcium/calmodulin-dependent protein kinase II. *Anesthesiology* 2009;110:516-28.
17. Halestrap AP, Clarke SJ, Javadov S. Mitochondrial permeability transition pore opening during myocardial reperfusion--a target for cardioprotection. *Cardiovasc Res* 2004;61:372-85.
18. Di Lisa F, Menabò R, Canton M, Barile M, Bernardi P. Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD⁺ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. *J Biol Chem* 2001;276:2571-5.
19. Landoni G, Lopez-Delgado JC, Sartini C, Tamà S, Zangrillo A. Halogenated agents and cardiovascular surgery: has mortality really decreased? *Curr Vasc Pharmacol* 2018;16:336-43.

20. Garcia-Dorado D, Rodriguez-Sinovas A, Ruiz-Meana M, Inseste J, Agulló L, et al. The end-effectors of preconditioning protection against myocardial cell death secondary to ischemia reperfusion. *Cardiovasc Res* 2006;70:274-85.
21. Novalija E, Varadarajan SG, Camara AK, An J, Chen Q, et al. Anesthetic preconditioning: triggering role of reactive oxygen and nitrogen species in isolated hearts. *Am J Physiol Heart Circ Physiol* 2002;283:H44-52.
22. Zweier JL, Talukder MA. The role of oxidants and free radicals in reperfusion injury. *Cardiovasc Res* 2006;70:181-90.
23. Heindl B, Becker BF, Zahler S, Conzen PF. Volatile anaesthetics reduce adhesion of blood platelets under low-flow conditions in the coronary system of isolated guinea pig hearts. *Acta Anaesthesiol Scand* 1998;42:995-1003.
24. Das DK, Maulik N. Cardiac genomic response following preconditioning stimulus. *Cardiovasc Res* 2006;70:254-63.
25. Heusch G, Schulz R. Remote preconditioning. *J Mol Cell Cardiol* 2002;34:1279-81.
26. Hartman JC, Pagel PS, Proctor LT, Kampine JP, Schmeling WT, et al. Influence of desflurane, isoflurane and halothane on regional tissue perfusion in dogs. *Can J Anaesth* 1992;39:877-87.
27. Fortis S, Spieth PM, Lu WY, Parotto M, Haitsma JJ, et al. Effects of anesthetic regimes on inflammatory responses in a rat model of acute lung injury. *Intensive Care Med* 2012;38:1548-55.
28. Schilling T, Kozian A, Senturk M, Huth C, Reinhold A, et al. Effects of volatile and intravenous anesthesia on the alveolar and systemic inflammatory response in thoracic surgical patients. *Anesthesiology* 2011;115:65-74.
29. Yang Q, Dong H, Deng J, Wang Q, Ye R, et al. Sevoflurane preconditioning induces neuroprotection through reactive oxygen species-mediated up-regulation of antioxidant enzymes in rats. *Anesth Analg* 2011;112:931-7.
30. Beck-Schimmer B, Breitenstein S, Bonvini JM, Lesurtel M, Ganter M, et al. Protection of pharmacological postconditioning in liver surgery: results of a prospective randomized controlled trial. *Ann Surg* 2012;256:837-44.
31. Lee HT, Kim M, Kim J, Kim N, Emala CW. TGF-beta1 release by volatile anesthetics mediates protection against renal proximal tubule cell necrosis. *Am J Nephrol* 2007;27:416-24.
32. Landoni G, Biondi-Zoccai GG, Zangrillo A, Bignami E, D'Avolio S, et al. Desflurane and sevoflurane in cardiac surgery: a meta-analysis of randomized clinical trials. *J Cardiothorac Vasc Anesth* 2007;21:502-11.
33. Pasin L, Landoni G, Cabrini L, Borghi G, Taddeo D, et al. Propofol and survival: a meta-analysis of randomized clinical trials. *Acta Anaesthesiol Scand* 2015;59:17-24.
34. Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, et al. A multicenter trial of remote ischemic preconditioning for heart surgery. *N Engl J Med* 2015;373:1397-407.
35. Landoni G, Baiardo Redaelli M, Votta CD. Remote ischemic preconditioning and cardiac surgery. *N Engl J Med* 2016;374:489.
36. Jakobsen CJ, Berg H, Hindsholm KB, Faddy N, Sloth E. The influence of propofol versus sevoflurane anesthesia on outcome in 10,535 cardiac surgical procedures. *J Cardiothorac Vasc Anesth* 2007;21:664-71.
37. Uhlig C, Bluth T, Schwarz K, Deckert S, Heinrich L, et al. Effects of volatile anesthetics on mortality and postoperative pulmonary and other complications in patients undergoing surgery: a systematic review and meta-analysis. *Anesthesiology* 2016;124:1230-45.
38. Zangrillo A, Musu M, Greco T, Di Prima AL, Matteazzi A, et al. Additive effect on survival of anaesthetic cardiac protection and remote ischemic preconditioning in cardiac surgery: a bayesian network meta-analysis of randomized trials. *PLoS One* 2015;10:e0134264.
39. Zhou C, Liu Y, Yao Y, Zhou S, Fang N, et al. β -blockers and volatile anesthetics may attenuate cardioprotection by remote preconditioning in adult cardiac surgery: a meta-analysis of 15 randomized trials. *J Cardiothorac Vasc Anesth* 2013;27:305-11.
40. Yang L, Wang G, Du Y, Ji B, Zheng Z. Remote ischemic preconditioning reduces cardiac troponin I release in cardiac surgery: a meta-analysis. *J Cardiothorac Vasc Anesth* 2014;28:682-9.
41. Thielmann M, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, et al. Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial. *Lancet* 2013;382:597-604.
42. De Hert S, Vlasselaers D, Barbé R, Ory JP, Dekegel D, et al. A comparison of volatile and non volatile agents for cardioprotection during on-pump coronary surgery. *Anaesthesia* 2009;64:953-60.
43. Bignami E, Biondi-Zoccai G, Landoni G, Fochi O, Testa V, et al. Volatile anesthetics reduce mortality in cardiac surgery. *J Cardiothorac Vasc Anesth* 2009;23:594-9.
44. Landoni G, Greco T, Biondi-Zoccai G, Nigro Neto C, Febres D, et al. Anaesthetic drugs and survival: a Bayesian network meta-analysis of randomized trials in cardiac surgery. *Br J Anaesth* 2013;111:886-96.
45. Marik PE. Propofol: an immunomodulating agent. *Pharmacotherapy* 2005;25:28S-33S.
46. Meybohm P, Bein B, Brosteanu O, Cremer J, Gruenewald M, et al. A multicenter trial of remote ischemic preconditioning for heart surgery. *N Engl J Med* 2015;373:1397-407.
47. Cromheecke S, Pepermans V, Hendrickx E, Lorsomradee S, Ten Broecke PW, et al. Cardioprotective properties of sevoflurane in patients undergoing aortic valve replacement with cardiopulmonary bypass. *Anesth Analg* 2006;103:289-96.
48. Pinaud F, Corbeau JJ, Baufreton C, Binuani JP, De Brux JL, et al. Remote ischemic preconditioning in aortic valve surgery: results of a randomized controlled study. *J Cardiol* 2016;67:36-41.
49. De Hert SG, Turani F, Mathur S, Stowe DF. Cardioprotection with volatile anesthetics: mechanisms and clinical implications. *Anesth Analg* 2005;100:1584-93.
50. Goldfine H, Aurigemma GP, Zile MR, Gaasch WH. Left ventricular length-force-shortening relations before and after surgical correction of chronic mitral regurgitation. *J Am Coll Cardiol* 1998;31:180-5.
51. Çaparlar CÖ, Özhan MÖ, Sützer MA, Yazicioğlu D, Eşkin MB, et al. Fast-track anesthesia in patients undergoing outpatient laparoscop-

- ic cholecystectomy: comparison of sevoflurane with total intravenous anesthesia. *J Clin Anesth* 2017;37:25-30.
52. Jain A, Gombar S, Ahuja V. Recovery profile after general anaesthesia in paediatric ambulatory surgeries: desflurane versus propofol. *Turk J Anaesthesiol Reanim* 2018;46:21-7.
53. Liu TC, Lai HC, Lu CH, Huang YS, Hung NK, et al. Analysis of anesthesia-controlled operating room time after propofol-based total intravenous anesthesia compared with desflurane anesthesia in functional endoscopic sinus surgery. *Medicine (Baltimore)* 2018;97:e9805.
54. Landoni G, Pisano A, Lomivorotov V, Alvaro G, Hajjar L, et al. Randomized evidence for reduction of perioperative mortality: an updated consensus process. *J Cardiothorac Vasc Anesth* 2017;31:719-30.

AUTHOR INSTRUCTIONS

1. Submission Overview

Before you decide to publish with us, please read the following items carefully and make sure that you are well aware of Editorial Policies and the following requirements.

1.1 Topic Suitability

The topic of the manuscript must fit the scope of the journal. Please refer to Aims and Scope for more information.

1.2 Open Access and Copyright

The journal adopts Gold Open Access publishing model since its establishment and has been distributing contents under Attribution 4.0 International License. Please make sure that you are well aware of these policies.

1.3 Publication Fees

There are no fees for submission, processing, and publication.

1.4 Language Editing

All submissions are required to be presented clearly and cohesively in good English. Authors whose first language is not English are advised to have their manuscripts checked or edited by a native English speaker before submission to ensure the high quality of expression. A well-organized manuscript in good English would make the peer review even the whole editorial handling more smooth and efficient.

If needed, authors are recommended to consider the language editing services provided by Charlesworth to ensure that the manuscript is written in correct scientific English before submission. Authors who publish with OAE journals enjoy a special discount for the services of Charlesworth via the following two ways.

Submit your manuscripts directly at <http://www.charlesworthauthorservices.com/~OAE>;

Open the link <http://www.charlesworthauthorservices.com/>, and enter Promotion Code “OAE” when you submit.

1.5 Work Funded by the National Institutes of Health

If an accepted manuscript was funded by National Institutes of Health (NIH), the author may inform editors of the NIH funding number. The editors are able to deposit the paper to the NIH Manuscript Submission System on behalf of the author.

2. Submission Preparation

2.1 Cover Letter

A cover letter is required to be submitted accompanying each manuscript. It should be concise and explain why the study is significant, why it fits the scope of the journal, and why it would be attractive to readers, *etc.*

Here is a guideline of a cover letter for authors' consideration:

In the first paragraph: include the title and type (e.g., Original Article, Review, Case Report, *etc.*) of the manuscript, a brief on the background of the study, the question the author sought out to answer and why;

In the second paragraph: concisely explain what was done, the main findings and why they are significant;

In the third paragraph: indicate why the manuscript fits the Aims and Scope of the journal, and why it would be attractive to readers;

In the fourth paragraph: confirm that the manuscript has not been published elsewhere and not under consideration of any other journal. All authors have approved the manuscript and agreed on its submission to the journal. Journal's specific requirements have been met if any.

If the manuscript is contributed to a special issue, please also mention it in the cover letter.

If the manuscript was presented partly or entirely in a conference, the author should clearly state the background information of the event, including the conference name, time and place in the cover letter.

2.2 Types of Manuscripts

There is no restriction on the length of manuscripts, number of figures, tables and references, provided that the manuscript is concise and comprehensive. The journal publishes Original Article, Review, Meta-Analysis, Case Report, Commentary, *etc.* For more details about paper type, please refer to the following table.

Manuscript Type	Definition	Abstract	Keywords	Main Text Structure
Original Article	An Original Article describes detailed results from novel research. All findings are extensively discussed.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Review	A Review paper summarizes the literature on previous studies. It usually does not present any new information on a subject.	Unstructured abstract. No more than 250 words.	3-8 keywords	The main text may consist of several sections with unfixed section titles. We suggest that the author includes an "Introduction" section at the beginning, several sections with unfixed titles in the middle part, and a "Conclusion" section in the end.
Case Report	A Case Report details symptoms, signs, diagnosis, treatment, and follows up an individual patient. The goal of a Case Report is to make other researchers aware of the possibility that a specific phenomenon might occur.	Unstructured abstract. No more than 150 words.	3-8 keywords	The main text consists of three sections with fixed section titles: Introduction, Case Report, and Discussion.
Meta-Analysis	A Meta-Analysis is a statistical analysis combining the results of multiple scientific studies. It is often an overview of clinical trials.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Systematic Review	A Systematic Review collects and critically analyzes multiple research studies, using methods selected before one or more research questions are formulated, and then finding and analyzing related studies and answering those questions in a structured methodology.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Technical Note	A Technical Note is a short article giving a brief description of a specific development, technique or procedure, or it may describe a modification of an existing technique, procedure or device applied in research.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Commentary	A Commentary is to provide comments on a newly published article or an alternative viewpoint on a certain topic.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Editorial	An Editorial is a short article describing news about the journal or opinions of senior editors or the publisher.	None required	None required	/
Letter to Editor	A Letter to Editor is usually an open post-publication review of a paper from its readers, often critical of some aspect of a published paper. Controversial papers often attract numerous Letters to Editor	Unstructured abstract (optional). No more than 250 words.	3-8 keywords (optional)	/
Opinion	An Opinion usually presents personal thoughts, beliefs, or feelings on a topic.	Unstructured abstract (optional). No more than 250 words.	3-8 keywords	/
Perspective	A Perspective provides personal points of view on the state-of-the-art of a specific area of knowledge and its future prospects. Links to areas of intense current research focus can also be made. The emphasis should be on a personal assessment rather than a comprehensive, critical review. However, comments should be put into the context of existing literature. Perspectives are usually invited by the Editors.	Unstructured abstract. No more than 150 words.	3-8 keywords	/

2.3 Manuscript Structure

2.3.1 Front Matter

2.3.1.1 Title

The title of the manuscript should be concise, specific and relevant, with no more than 16 words if possible. When gene or protein names are included, the abbreviated name rather than full name should be used.

2.3.1.2 Authors and Affiliations

Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email addresses for all authors should be listed. At least one author should be designated as corresponding author. In addition, corresponding authors are suggested to provide their Open Researcher and Contributor ID upon submission. Please note that any change to authorship is not allowed after manuscript acceptance.

2.3.1.3 Abstract

The abstract should be a single paragraph with word limitation and specific structure requirements (for more details please refer to Types of Manuscripts). It usually describes the main objective(s) of the study, explains how the study was done, including any model organisms used, without methodological detail, and summarizes the most important results and their significance. The abstract must be an objective representation of the study: it is not allowed to contain results which are not presented and substantiated in the manuscript, or exaggerate the main conclusions. Citations should not be included in the abstract.

2.3.1.4 Keywords

Three to eight keywords should be provided, which are specific to the article, yet reasonably common within the subject discipline.

2.3.2 Main Text

Manuscripts of different types are structured with different sections of content. Please refer to Types of Manuscripts to make sure which sections should be included in the manuscripts.

2.3.2.1 Introduction

The introduction should contain background that puts the manuscript into context, allow readers to understand why the study is important, include a brief review of key literature, and conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved. Relevant controversies or disagreements in the field should be introduced as well.

2.3.2.2 Methods

Methods should contain sufficient details to allow others to fully replicate the study. New methods and protocols should be described in detail while well-established methods can be briefly described or appropriately cited. Experimental participants selected, the drugs and chemicals used, the statistical methods taken, and the computer software used should be identified precisely. Statistical terms, abbreviations, and all symbols used should be defined clearly. Protocol documents for clinical trials, observational studies, and other non-laboratory investigations may be uploaded as supplementary materials.

2.3.2.3 Results

This section contains the findings of the study. Results of statistical analysis should also be included either as text or as tables or figures if appropriate. Authors should emphasize and summarize only the most important observations. Data on all primary and secondary outcomes identified in the section Methods should also be provided. Extra or supplementary materials and technical details can be placed in supplementary documents.

2.3.2.4 Discussion

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study. Future research directions may also be mentioned.

2.3.2.5 Conclusion

It should state clearly the main conclusions and include the explanation of their relevance or importance to the field.

2.3.3 Back Matter

2.3.3.1 Acknowledgments

Anyone who contributed towards the article but does not meet the criteria for authorship, including those who provided professional writing services or materials, should be acknowledged. Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgments section. This section is not added if the author does not have anyone to acknowledge.

2.3.3.2 Authors' Contributions

Each author is expected to have made substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data, or the creation of new software used in the work, or have drafted the work or substantively revised it.

Please use Surname and Initial of Forename to refer to an author's contribution. For example: made substantial contributions to conception and design of the study and performed data analysis and interpretation: Salas H, Castaneda WV; performed data acquisition, as well as provided administrative, technical, and material support: Castillo N, Young V.

If an article is single-authored, please include "The author contributed solely to the article." in this section.

2.3.3.3 Availability of Data and Materials

In order to maintain the integrity, transparency and reproducibility of research records, authors should include this section in their manuscripts, detailing where the data supporting their findings can be found. Data can be deposited into data repositories or published as supplementary information in the journal. Authors who cannot share their data should state that the data will not be shared and explain it. If a manuscript does not involve such issue, please state "Not applicable." in this section.

2.3.3.4 Financial Support and Sponsorship

All sources of funding for the study reported should be declared. The role of the funding body in the experiment design, collection, analysis and interpretation of data, and writing of the manuscript should be declared. Any relevant grant numbers and the link of funder's website should be provided if any. If the study is not involved with this issue, state "None." in this section.

2.3.3.5 Conflicts of Interest

Authors must declare any potential conflicts of interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. If there are no conflicts of interest, please state "All authors declared that there are no conflicts of interest." in this section. Some authors may be bound by confidentiality agreements. In such cases, in place of itemized disclosures, we will require authors to state "All authors declare that they are bound by confidentiality agreements that prevent them from disclosing their conflicts of interest in this work." If authors are unsure whether conflicts of interest exist, please refer to the "Conflicts of Interest" of OAE Editorial Policies for a full explanation.

2.3.3.6 Ethical Approval and Consent to Participate

Research involving human subjects, human material or human data must be performed in accordance with the Declaration of Helsinki and approved by an appropriate ethics committee. An informed consent to participate in the study should also be obtained from participants, or their parents or legal guardians for children under 16. A statement detailing the name of the ethics committee (including the reference number where appropriate) and the informed consent obtained must appear in the manuscripts reporting such research.

Studies involving animals and cell lines must include a statement on ethical approval. More information is available at Editorial Policies.

If the manuscript does not involve such issue, please state "Not applicable." in this section.

2.3.3.7 Consent for Publication

Manuscripts containing individual details, images or videos, must obtain consent for publication from that person, or in the case of children, their parents or legal guardians. If the person has died, consent for publication must be obtained from the next of kin of the participant. Manuscripts must include a statement that a written informed consent for publication was obtained. Authors do not have to submit such content accompanying the manuscript. However, these documents must be available if requested. If the manuscript does not involve this issue, state "Not applicable." in this section.

2.3.3.8 Copyright

Authors retain copyright of their works through a Creative Commons Attribution 4.0 International License that clearly states how readers can copy, distribute, and use their attributed research, free of charge. A declaration "© The Author(s) 2018." will be added to each article. Authors are required to sign License to Publish before formal publication.

2.3.3.9 References

References should be numbered in order of appearance at the end of manuscripts. In the text, reference numbers should be placed in square brackets and the corresponding references are cited thereafter. Only the first five authors' names are required to be listed in the references, other authors' names should be omitted and replaced with "et al.". Abbreviations of the journals should be provided on the basis of Index Medicus. Information from manuscripts accepted but not published should be cited in the text as "Unpublished material" with written permission from the source.

References should be described as follows, depending on the types of works:

Types	Examples
Journal articles by individual authors	Weaver DL, Ashikaga T, Krag DN, Skelly JM, Anderson SJ, et al. Effect of occult metastases on survival in node-negative breast cancer. <i>N Engl J Med</i> 2011;364:412-21. [PMID: 21247310 DOI: 10.1056/NEJMoal008108]
Organization as author	Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. <i>Hypertension</i> 2002;40:679-86. [PMID: 12411462]
Both personal authors and organization as author	Vallancien G, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1,274 European men suffering from lower urinary tract symptoms. <i>J Urol</i> 2003;169:2257-61. [PMID: 12771764 DOI: 10.1097/01.ju.0000067940.76090.73]
Journal articles not in English	Zhang X, Xiong H, Ji TY, Zhang YH, Wang Y. Case report of anti-N-methyl-D-aspartate receptor encephalitis in child. <i>J Appl Clin Pediatr</i> 2012;27:1903-7. (in Chinese)
Journal articles ahead of print	Odibo AO. Falling stillbirth and neonatal mortality rates in twin gestation: not a reason for complacency. <i>BJOG</i> 2018; Epub ahead of print [PMID: 30461178 DOI: 10.1111/1471-0528.15541]
Books	Sherlock S, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub; 1993. pp. 258-96.
Book chapters	Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. <i>The genetic basis of human cancer</i> . New York: McGraw-Hill; 2002. pp. 93-113.
Online resource	FDA News Release. FDA approval brings first gene therapy to the United States. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm . [Last accessed on 30 Oct 2017]
Conference proceedings	Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.
Conference paper	Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. <i>Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming</i> ; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. pp. 182-91.
Unpublished material	Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. <i>Proc Natl Acad Sci U S A</i> . Forthcoming 2002.

For other types of references, please refer to U.S. National Library of Medicine.

The journal also recommends that authors prepare references with a bibliography software package, such as EndNote to avoid typing mistakes and duplicated references.

2.3.3.10 Supplementary Materials

Additional data and information can be uploaded as Supplementary Material to accompany the manuscripts. The supplementary materials will also be available to the referees as part of the peer-review process. Any file format is acceptable, such as data sheet (word, excel, csv, cdx, fasta, pdf or zip files), presentation (powerpoint, pdf or zip files), image (cdx, eps, jpeg, pdf, png or tiff), table (word, excel, csv or pdf), audio (mp3, wav or wma) or video (avi, divx, flv, mov, mp4, mpeg, mpg or wmv). All information should be clearly presented. Supplementary materials should be cited in the main text in numeric order (e.g., Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2, *etc.*). The style of supplementary figures or tables complies with the same requirements on figures or tables in main text. Videos and audios should be prepared in English, and limited to a size of 500 MB or a duration of 3 minutes.

2.4 Manuscript Format

2.4.1 File Format

Manuscript files can be in DOC and DOCX formats and should not be locked or protected.

2.4.2 Length

There are no restrictions on paper length, number of figures, or amount of supporting documents. Authors are encouraged to present and discuss their findings concisely.

2.4.3 Language

Manuscripts must be written in English.

2.4.4 Multimedia Files

The journal supports manuscripts with multimedia files. The requirements are listed as follows:

Videos or audio files are only acceptable in English. The presentation and introduction should be easy to understand. The frames should be clear, and the speech speed should be moderate.

A brief overview of the video or audio files should be given in the manuscript text.

The video or audio files should be limited to a duration of 3 min and a size of up to 500 MB.

Please use professional software to produce high-quality video files, to facilitate acceptance and publication along with the submitted article. Upload the videos in mp4, wmv, or rm format (preferably mp4) and audio files in mp3 or wav format.

2.4.5 Figures

Figures should be cited in numeric order (e.g., Figure 1, Figure 2) and placed after the paragraph where it is first cited;

Figures can be submitted in format of tiff, psd, AI or jpeg, with resolution of 300-600 dpi;

Figure caption is placed under the Figure;

Diagrams with describing words (including, flow chart, coordinate diagram, bar chart, line chart, and scatter diagram, *etc.*) should be editable in word, excel or powerpoint format. Non-English information should be avoided;

Labels, numbers, letters, arrows, and symbols in figure should be clear, of uniform size, and contrast with the background; Symbols, arrows, numbers, or letters used to identify parts of the illustrations must be identified and explained in the legend;

Internal scale (magnification) should be explained and the staining method in photomicrographs should be identified;

All non-standard abbreviations should be explained in the legend;

Permission for use of copyrighted materials from other sources, including re-published, adapted, modified, or partial figures and images from the internet, must be obtained. It is authors' responsibility to acquire the licenses, to follow any citation instruction requested by third-party rights holders, and cover any supplementary charges.

2.4.6 Tables

Tables should be cited in numeric order and placed after the paragraph where it is first cited;

The table caption should be placed above the table and labeled sequentially (e.g., Table 1, Table 2);

Tables should be provided in editable form like DOC or DOCX format (picture is not allowed);

Abbreviations and symbols used in table should be explained in footnote;

Explanatory matter should also be placed in footnotes;

Permission for use of copyrighted materials from other sources, including re-published, adapted, modified, or partial tables from the internet, must be obtained. It is authors' responsibility to acquire the licenses, to follow any citation instruction requested by third-party rights holders, and cover any supplementary charges.

2.4.7 Abbreviations

Abbreviations should be defined upon first appearance in the abstract, main text, and in figure or table captions and used consistently thereafter. Non-standard abbreviations are not allowed unless they appear at least three times in the text. Commonly-used abbreviations, such as DNA, RNA, ATP, *etc.*, can be used directly without definition. Abbreviations in titles and keywords should be avoided, except for the ones which are widely used.

2.4.8 Italics

General italic words like *vs.*, *et al.*, *etc.*, *in vivo*, *in vitro*; *t* test, *F* test, *U* test; related coefficient as *r*, sample number as *n*, and probability as *P*; names of genes; names of bacteria and biology species in Latin.

2.4.9 Units

SI Units should be used. Imperial, US customary and other units should be converted to SI units whenever possible. There is a space between the number and the unit (i.e., 23 mL). Hour, minute, second should be written as h, min, s.

2.4.10 Numbers

Numbers appearing at the beginning of sentences should be expressed in English. When there are two or more numbers in a paragraph, they should be expressed as Arabic numerals; when there is only one number in a paragraph, number < 10 should be expressed in English and number > 10 should be expressed as Arabic numerals. 12345678 should be written as 12,345,678.

2.4.11 Equations

Equations should be editable and not appear in a picture format. Authors are advised to use either the Microsoft Equation Editor or the MathType for display and inline equations.

2.5 Submission Link

Submit an article via <https://www.oaemesas.com/vp>.



OAE Publishing Inc.

www.oaepublish.com

**Vessel Plus
(VP)**

Los Angeles Office

245 E Main Street ste122, Alhambra,
CA 91801, USA

Tel: +1 323 9987086

E-mail: editorialoffice@vpjournal.net

Website: www.vpjournal.net

