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CONTENTS

- 1 Dyslipidemia and atherosclerotic carotid artery stenosis**
Yoichi Miura, Hidenori Suzuki
Vessel Plus 2019;3:1 <http://dx.doi.org/10.20517/2574-1209.2018.69>
- 2 Dysfunctional high-density lipoprotein and atherogenesis**
Constantine E. Kosmas, Delia Silverio, Andreas Sourlas, Peter D. Montan, Eliscer Guzman
Vessel Plus 2019;3:2 <http://dx.doi.org/10.20517/2574-1209.2018.79>
- 3 Low density lipoprotein-induced lipid accumulation is a key phenomenon of atherogenesis at the arterial cell level**
Alexander N. Orekhov, Veronika A. Myasoedova
Vessel Plus 2019;3:3 <http://dx.doi.org/10.20517/2574-1209.2018.80>
- 4 Bridging aortic valve surgery to 21st century: what can a surgeon do?**
Mizar D'Abramo, Luisa Ferrante, Manuel Guerrero, Wael Saade, Ernesto Greco, Fabio Miraldi, Antonino Marullo, Mariangela Peruzzi, Antonio Barretta, Piero Proietti, Giuseppe Biondi-Zoccai, Sebastiano Sciarretta, Giacomo Frati, Alessandra Iaccarino
Vessel Plus 2019;3:4 <http://dx.doi.org/10.20517/2574-1209.2018.41>
- 5 MtDNA mutations linked with left ventricular hypertrophy**
Margarita A. Sazonova, Vasily V. Sinyov, Anastasia I. Ryzhkova, Marina D. Sazonova, Zukhra B. Khasanova, Igor A. Sobenin
Vessel Plus 2019;3:5 <http://dx.doi.org/10.20517/2574-1209.2018.56>
- 6 Successful plasmapheresis treatment of severe hypertriglyceridemia during late pregnancy**
Noémi Zsíros, Beáta Kovács, György Paragh, József Balla, Mariann Harangi
Vessel Plus 2019;3:6 <http://dx.doi.org/10.20517/2574-1209.2018.78>
- 7 Trigger mechanisms in insulin resistance and diabetes mellitus development**
Vira Zlatkina, Olena Karaya, Natalia Yarmish, Anna Shalimova
Vessel Plus 2019;3:7 <http://dx.doi.org/10.20517/2574-1209.2019.03>
- 8 Mitochondrial mutations associated with cardiac angina**
Margarita A. Sazonova, Anastasia I. Ryzhkova, Vasily V. Sinyov, Marina D. Sazonova, Nadezhda N. Nikitina, Tatiana P. Shkurat, Igor A. Sobenin, Alexander N. Orekhov
Vessel Plus 2019;3:8 <http://dx.doi.org/10.20517/2574-1209.2019.01>
- 9 The use of lipoprotein apheresis for the treatment of high-risk patients with elevated lipoprotein(a) and hypercholesterolemia**
Michael Yaroustovsky, Marina Abramyan, Ekaterina Rogalskaya, Ekaterina Komardina
Vessel Plus 2019;3:9 <http://dx.doi.org/10.20517/2574-1209.2019.02>

- 10 Monocyte differentiation and macrophage polarization**
Alexander N. Orekhov, Varvara A. Orekhova, Nikita G. Nikiforov, Veronika A. Myasoedova, Andrey V. Grechko, Elena B. Romanenko, Dongwei Zhang, Dimitry A. Chistiakov
Vessel Plus 2019;3:10 <http://dx.doi.org/10.20517/2574-1209.2019.04>
- 11 Vascular remodeling 2018: the updates**
Evgenia Gerasimovskaya, Alexander Verin
Vessel Plus 2019;3:11 <http://dx.doi.org/10.20517/2574-1209.2019.11>
- 12 Usefulness of chronic total occlusion devices and techniques in other complex lesion subsets**
Lazzaro Paraggio, Francesco Burzotta, Cristina Aurigemma, Carlo Trani
Vessel Plus 2019;3:12 <http://dx.doi.org/10.20517/2574-1209.2018.72>
- 13 Computational evaluation of mitral valve repair with MitraClip**
Brandon Prescott, Chad J. Abunassar, Konstantinos P. Baxevanakis, Liguao Zhao
Vessel Plus 2019;3:13 <http://dx.doi.org/10.20517/2574-1209.2018.70>
- 14 Carotid atherosclerosis-related mutations of mitochondrial DNA do not explain the phenotype of metabolic syndrome**
Igor A. Sobenin, Jukka T. Salonen, Zuhra B. Khasanova, Vasily V. Sinyov, Tatiana V. Kirichenko, Alexandra A. Melnichenko, Alexandra I. Prokudina, Varvara A. Orekhova, Andrey V. Grechko
Vessel Plus 2019;3:14 <http://dx.doi.org/10.20517/2574-1209.2018.63>
- 15 Profiling of risk of subclinical atherosclerosis: possible interplay of genetic and environmental factors as the update of conventional approach**
Igor A. Sobenin, Veronika A. Myasoedova, Tatiana V. Kirichenko, Varvara A. Orekhova, Zuhra B. Khasanova, Vasily V. Sinyov, Alexandra A. Melnichenko, Andrey V. Grechko, Alexander N. Orekhov
Vessel Plus 2019;3:15 <http://dx.doi.org/10.20517/2574-1209.2019.09>
- 16 Lipid profile of children with glycogen storage disease**
Tatyana Victorovna Strokova, Elena Vyacheslavovna Pavlovskaya, Andrey Igorevich Zubovich, Yurgita Ruslanovna Varaeva, Natalia Valerievna Polenova, Elena Nikolaevna Livantsova, Madlena Enverovna Bagaeva, Alexander Gennadievich Surkov, Svetlana Dmitrievna Kosyura, Antonina Vladimirovna Starodubova
Vessel Plus 2019;3:16 <http://dx.doi.org/10.20517/2574-1209.2019.08>
- 17 Subcellular anti-atherosclerotic therapy**
Anastasia V. Ryabova, Igor D. Romanishkin, Aleksey S. Skobeltsin, Arkadii S. Moskalev, Vladimir I. Makarov, Victor B. Loschenov, Nikita G. Nikiforov, Igor A. Sobenin, Alexander N. Orekhov
Vessel Plus 2019;3:17 <http://dx.doi.org/10.20517/2574-1209.2019.10>
- 18 Clinical and perioperative applications of three-dimensional echocardiography**
Lisa Q. Rong, Antonino Di Franco
Vessel Plus 2019;3:18 <http://dx.doi.org/10.20517/2574-1209.2019.007>
- 19 The link between hypertension and preeclampsia/eclampsia-life-long cardiovascular risk for women**
Tamar Vakhtangadze, Nino Gakhokidze, Magda Khutsishvili, Salome Mosidze
Vessel Plus 2019;3:19 <http://dx.doi.org/10.20517/2574-1209.2019.07>

- 20 **Type III coronary perforation during chronic total occlusion percutaneous coronary interventions treated with Cyanoacrylate glue embolization: case report and review of the technique**
Carlo Tumscitz, Valerio Lanzillotti, Lucia Pirani, Anna Maria Di Cesare, Alessandra Scoccia, Francesco Gallo
Vessel Plus 2019;3:20 <http://dx.doi.org/10.20517/2574-1209.2018.71>
- 21 **Moderator band connections: an unusual route in retrograde chronic total occlusion procedure: moderator band connections as options to right coronary artery chronic total occlusion**
Marcelo H. Ribeiro, Luis A. P. Dallan, Expedito E. Ribeiro da Silva, Carlos M. Campos, Marouane Boukhris, Alfredo R. Galassi, Daniel Weilenmann, João Antônio Brum da Silveira, Satoru Sumitsuji
Vessel Plus 2019;3:21 <http://dx.doi.org/10.20517/2574-1209.2018.66>
- 22 **Del Nido cardioplegia: from an infant conceive to an adult life - a brief review of the current evidence in adult patients**
Claudio Pragliola, Essam Hassan, Abdulaziz Al Hossan, Khaled Al Otaibi, Juan J. T. Alfonso, Afnan Al Khalaf, Khalid D. Al Garni
Vessel Plus 2019;3:22 <http://dx.doi.org/10.20517/2574-1209.2019.003>
- 23 **Total arterial coronary grafting: outcomes, concerns and controversies**
Shahzad G. Raja
Vessel Plus 2019;3:23 <http://dx.doi.org/10.20517/2574-1209.2019.05>
- 24 **Fractional flow reserve guided coronary artery bypass grafting - new developments and future perspectives**
Johan Van der Merwe, Filip Casselman
Vessel Plus 2019;3:24 <http://dx.doi.org/10.20517/2574-1209.2019.17>
- 25 **Complete surgical myocardial revascularization: shift of paradigm of the gold standard in the current era**
Venkat R. Machiraju
Vessel Plus 2019;3:25 <http://dx.doi.org/10.20517/2574-1209.2019.008>
- 26 **Contemporary indications for percutaneous revascularization of coronary chronic total occlusions**
Milan Dobric, Sinisa Stojkovic
Vessel Plus 2019;3:26 <http://dx.doi.org/10.20517/2574-1209.2018.77>
- 27 **Transthoracic vs. transesophageal echocardiography in transcatheter aortic valve implantation: a systematic review and meta-analysis**
Tiffany K. Lam, Derrick Y. Tam, Apurva R. Dixit, Stephen E. Fremes
Vessel Plus 2019;3:27 <http://dx.doi.org/10.20517/2574-1209.2019.009>
- 28 **Respite for hybrid coronary revascularization**
Vincent Gacad, Twinkle Singh, Ayush Motwani, Rohan Samson, Thierry H. Le Jemtel
Vessel Plus 2019;3:28 <http://dx.doi.org/10.20517/2574-1209.2019.011>

- 29 **Chronic total occlusion percutaneous coronary intervention complications: prevention and management**
Francesco Colombo, Alessandro Bernardi, Roberto Garbo
Vessel Plus 2019;3:29 <http://dx.doi.org/10.20517/2574-1209.2019.005>
- 30 **The hybrid algorithm for chronic total occlusions**
Carlo Zivelonghi, Simone Budassi, Pierfrancesco Agostoni
Vessel Plus 2019;3:30 <http://dx.doi.org/10.20517/2574-1209.2019.06>
- 31 **Atrial septal defect repair in the age of transcatheter devices**
Eric Zimmermann, Hafiz Hussain, Berhane Worku, Dimitrios Dougenis, Dimitrios Avgerinos
Vessel Plus 2019;3:31 <http://dx.doi.org/10.20517/2574-1209.2019.010>
- 32 **Characterizing the mechanical properties of the aortic wall**
Sonja Pejicic, Syed M. Ali Hassan, David E. Rival, Gianluigi Bisleri
Vessel Plus 2019;3:32 <http://dx.doi.org/10.20517/2574-1209.2019.18>
- 33 **Mechanical ventilation and cardiopulmonary bypass: a narrative review of the mechanistic lung protective measures**
Marco Echeverria-Villalobos, Dolly M. Munlemvo, Juan Fiorda-Diaz, Michael K. Essandoh
Vessel Plus 2019;3:33 <http://dx.doi.org/10.20517/2574-1209.2019.12>
- 34 **Surgical treatment for heart failure: cell-based therapy with engineered tissue**
Jordan J. Lancaster, Jen Watson Koevary, Ikeotunye Royal Chinyere, Sherry L. Daugherty, Kenneth A. Fox, Steven Goldman
Vessel Plus 2019;3:34 <http://dx.doi.org/10.20517/2574-1209.2019.16>
- 35 **Finite element analysis of polyether ether ketone 450G biomaterial used as cardiovascular stent implant**
Vasanth Kumar, C. M. Ramesha, V. Sharanraj
Vessel Plus 2019;3:35 <http://dx.doi.org/10.20517/2574-1209.2019.006>
- 36 **Outcomes of long left coronary endarterectomy in patients with diffuse coronary artery disease**
Kamaraj Radhakrishnan, Sean D. Galvin, Adam El-Gamel
Vessel Plus 2019;3:36 <http://dx.doi.org/10.20517/2574-1209.2019.23>
- 37 **Acute mechanical complications in patients suffering from acute myocardial infarction**
Francesco Formica, Serena Mariani, Stefano D'Alessandro
Vessel Plus 2019;3:37 <http://dx.doi.org/10.20517/2574-1209.2019.19>
- 38 **The frontier in Cardiac Surgery is intellectual**
Carlos A. Mestres, Alberto Pozzoli, Maurizio Taramasso, Michel Zuber, Francesco Maisano
Vessel Plus 2019;3:38 <http://dx.doi.org/10.20517/2574-1209.2019.20>
- 39 **Percutaneous edge-to-edge mitral valve repair for secondary mitral regurgitation: perspectives on COAPT and MITRA-FR trials**
Nirvik Pal, Mark Nelson, John Butterworth
Vessel Plus 2019;3:39 <http://dx.doi.org/10.20517/2574-1209.2019.24>

40 Deletion of retinoic acid-related orphan receptor gamma reduces body weight and hepatic lipids in mice by modulating the expression of lipid metabolism genes

Jahangir Iqbal, Zainab Jahangir, Divya Veluru, Abeer Al Otaibi, Sindiyan A. Mubarak, Badr Al Subie, Ahmad F. Alghanem, Ali Al Qarni, Ahmed Bakillah, Abbas Hawwari

Vessel Plus 2019;3:40 <http://dx.doi.org/10.20517/2574-1209.2019.28>

Review

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Dyslipidemia and atherosclerotic carotid artery stenosis

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Abstract

Carotid artery atherosclerosis or stenosis is frequently present at the carotid bifurcation or the internal carotid artery, accounting for at least 20% of all ischemic strokes. High levels of serum total cholesterol and low-density lipoprotein cholesterol are established risk factors for genesis and progression of atherosclerotic lesions through various mechanisms. In addition, accumulating evidence has shown that a high level of triglyceride is associated with increased atherosclerosis risks. The so-called “vulnerable plaque” with a large lipid core, thin fibrous cap and intra-plaque hemorrhage tends to cause subsequent thromboembolic ischemic events. Statins are known not only to lower serum cholesterol levels but also to promote plaque stabilization via pleiotropic effects such as reducing subclinical systemic inflammation, endothelial activation, leukocyte intra-plaque infiltration, and increasing intimal smooth muscle cell migration. This article discusses the mechanisms of atherosclerosis formation induced by dyslipidemia and the role of lipid-lowering agents including statins in patients with symptomatic and asymptomatic atherosclerotic carotid artery stenosis.

Keywords: Atherosclerosis, carotid artery stenosis, lipid-lowering agent

INTRODUCTION

Carotid artery (CA) stenosis is caused by local thickening of CA wall due to atherosclerosis, and has a predilection for the CA bifurcation or the internal CA. The prevalence of significant CA stenosis is reported to be 7%-9% in the general population^[1]. The high prevalence was observed in association with acute ischemic stroke (60%), coronary heart disease (18%), and atherosclerosis (11%)^[2]. A thromboembolism from CA atherosclerotic plaque causes at least 20% of all ischemic strokes^[3]. The progression of CA atherosclerosis was promoted by dyslipidemia, hypertension, smoking, diabetes, and certain hemodynamic features



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including turbulent blood flow or low wall shear stress^[4]. Accumulating evidences indicate that symptomatic $\geq 70\%$ CA stenosis should undergo expedited surgery, and most of the guidelines recommend carotid endarterectomy (CEA) within 2 weeks of minor ischemic strokes or transient ischemic attacks (TIAs). In contrast, some guidelines also recommend CEA for asymptomatic CA stenosis, but surgical or interventional therapies for asymptomatic CA stenosis are a weaker recommendation^[5]. This is because recent advance in medical therapies has reduced the stroke risk in an asymptomatic CA stenosis. Indeed, a large prospective cohort study reported that asymptomatic $\geq 50\%$ CA stenosis was associated with an annual rate of $< 1\%$ ischemic strokes^[6]. According to meta-analyses, an annual rate of ischemic strokes in asymptomatic severe CA stenosis was decreased to 1.13% after 2000 with medical therapy alone, while that was 2.83% before 2000^[7]. The remarkable thing is that the time of decreases in ischemic stroke risks in CA stenosis overlaps with that of increases in modern intensive medical treatment including statin medications and the improved control of hypertension.

Several lipid-lowering agents such as a statin are well known to be effective for the primary or secondary stroke prevention^[8-10]. Statin exerts not only lipid-lowering effects but also pleiotropic effects including the reduction of inflammatory reactions, endothelial cell (EC) activation, and smooth muscle cell (SMC) proliferation^[11]. As for the association between statins and atherosclerotic CA stenosis, statins are well known to decrease CA intima-media thickness (IMT)^[12-15], suppress CA plaque progression^[16,17], and improve CA plaque vulnerability^[18,19].

This article focuses on mechanisms of CA atherosclerosis development, by which dyslipidemia induces, and discusses potential therapeutic roles of statins as well as non-statin lipid-lowering agents and non-drug therapies for atherosclerotic CA stenosis.

THE FORMATION OF ATHEROSCLEROTIC CA PLAQUE [Figure 1]

The mechanisms of atherosclerotic formation in CA are similar to those in other arteries. A hemodynamic shear stress triggers EC dysfunction and induces SMC accumulations in the subendothelial space, initiating atherosclerosis formation at arterial branch sites as intimal cell masses^[20,21]. Physical or metabolic injury-induced disturbance of EC integrity makes ECs transduce hemodynamic stress into biochemical signals, which change the expressions of cell adhesion molecules (CAMs) and other cell surface receptors to alter blood cell adhesion^[20]. Several mediators including reactive oxygen species induce CAMs such as intercellular adhesion molecule (ICAM)-1, vascular CAM (VCAM)-1, and endothelial-leukocyte adhesion molecules on ECs^[21]. The first step of atherogenesis is that low-density lipoprotein (LDL) cholesterol (Cho) (LDL-C) enters into the subendothelial spaces, and is trapped by a high affinity to the glycoprotein molecules at the lesion^[22]. Although LDL particles cannot penetrate the junctions between ECs due to their too large molecular size, most of circulating LDLs can be transported across the EC by receptor-mediated or nonspecific uptake into micropinocytic channels. Each EC has receptors for both LDLs and the modified forms. A free receptor modifies and oxidizes LDL into modified forms of LDL such as oxidized LDLs (ox-LDLs), and ox-LDLs promote transendothelial migration of monocytes into the subendothelial spaces, which is guided by chemokines. Moreover, ox-LDLs induce the differentiation of monocytes into macrophages. Macrophages develop receptors for ox-LDLs to become lipid-laden foam cells through the receptor-mediated incorporation of ox-LDLs^[21]. Thus, Cho is trapped within the arterial walls, which is the hallmark of early-stage atherosclerotic lesion formation. Macrophage also induces local inflammatory reactions within the vessel walls, which are promoted by cytokines, activated helper T cells, and activators of scavenger receptors^[23]. Accumulated lipid-laden foam cells in the tunica intima in the artery wall mature fatty streaks and produce a lesion, which is covered with a fibrous cap consisting of macrophages, SMCs, and extracellular matrix (ECM) components including collagen, elastin and proteoglycans^[24]. The fibrous cap is a layer of connective tissues, and separates a lipid-rich core from arterial lumen, forming atherosclerotic plaques. When chronic inflammation is present, foam cells are persistently recruited, and apoptosis and

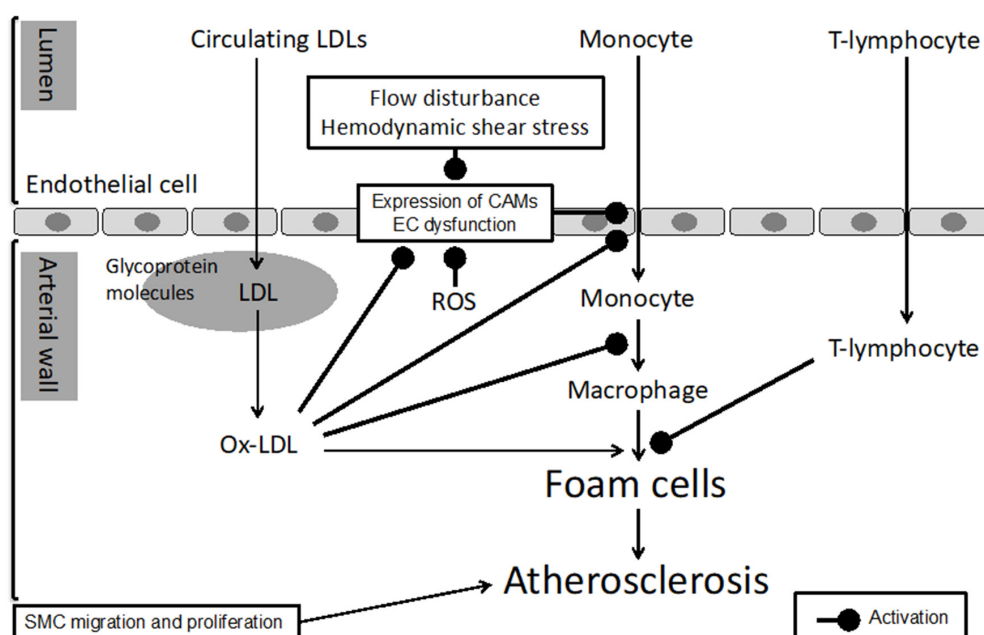


Figure 1. Formation of atherosclerosis. Atherosclerosis begins at the sites of EC damage or dysfunction, and develops with inflammatory reactions and dynamic interactions among plasma molecules including LDL and Ox-LDL, monocytes and macrophages. EC: endothelial cell; LDL: low density lipoprotein; Ox-LDL: oxidized LDL; CAM: cell adhesion molecule; ROS: reactive oxygen species; SMC: smooth muscle cell

ECM degradation by matrix metalloproteinases (MMPs) are promoted and associated with more necrotic environment within atherosclerotic plaques. As a result, atherosclerotic lesions are furthermore developed and matured.

CA PLAQUE VULNERABILITY

An atherosclerotic CA plaque consists of a lipid core with inflammatory cell infiltrations and a fibrous cap, and is classified into stable and unstable or vulnerable ones. A stable plaque is characterized by a thicker fibrous cap, which prevents plaques from rupture. In contrast, the characteristics of unstable or vulnerable plaque is intra-plaque hemorrhage and a large lipid core covered with a thin fibrous cap, which contains less ECM and SMCs, and is often associated with the infiltration of inflammatory cells and the secretion of MMPs and cytokines^[25]. Several reports have shown that a rich network of small vessels, that is, vasa vasorum, is interweaved into the ECM of most of mature plaques^[26]. Unstable or vulnerable plaque is more likely to rupture, causing thromboembolic strokes. Intra-plaque hemorrhage is also known as a predictor for thromboembolic strokes and the recurrence. As well, strong correlations are observed between intra-plaque hemorrhage and plaque rupture, and symptomatic CA stenosis was more frequently associated with intra-plaque hemorrhage compared with asymptomatic CA stenosis (74% vs. 32%)^[27].

Many atherosclerotic mediators play a role in CA plaque vulnerability^[28-30]. Morgan *et al.*^[28] reported the relationships between MMPs-1 or -12 and CA plaque instability: MMP-1 was upregulated more in a CA plaque with a thin fibrous cap compared with that with a thick fibrous cap, and MMP-12 was induced more in a ruptured CA plaque than a CA plaque with no disruption of a fibrous cap. Montecucco *et al.*^[29] demonstrated that the down-regulation of cannabinoid receptor type 2 that prevents neutrophil release of MMP-9 caused an increase in vulnerability in a symptomatic CA plaque. It was also found that anti-apolipoprotein (Apo) A-1 auto-antibodies played a role in an increase in histological features of plaque vulnerability in severe CA stenosis^[29]. More recently, Rao *et al.*^[30] revealed that triggering receptor expressed on myeloid cells (TREM)-1 related to MMPs-1 and -9 was increased in a symptomatic CA plaque, suggesting a potential role of TREM-1 in CA plaque destabilization.

DYSLIPIDEMIA AND ATHEROSCLEROSIS

Total Cho

Numerous epidemiology and clinical studies have demonstrated that dyslipidemia is a major risk factor for atherosclerosis formation^[2,21]. Hypercholesterolemia increases levels of cellular free Cho about 2- to 4-fold in vascular ECs^[31]. As a result, a high level of free Cho in ECs induces changes in the plasma membrane Cho content and the composition of lipid rafts, leading to altering membrane function and therefore affecting cell function in SMCs^[32]. High levels of free Cho also increase reactive oxygen species generation via various mechanisms^[31,33]. Increased reactive oxygen species generation causes EC dysfunction in terms of reducing nitric oxide bioavailability and uncoupling endothelial nitric oxide synthase (eNOS). In addition, hypercholesterolemia activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidases and myeloperoxidases, resulting in reactive oxygen species generation^[21]. Hypercholesterolemia also promotes the secretion of a proinflammatory cytokine such as tumor necrosis factor- α , interleukin-1 β , and interferon- γ , as well as mitochondrial and NADPH oxidase-generated reactive oxygen species^[21]. In the presence of hypercholesterolemia, caveolin, an essential protein component of caveolae, binds to eNOS, leading to an inactivity of eNOS^[21].

LDL

LDL interactions with the endothelium have been closely associated with accelerated atherosclerosis^[20,21]. LDLs and ox-LDLs activate EC NADPH oxidases, generating reactive oxygen species^[21,33]. LDLs and ox-LDLs also cause increases in the binding of eNOS to CD36, and consequently the attenuation of eNOS activity and the displacement of the protein from EC caveolae are induced^[34]. Additionally, the interactions between eNOSs and NADPH oxidases determine the production of nitric oxide and reactive oxygen species, because nitric oxide produced adjacent to NADPH oxidases is scavenged by reactive oxygen species^[35]. Furthermore, native LDLs increase the expressions of ICAM-1, VCAM-1, and p-selectin^[21].

Triglyceride and TG-rich lipoprotein

Triglyceride (TG) is a major component of TG-rich lipoprotein (TGRL) including chylomicrons (CMs), very LDLs (VLDLs) and their remnants. TG and TGRL contribute directly to the development and progression of atherosclerotic plaque. While CMs and larger VLDLs cannot penetrate the arterial walls, their smaller TGRL remnants not only penetrate the arterial intima, but also promote the binding and retention to connective tissue matrix^[36]. This transcytosis involvement in the transport system is restricted to a lipoprotein with a diameter of ≤ 60 -70 nm. TGRL remnants carry about 40 times more Cho per particle compared with LDLs, due to their larger size^[36]. Accumulation of CMs and VLDLs with abundant Apo E have been shown in an atherosclerotic plaque. Macrophages directly take up such particles, and resultantly have massive Cho loading, leading to the formation of foam cells.

TG and TGRL also accelerate the atherogenesis through an indirect mechanism, particularly that involving binding and lipolysis at the arterial walls^[37-40]. TGRL increases the production of reactive oxygen species, secretion of tumor necrosis factor- α , and expressions of CAMs^[37]. A high level of TGs leads to TGRLs enriched with Apo C-III, and influences signaling pathways, which lead to activation of nuclear factor (NF)- κ B and upregulation of inflammatory mediators, causing the development of fatty streaks and the advancement of atherosclerosis^[38]. CM remnants migrate to the subendothelial space, activate leukocytes, and accelerate the formation of foam cells: CM also activates monocytes, and enhances migration of monocytes and postprandial neutrophils^[39]. TG in the presence of an elevated concentration of VLDL generates small dense LDL, which is particularly atherogenic, since these particles are retained preferentially by the arterial walls. Furthermore, elevated TG is linked with procoagulant states by increasing factor VII, and activating factor VII phospholipid complexes, factor X, factor XII, tissue plasminogen activator inhibitor, and thrombin generation^[21,40].

The proatherogenic mechanisms associated with TG and TGRL are rather complicated and need to be further explored. However, current knowledge and accumulating evidences of clinical studies indicate that therapies lowering TG levels can be one of important treatment strategies to suppress atherosclerotic formation and ischemic stroke risks.

High-density lipoprotein Cho

High-density lipoprotein Cho (HDL-C) is potentially highly protective for atherosclerosis. HDL-C is classically known to reverse Cho transport: that is, HDL-C removes excess Cho from foam cells within peripheral tissues, and delivers it to liver for metabolism^[41]. In addition, recent reports have shown that HDL-C exerts a lot of other functions, which reduce atherosclerotic development^[41-43]. Beneficial effects of HDL-C against atherogenesis may come from its protective effects against the generation of reactive oxygen species and VCAMs^[42]. Reconstituted HDL-C suppresses the activation of leukocyte NADPH oxidases^[43]. The effects of HDL-C may contribute to protecting vascular injuries, resulting in the suppression of atherosclerosis. HDL-C inhibits the expressions of cytokine-induced CAMs, and then suppresses the adhesion of monocytes to ECs^[44]. Paraoxonase-containing HDL-C suppresses oxidation of LDL-C, and prevents expressions of monocyte chemotactic protein-1^[45]. As well, LDL-C was reported to increase monocyte chemotactic protein-1 expressions and cause 7-fold increases in monocyte migration into the subendothelial space, whereas purified HDLs inhibited the monocyte migration by 91%^[46].

HDL-C protects erythrocytes against the generation of procoagulant activity, and enhances anticoagulant activities of protein S^[47]. HDL-C is also positively correlated with plasminogen activator inhibitor-1 activity^[21]. Furthermore, HDL-C inhibits thrombin-induced binding of fibrinogen to platelets and suppresses the platelet aggregation^[48]. Thus, HDL-C can reduce blood clot formation, and therefore high levels of HDL-C can prevent ischemic stroke.

DYSLIPIDEMIA AND STROKE

A high level of serum total Cho (TC) and LDL-C is well known to be major risk factors for ischemic stroke. LDL-C-lowering therapies have attracted extensive attention, and have been a cornerstone of the primary and secondary prevention for ischemic stroke^[49]. Recently, higher TG levels also have been repeatedly reported to increase atherosclerotic risks^[50]. A meta-analysis study was conducted to investigate the association between lipid levels induced by lipid-modifying drugs and stroke risks, and revealed that higher levels of TG at baseline had a higher incidence of ischemic stroke occurrence (adjusted relative risk, 1.05 per 10 mg/dL increase) in placebo groups in 64 randomized clinical trials^[50]. In contrast, HDL-C levels are inversely associated with risks for the development of atherosclerosis and ischemic stroke^[41,51,52]. According to a systematic review of 18 prospective studies investigating relationships between HDL-C levels and stroke risks, each 10 mg/dL increase in HDL-C levels decreased risks for ischemic strokes ranging from 11%-15%^[52]. Some reports suggested that the negative association between HDL-C levels and ischemic stroke risks is more dependent on HDL-C sub-fractions rather than total levels of HDL-C. HDL sub-fractions have been classified on their size and relative concentrations of Cho, and each sub-fraction is known to have different biological activities, biochemical properties, and vascular metabolisms^[41,53]. HDL-2 and HDL-3 are major circulating sub-fractions in the peripheral blood^[53]. In contrast with HDL-2, smaller dense protein-rich HDL-3 appears to suppress oxidation of LDLs and thereby to prevent atherosclerosis^[53]. It was demonstrated that HDL sub-fractions differently influenced CA diseases in the Northern Manhattan study: HDL-2 and plaque thickness were positively related, while HDL-3 and plaque area were inversely associated^[54]. In another prospective study, however, small-size HDL-C and medium-size HDL-C were both associated with lower incidences of stroke occurrence, in particular as to lacunar infarcts and hemorrhagic strokes^[55]. On the other hand, large-size HDL-C did not influence the stroke risks^[55]. More studies are thus needed to clarify and understand the association between HDL sub-fractions and the risk of strokes. Another important finding is that lower levels of TC and LDL-C were associated with higher risks of intracranial

hemorrhage^[56,57]. In a previous meta-analysis study, an inverse dose-response association was shown between TC levels and hemorrhagic stroke risks [odds ratio (OR), 0.85 per 1 mmol/L increase]^[56]. A more recent systematic review also showed that hemorrhagic stroke was increased under low TC conditions: East Asian ethnic status favors the development of subarachnoid hemorrhage, and non-East Asian ethnic status is predisposed to intracerebral hemorrhage^[57].

As to the relationships between dyslipidemia and the subtypes of ischemic strokes, dyslipidemia has a stronger association with ischemic stroke due to atherosclerotic large artery diseases: it was reported that the association between Cho levels and risks of atherosclerotic stroke was the highest (OR, 3.2)^[51], and the findings were confirmed in other studies^[49]. On the other hand, there are no or obscure associations between dyslipidemia and other ischemic stroke subtypes: an association between dyslipidemia and lacunar strokes was shown in some studies but not all studies^[51], and most studies failed to show any association of dyslipidemia with cardioembolic infarction^[49,51].

DYSLIPIDEMIA AND CA STENOSIS

The risk factors for CA stenosis are similar to those for other vascular diseases, and the relationship between dyslipidemia and CA stenosis is well known. Mathiesen *et al.*^[58] reported that TC, HDL-C (inversely), fibrinogen, systolic blood pressure, and current smoking were independent factors for the development of CA stenosis. The determinants of CA IMT were LDL-C levels, systolic blood pressure, body mass index and smoking in childhood, and systolic blood pressure, body mass index and smoking in adulthood^[59]. Other previous studies reported that the progression of CA atherosclerosis was associated with a higher level of TC, LDL-C, or a lower level of HDL-C^[60]. In patients with type 2 diabetes, regression of CA IMT was observed when LDL-C and systolic blood pressure were reduced to a lower target^[61].

Several studies investigated if TG can be a risk factor for the progression of CA stenosis^[60,62]. In patients with diabetes, the CA atherosclerosis progression tended to occur more frequently when fasting TG levels were higher^[60]. Recently, Kitagami *et al.*^[62] reported that a higher level of TG was an independent risk factor for the progression of CA atherosclerosis in patients with moderate to severe CA stenosis who were treated with CA stenting (CAS), CEA, or other treatments under well-controlled LDL-C levels. The findings suggest that to control TG levels at least within the normal limits is an important management strategy for CA stenosis^[62]. However, to our knowledge, no studies have revealed the relationships between the degree of changes in TG levels and the rate of CA IMT progression. It is expected that some studies investigate if therapeutic reduction of TG levels can suppress the risks of progression of CA atherosclerosis or stenosis.

LIPID-LOWERING AGENTS AND CA STENOSIS [Figure 2]

Statin

Statin inhibits the synthesis of Cho in liver and thereby increases the uptake of Cho by liver, resulting in a decrease in circulating lipid levels: thus, statin has been extensively reported to exert a lipid-lowering effect.

Experimental studies have demonstrated protective effects of statins on ischemic stroke^[63-65]. For example, rosuvastatin upregulated eNOS and protected ischemic brain in middle cerebral artery occlusion (MCAO) mice^[63]; atorvastatin reduced brain edema via the suppression of aquaporin 4 expression in MCAO rats^[64]; and simvastatin protected cerebrum from ischemic and reperfusion injury by decreasing the expressions of Ca²⁺/calmodulin-dependent protein kinase II and aquaporin 4 in MCAO rats^[65].

In clinical settings, there are good evidences that statin reduces risks of ischemic strokes by about 30%, and that statins are effective for the primary prevention of ischemic strokes in the elderly and patients with diabetics, or hypertension^[8]. In addition, statins are reported to have beneficial effects on the secondary prevention for TIAs and ischemic strokes: statin administration significantly reduced the risk of fatal or

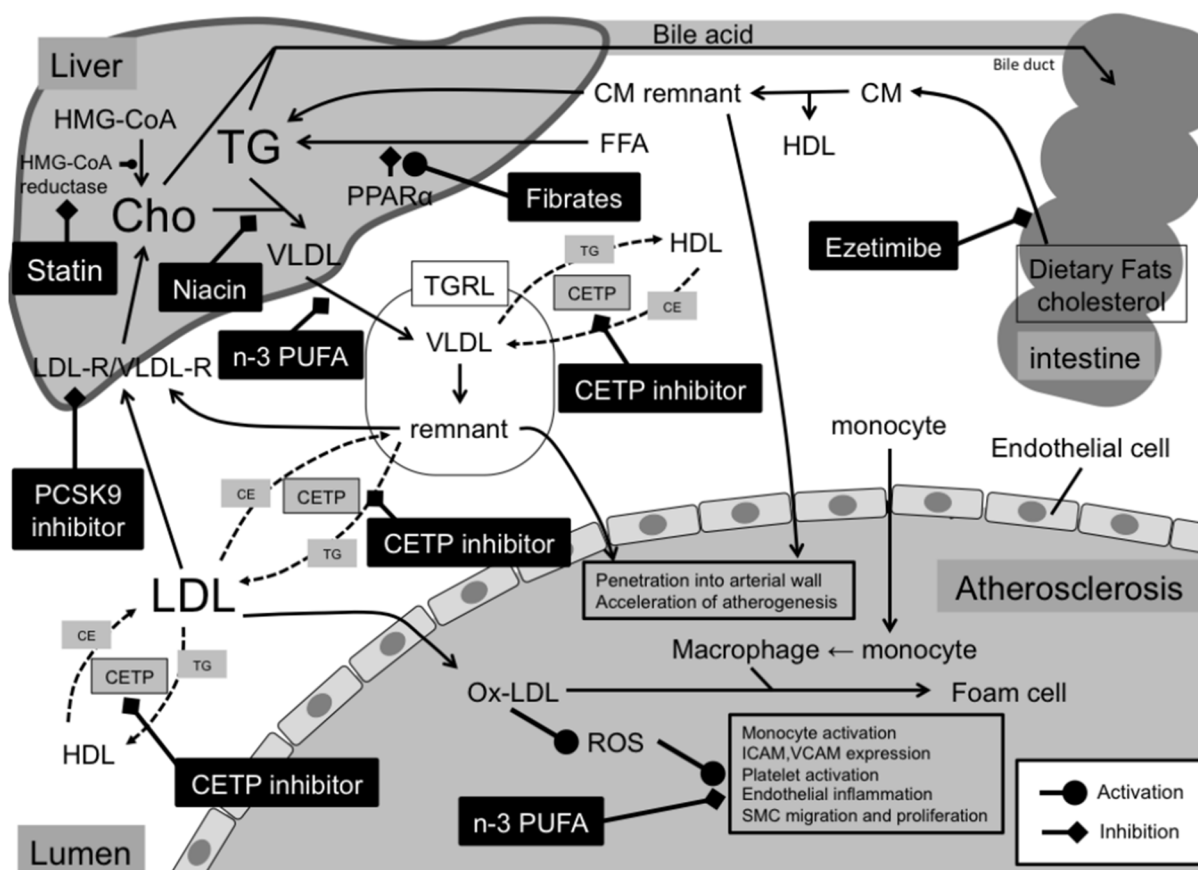


Figure 2. Metabolic pathways for cholesterol (Cho) and the main acting points of lipid-lowering agents. Statin is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitor, and exerts lipid-lowering effects by inhibiting the synthesis of Cho in liver and pleiotropic effects such as anti-inflammation and improvement of endothelial dysfunction. Niacins modify plasma lipid levels by inhibition of lipolysis in adipose tissues. Fibrates reduce triglyceride (TG) levels and increase high-density lipoprotein (HDL) Cho (HDL-C) levels via various mechanisms including the inactivation of peroxisome proliferator activated receptor (PPAR) α , which increases the oxidation of free fatty acid (FFA) in liver and reduces the hepatic synthesis of TG and expression of lipoprotein lipase. Omega-3 polyunsaturated fatty acids (PUFAs), the major component of fish oil, are widely used as a TG-lowering therapy and have been found against oxidative stress and inflammation. Ezetimibes reduce low-density lipoprotein (LDL) Cho (LDL-C) levels by inhibition of the absorption of Cho from intestines. Cho ester (CE) transfer protein (CETP) inhibitors increase HDL-C levels by the inhibition of CETP, which promotes the net effects on the equilibration of both CE and TG among all lipoprotein particles. Proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors remove LDL-C from plasma by the inhibition of PCSK9, which is a hepatic protease that decreases hepatic LDL receptor (LDL-R) leading to an increase in the plasma concentration of LDL-C. CM: chylomicron; ICAM: intercellular adhesion molecule; Ox-LDL: oxidized LDL; ROS: reactive oxygen species; SMC: smooth muscle cell; TGRL: TG-rich lipoprotein; VCAM: vascular cell adhesion molecule; VLDL: very LDL; VLDL-R: VLDL receptor

nonfatal strokes by 16%^[9] and prevented the development of atherothrombotic infarction even after adjusting LDL-C levels at baseline [adjusted hazard ratio (HR), 0.39]^[10]. The American Heart Association guideline states that statin treatment is reasonable for reducing LDL-C levels to less than or equal to 70 mg/dL in a patient with an atherosclerotic disease at the extracranial CA or vertebral artery who has sustained ischemic strokes^[66].

Statins are well known not only to decrease IMT and to suppress plaque progression in the CA, but also to improve plaque vulnerability in the CA as described below. These effects might be exerted by their pleiotropic effects, which include the inhibition of subclinical systemic inflammation, EC activation, and intra-plaque infiltrations of leukocytes, as well as an increase in protective SMC migration into plaques^[11].

Statin and CA IMT

Statins were for the first time reported to play a role in reducing CA IMT in coronary heart disease patients with hypercholesterolemia^[12]. After that, several randomized clinical trials revealed that statins have

beneficial and preventive effects against the progression of CA IMT^[13-15]. According to meta-analyses of 7 clinical trials of statins, statin treatments contributed to a mean of -0.012 mm/year reduction in CA IMT^[13]. In the subsequent meta-analyses of 11 clinical trials of statins, the mean differences of changes in carotid IMT between statin and placebo therapies were -0.040 mm/25.6 months ($P < 0.001$)^[14]. As regards dosages, intensive lipid-lowering treatments may result in a more regression of CA IMT compared with moderate lipid-lowering treatments in patients with familial hypercholesterolemia^[67].

Statin and CA plaque volume

Ainsworth *et al.*^[17] used 3-dimensional ultrasound scans and evaluated CA plaque volume after 3 months treatment with randomly assigned placebo or 80 mg/day of atorvastatin in 38 patients with asymptomatic > 60% CA stenosis: atorvastatin treatments decreased the plaque volume, although placebo treatments caused a tendency for the plaque volume to increase. Corti *et al.*^[16] used magnetic resonance imaging (MRI), and found that 2 years simvastatin treatments induced a significant regression in an asymptomatic plaque in both aorta and CA in hypercholesterolemic patients. In regard to dosages, the same group investigated effects of doses of 20 or 80 mg/day simvastatin treatments for a mean follow-up of 18.1 months on CA plaques in 51 patients, and showed no differences in vessel wall changes between the two dosages^[68]. In another clinical trial, 43 patients with asymptomatic 16%-79% CA stenosis were treated with randomly assigned low (5 mg/day) or high (40 or 80 mg/day) dosages of rosuvastatin for 2 years, and showed no changes in CA plaque volume between the 2 treatment groups on duplex ultrasound^[69]. In the trial, however, all plaques with a lipid-rich necrotic core at baseline were decreased by a mean of 41% with the statin treatments^[69]. It was also demonstrated in patients with 24 statin-naïve newly diagnosed stable coronary artery diseases that 3 months open-label statin treatments induced a 3.1% reduction in the carotid plaque index (normalized vessel wall area)^[70].

Statin and CA plaque vulnerability

The relationships between statin treatments and CA plaque vulnerability were investigated previously^[18,19]. Tang *et al.*^[18] estimated the extent of inflammations in CA plaques using ultra-small supermagnetic iron oxide enhanced carotid MRI, which visualizes the infiltrations of macrophages in human CA atheroma *in vivo*, and reported that 3 months treatments with a high dose (80 mg/day), but not a low dose (10 mg/day) of atorvastatin decreased intra-plaque inflammations. Mujaj *et al.*^[19] reported that high-dosage statins have beneficial influences on the compositions of CA atherosclerosis: that is, statins shifted vulnerable plaques with a lipid core to more stable calcified plaques. Lenglet *et al.*^[71] revealed that statin treatment reduced serum levels of neutrophilic products, receptor activator of NF- κ B ligand/osteoprotegerin ratio, osteopontin, and MMP-9 /pro-MMP-9 activity in severe CA stenosis patients having no history of ischemic stroke. Moreover, CA plaques on statin therapy exhibited an increase in collagen, and reduced levels of neutrophil infiltration and MMP-9 compared with untreated patients with asymptomatic severe CA stenosis^[71].

Statin in CEA and CAS

Statin therapy is reported to reduce perioperative risks of ischemic stroke, and to improve clinical outcomes in patients with CA stenosis undergoing CEA and CAS^[72]. McGirt *et al.*^[72] investigated effects of statin treatment on the incidences of perioperative strokes and mortality in 1,566 patients with CA stenosis undergoing CEA, and showed that statins had a benefit to reduce perioperative strokes, TIAs and all-cause mortality related to CEA. In the report, the usage of statins independently reduced the odds of stroke threefold (OR, 0.35) and death fivefold (OR, 0.20)^[72].

Periprocedural usage of statins may be more beneficial in patients with CA stenosis undergoing CAS, as high-pressure balloons and stents are applied during CAS and therefore thromboses and inflammations are likely to be developed within the vessel walls after CAS. Tanemura *et al.*^[73] investigated the association between cerebral embolisms related to CAS and CA plaque characteristics using 3-dimensional T1-weighted gradient echo MRI, and revealed that vulnerable and large CA plaques had high risks for cerebral embolisms

during CAS. In a recent meta-analysis of 8 studies that investigated effects of pre-CAS treatments with statins on periprocedural adverse events, statin treatments statistically reduced periprocedural risks of ischemic strokes (OR, 0.39) and death (OR, 0.30) in CA stenosis patients treated with CAS^[74].

OTHER LIPID-LOWERING AGENTS [Figure 2]

Classical non-statin lipid-lowering agents including niacin acids, fibrates and omega-3 fatty acids can improve lipid profiles. However, the benefit of these non-statin lipid-lowering agents has not been well established for the primary and secondary prevention of strokes. A meta-analysis including 78 clinical trials revealed that non-statin lipid-lowering agents such as fibrates, diet and other treatments did not reduce the risks of strokes (fibrates: OR, 0.98; diet: OR, 0.92; other treatments: OR, 0.81)^[75].

Niacin can reduce peripheral blood levels of TG by approximately 35%, decrease LDL-C levels by 10%-15%, and increase HDL-C levels by up to 25%^[76]. Niacins may also enhance a beneficial effect such as vasoprotection and anti-inflammatory actions independent of its lipid-modifying activities^[76]. Although Villines *et al.*^[77] revealed the regression of CA IMT thickening induced by niacin, its beneficial effects on the reduction of ischemic stroke risks remain uncertain. In a meta-analysis of 11 clinical studies including 9,959 patients, no significant association was found between niacin therapy and the risks of strokes (OR, 0.88)^[78].

Fibrate also lowers TG levels and increases HDL-C levels. Fibrate furthermore may exert pleiotropic effects via regulating the interaction with peroxisome proliferator activated receptor (PPAR) alpha, which influences vascular inflammation and thrombogenesis^[79,80]. However, it is uncertain if fibrates can reduce risks of stroke event. In the clinical trial consisting of 2,531 men with coronary artery diseases and low levels of HDL-C, benzofibrate showed a 31% reduction in the risks of stroke^[79]. On the other hand, a meta-analysis including 18 clinical trials with 45,058 patients showed no preventive effects of fibrates on stroke development^[80]. Recently, pemafibrate has been drawn attention to as a novel selective PPAR alpha modulator. In the phase III clinical trial, pemafibrate significantly reduced TG levels from baseline by up to 45% with low incidence rates of adverse drug reactions^[81]. In addition, the combination therapy of pemafibrate with pitavastatin exerted a robust reduction in a fasting level of TG by about 50% compared with the statin-monotherapy^[81]. Another trial is ongoing to investigate the efficacy of pemafibrate on cerebrovascular outcomes in patients with diabetes, and the results are awaited. It will be also necessary to verify whether pemafibrate has beneficial effects on the primary and secondary prevention of strokes in patients with CA stenosis.

Omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acids (EPAs) and docosahexaenoic acids (DHAs) are approved as an adjunct to diet for lowering plasma TG via multiple metabolic pathways^[82]. In a previous randomized clinical trial, 114 patients awaiting CEA were randomized to 3 groups taking placebo, fish oils (n-3 PUFA), and sunflower oils (n-6 PUFA): CA plaques in the n-3 PUFA treatment group had higher proportions of EPAs and DHAs, reduced infiltration of monocyte and macrophage, and a thicker fibrous cap compared with other groups^[83]. In another randomized clinical trial including 121 patients awaiting CEA, the proportion of EPAs was higher in CA plaques treated with n-3 PUFAs compared to placebo^[84]. The remarkable thing is that the EPA content in plaque phospholipids had inverse association with plaque instability, plaque inflammation, and the number of T cells in the plaques^[84]. CA atherosclerotic plaque in patients treated with n-3 PUFAs showed a lower level of messenger ribonucleic acids (mRNAs) for MMPs-7, -9, -12, interleukin-6 and ICAM-1^[84]. Thus, the stability of plaques by increased n-3 PUFAs intake may induce the reductions in the risk of ischemic stroke.

Ezetimibe prevents the absorption of Cho from intestines, and therefore reduces a level of TC. In the clinical trial including 18,144 patients hospitalized for acute coronary syndrome, the combination therapy of 10 mg/day ezetimibe with 40 mg/day simvastatin resulted in a significant reduction in stroke risks (HR, 0.936)^[85].

Furthermore, in patients stabilized after acute coronary syndrome, the addition of ezetimibe to simvastatin reduced the incidence of ischemic strokes (HR, 0.52): the preventive effects on ischemic strokes were remarkable in patients with prior strokes^[86].

Cholesteryl ester transfer protein (CETP) inhibitors increase a level of HDL-C, and some of them decrease levels of LDL-C and TG in addition to increasing HDL-C^[87]. However, the first clinical trial to investigate the effects of torcetrapib on atherosclerotic events was halted because of statistically higher incidences of death: hypertension due to activation of the renin-angiotensin-aldosterone system was considered to be a cause of the adverse events^[87]. In the subsequent randomized clinical trials of CETP inhibitors including dalcetrapib^[88] and evacetrapib^[89], serious off-target adverse events did not occur, but no risk reduction for cardiovascular events was revealed. In a more recent randomized clinical trial^[90], ≥ 4 years anacetrapib treatments significantly prevented the development of coronary artery events (HR, 0.91) associated with no increased risks of death, cancer, and other serious adverse events. It is necessary to examine whether CETP inhibitors are effective for preventing stroke.

Proprotein convertase subtilisin-kexin type 9 (PCSK9) is a hepatic protease, which decreases receptors for LDL in liver and causes an increase in the plasma concentration of LDL-C. A monoclonal antibody inhibitor of PCSK9 is a novel lipid-lowering agent, which has been reported to suppress LDL-C levels by 60%-70% in patients taking a statin^[91-93]. A meta-analysis of 24 randomized clinical trials including 10,159 patients reported that a PCSK9 inhibitor significantly reduced all-cause mortality (OR, 0.45) and myocardial infarction (OR, 0.49)^[91]. In a randomized clinical trial including 4,465 patients, evolocumab decreased LDL-C levels by 61% as well as the incidence of cardiovascular events (HR, 0.47)^[92]. However, evolocumab treatment exhibited neurocognitive adverse events more frequently compared with the control group regardless of LDL-C levels during the treatment^[92]. Another phase III randomized clinical trial including 2,341 patients with high risks for cardiovascular events revealed that alirocumab treatment induced a 62% reduction in a level of LDL-C, which in turn reduced a major adverse cardiovascular event compared with placebo treatment (HR, 0.52)^[93]. However, alirocumab treatment did not decrease risks of ischemic strokes, and rather had higher rates of injection-site reactions, myalgia, and neurocognitive side effects including memory impairments, confusional states and ophthalmologic events^[93]. The mechanisms of neurocognitive changes associated with PCSK9 inhibitors are not certain and the future clarification is required.

Randomized clinical trials have not been conducted and are needed to test the safety and efficacy of non-statin lipid-lowering agents as add-on treatments to a statin in patients with CA stenosis.

NON-DRUG THERAPIES

In addition to medications, lifestyle changes are a requisite component of lipid-lowering therapies, including diet and exercise. According to the 2016 guidelines of the European Society of Cardiology and the European Atherosclerosis Society for the management of dyslipidemia, diet should include a reducing intake of saturated fats and increasing intake of polyunsaturated and monounsaturated fats, cereals, fruits, vegetables and fat free dairy products^[94]. In addition, patients with a body mass index greater than 25 kg/m² should follow low-calorie diets. Moreover, at least 30 min a day of moderate-intensity aerobic activity for five days a week are recommended. The intake of dietary fiber, monounsaturated and PUFAs has been reported to decrease stroke risks by reducing LDL-C and postprandial lipids levels with some discrepancies^[95]. Habitual exercise has also been revealed to have beneficial effects on risk factors of stroke, including hypertension, dyslipidemia, diabetes and body weight: moderate or high exercise was associated with a significant reduction in the incidence of stroke or its mortality^[96]. However, there is no evidence from published randomized controlled trials to support the secondary stroke prevention associated with post-stroke diet and exercise.

CONCLUSION

Dyslipidemia is a major player in the formation and progression of CA atherosclerosis. Statins can improve serum lipid profiles and are beneficial for both of the primary and secondary prevention of ischemic stroke. In addition, statins exert beneficial effects on CA plaque stability as well as regression of CA IMT and plaque volume, causing reduction in the risk of perioperative complications related with CAS and CEA. Non-statin lipid-lowering agents may have adjunctive effects as an add-on treatment to statin, and are expected further to suppress atherosclerotic CA diseases and to reduce stroke risks. However, the widespread effects of lipid-lowering agents on serum lipid profile and atherogenesis have not been fully elucidated. Further investigations to reveal the mechanisms of the effects and the randomized clinical trials to test the safety and efficacy of lipid-lowering agents are needed in patients with CA stenosis.

DECLARATIONS

Authors' contributions

Conception and design of the study, wrote the original draft: Miura Y
Provided administrative support, reviewed and edited the manuscript: Suzuki H

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Review

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Dysfunctional high-density lipoprotein and atherogenesis

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Abstract

High-density lipoprotein (HDL) plays a major role in reverse cholesterol transport (RCT) but also exhibits, anti-inflammatory, endothelial/vasodilatory, anti-thrombotic, antioxidant, anti-aggregating, anticoagulant and cytoprotective functions, which enhance its protective effect against cardiovascular disease. However, the function of HDL is dependent upon genetic, environmental and lifestyle factors. Modification of the protein or lipid components of HDL in certain conditions may convert the HDL particles from anti-inflammatory to pro-inflammatory and pro-atherogenic by limiting their ability to promote RCT and to prevent LDL modification. In our review, we will present the clinical and scientific data pertaining to the factors and conditions that impair HDL functionality and we will discuss the effects of dysfunctional HDL on atherogenesis.

Keywords: High-density lipoprotein, high-density lipoprotein functionality, atherogenesis, cardiovascular disease

INTRODUCTION

There is extensive clinical evidence showing that there is a clear inverse relationship between serum high-density lipoprotein-cholesterol (HDL-C) concentrations and the risk for cardiovascular disease (CVD), which is independent of the concentration of low-density-lipoprotein cholesterol (LDL-C)^[1]. Actually, in a large meta-analysis, which included 20 randomized controlled trials with 543,210 person-years of follow-up and 7,838 myocardial infarctions, it was shown that, after adjustment for on-treatment LDL-C levels, age, hypertension, diabetes, and tobacco use, statins do not affect the relationship between HDL-C concentration



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and cardiovascular risk, so that low HDL-C concentrations continue to exhibit a significant, independent association with increased CVD risk despite statin therapy^[2].

The cardioprotective effects of HDL are exerted mainly via its role in the reverse cholesterol transport (RCT) pathway, by promoting the removal of cholesterol from peripheral cells and thus inhibiting foam cell formation and preventing atherogenesis. Furthermore, HDL promotes endothelial repair, decreases the expression of endothelial adhesion molecules and possess anti-inflammatory, antioxidant, antiaggregatory and anticoagulant properties. Thus, it becomes evident that the cardioprotective effect of HDL goes beyond RCT^[3,4].

On the other hand, there is ample clinical evidence showing that HDL functionality, more than HDL-C concentration per se, plays a crucial role in atheroprotection^[5,6]. HDL functionality is assessed by the cholesterol efflux capacity (CEC), which determines the ability of HDL to accept cholesterol from macrophages for excretion into the liver. CEC has been shown to be an excellent predictor of atherosclerotic disease^[7].

Furthermore, it is known that under certain conditions, such as the oxidative environment of the acute-phase response, the HDL particles may lose their anti-inflammatory properties and become pro-inflammatory^[8].

In our review, we will present the clinical and scientific data pertaining to the factors and conditions that impair HDL functionality and we will discuss the effects of dysfunctional HDL on atherogenesis.

HDL STRUCTURE AND HETEROGENEITY

HDL is synthesized in the intestine and the liver and consists of a heterogeneous group of particles, which differ in density, size, electrophoretic mobility, and apolipoprotein content^[5,9]. Furthermore, the HDL particles present marked structural, physiochemical, compositional and functional heterogeneity and have significant differences in their biological properties^[5,10,11]. The major apolipoproteins of HDL are apolipoprotein A-I (ApoA-I), which constitutes approximately 70% of HDL protein and is present on virtually all HDL particles, and ApoA-II, which constitutes approximately 20% of HDL protein and is present on about two-thirds of HDL particles in humans^[5,12].

On the other hand, the structure of the HDL particles is very complex. Mass spectrometry studies have shown that the HDL particles carry an array of proteins, which are engaged in lipid metabolism but also affect complement regulation, acute-phase response and proteinase inhibition^[5,13]. Moreover, lipidomic studies have identified in excess of 200 molecular lipid species in normolipidemic HDL, including phospholipids, sphingolipids, steroids, cholesteryl esters (CEs), triglycerides, diacylglycerides, monoacylglycerides and free fatty acids^[5,14].

Given the above heterogeneity of HDL particles and their structural complexity, it becomes easily understandable that any modifications of the components of the HDL particles may alter their functionality and potentially render HDL dysfunctional.

FACTORS AFFECTING HDL FUNCTIONALITY

Certain genetic, environmental and pathophysiologic conditions can influence the HDL cardioprotective effects by disrupting its protein components, lipid content, or by promoting modifications in the enzymes responsible for HDL metabolism.

Systemic states, such as inflammation and its equivalent acute phase response (observed after surgery and during infection or trauma), can induce significant changes on the HDL particle. During acute phase response, pro-inflammatory cytokines promote changes in the structure of plasma proteins, including those of the HDL particle. Interleukin-1 beta (IL-1 β), IL-6 and tumor necrosis factor- α are released during acute phase response and promote the synthesis of serum amyloid A (SAA) and group IIA secretory phospholipase A2 (sPLA2-IIA), which act as pro-inflammatory molecules. SAA interacts with HDL and may result in a faster clearance of the HDL particle, resulting in reduced HDL and ApoA-I plasma levels. In addition, SAA promotes the loss of the anti-inflammatory activity of HDL and renders the HDL particle pro-inflammatory^[15-17]. With regard to sPLA2-IIA, its activation promotes the breakdown of HDL phospholipids with subsequent accumulation of two proatherogenic and pro-inflammatory lipid products, lysophospholipids and fatty acids^[18], which can also disrupt HDL protein structure^[19].

Furthermore, in pro-inflammatory states, Apo-AI becomes a substrate for myeloperoxidase (MPO), a protein released by macrophages, monocytes and neutrophils, which catalyzes the chlorination or nitration of tyrosyl residues of ApoA-I molecules in HDL. MPO promotes oxidative damage of the HDL particle, which leads to a significant reduction of its anti-inflammatory properties, thus rendering HDL dysfunctional^[20].

Oxidized LDL is a powerful inducer of atherogenesis due to its role in endothelial dysfunction and foam cell formation. The mechanism by which oxidized LDL promotes atherogenesis involves the promotion of monocyte adhesion to the endothelium via activation of macrophages and mast cells^[21]. As it was alluded to earlier in this review, under normal conditions, HDL has antioxidant properties and prevents oxidation of LDL, which contribute to its cardioprotective effect. However, in pro-inflammatory environments, HDL may also lose its ability to inhibit monocyte migration within the arterial wall and thus lose its antioxidative effects on LDL particles^[22,23].

Another factor that can modify the antiatherogenic properties of HDL is the alteration in the HDL lipid composition. Reorganization of HDL lipid components due to an upregulation of the activity of CE transfer protein, as observed in insulin resistance states, such as the metabolic syndrome, can modify the CE/triacylglyceride (TAG) ratio in HDL, which plays a crucial role for the antioxidant activity of HDL. Furthermore, increased TAG content in the lipid core may also cause dysregulation of CE transfer through scavenger receptor class B type I, therefore impairing RCT^[24].

It has been also shown that certain disease states may impair HDL function. Disorders such as atherosclerosis and type 2 diabetes mellitus promote a subclinical chronic inflammatory microenvironment at a biomolecular level, eliciting protein remodeling of HDL with subsequent disruption of its anti-atherogenic, antioxidative and anti-inflammatory properties^[25,26]. Furthermore, ApoA-I glycation impairs HDL functionality^[27], leading to the impairment of the anti-atherogenic^[28] and anti-inflammatory^[29] properties of HDL.

Environmental factors have also a significant impact on HDL function. Factors that alter HDL functionality include smoking, obesity and dietary habits. HDL is susceptible to oxidative modifications by cigarette smoking. As a result, HDL loses its atheroprotective properties in smokers and becomes dysfunctional^[30]. With regard to obesity, there is evidence that it may reduce CEC and impair HDL functionality^[31]. In addition, consumption of saturated fat has been shown to impair arterial endothelial function and reduce the anti-inflammatory activity of HDL. On the contrary, the anti-inflammatory activity of HDL is enhanced after consumption of polyunsaturated fat^[32].

IMPACT OF DYSFUNCTIONAL HDL ON CVD

There is extensive evidence from clinical studies confirming the adverse role of dysfunctional HDL on atherogenesis and the risk for CVD.

In a post hoc analysis of two large prospective studies, the IDEAL (Incremental Decrease in End Points through Aggressive Lipid Lowering) trial and the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk case-control study, it was shown that very high plasma HDL-C levels (≥ 70 mg/dL) and very large HDL particles (> 9.53 nm) conferred an increased risk for coronary artery disease (CAD) when levels of ApoA-I and ApoB were kept constant^[33]. This observation may be explained by the hypothesis that very large, cholesterol-enriched HDL particles, may at some point become cholesterol donors rather than acceptors and thus become pro-atherogenic^[33]. This hypothesis was supported in another community-based cohort study, in which it was clearly shown that cholesterol-overloaded HDL particles were independently associated with progression of carotid atherosclerosis in a population free of CVD. More specifically, participants with the highest estimated number of cholesterol molecules per HDL particle (≥ 53.0) had 1.56-fold increased progression of carotid atherosclerosis, as compared with those with the lowest estimated number of cholesterol molecules per HDL particle (< 41.0)^[5,34]. Furthermore, in a very recent study of two large population-based cohorts in Denmark (52,268 men and 64,240 women), it was clearly shown that in men and women in the general population extremely high HDL-C levels were paradoxically associated with high all-cause mortality risk^[35]. In addition, in a large-scale pooled analysis of 9 Japanese cohorts, which included 43,407 participants, it was again shown that extremely high HDL-C levels led to an increase of atherosclerotic CVD mortality^[36].

As it was mentioned earlier in this review, CEC from macrophages is currently considered an important metric of HDL function. In this regard, multiple studies have shown an inverse relationship between CEC and the incidence of cardiovascular events, independent to HDL-C levels^[7,37,38]. In the Dallas heart study, a multiethnic, population-based, cohort study, in which the HDL cholesterol level, HDL particle concentration, and CEC were measured at baseline in 2,924 adults free from CVD over a mean follow-up period of 9.4 years, there was a 67% reduction in the risk for cardiovascular events in the highest quartile of CEC, as compared to the lowest quartile^[7]. This again proves that dysfunctional HDL with low CEC may be an important factor in atherogenesis.

In addition, it has been shown that HDL and ApoA-I recovered from human atheroma are dysfunctional and are extensively oxidized by MPO. More specifically, while the amount of circulating ApoA-I that contains a 2-OH-Trp72 group (oxTrp72-ApoA-I) is minimal under normal conditions, it accounts for 20% of the ApoA-I in atherosclerotic arteries. Increased levels of oxTrp72-ApoA-I have been linked to an increased risk for CVD^[39]. Most importantly, there is evidence showing that dysfunctional HDLs with diminished anti-inflammatory activity are present in patients with CAD and they are actually found in higher abundance in patients with acute coronary syndrome (ACS) than in patients with stable angina^[40].

Furthermore, as it was alluded to earlier, HDL isolated from patients with CAD (but not HDL from healthy subjects) exhibits a pro-inflammatory rather than an anti-inflammatory effect when exposed to endothelial cells. In addition, HDL from patients with CAD (in contrast to HDL from healthy subjects) did not stimulate endothelial cell NO production, due to inhibition of the activation of endothelial NO synthase, leading to the loss of the endothelial anti-inflammatory and repair-stimulating effects of HDL in patients with CAD^[41,42].

The above were also corroborated in a large clinical study, which included 1,548 patients with CAD undergoing coronary artery bypass grafting. This study clearly demonstrated that higher pre-operative HDL-C levels were not associated with a reduction but rather with a clear tendency for an increase in the occurrence of major adverse cardiovascular events^[43]. This was attributed to the presence of dysfunctional HDL in patients with CAD, as it was clearly alluded above.

CONCLUSION

From the above review of the scientific and clinical data, it becomes evident that the HDL particles possess potent cardioprotective biological functions. In addition to their effect in the facilitation of RCT, the HDL

particles also possess cytoprotective, anti-inflammatory, antioxidant, antiaggregating and anticoagulant properties, which enhance their protective effect against CVD. These cardioprotective properties of HDL are not solely dependent on the HDL plasma concentration but also depend on HDL functionality. This was confirmed in a large meta-analysis of 108 randomized trials involving 299,310 participants at risk for CVD. In this meta-regression analysis, it was clearly shown that simply increasing the serum levels of HDL-C does not lower the risk of coronary heart disease events, coronary heart disease deaths, or total deaths^[44]. On the other hand, there is extensive evidence that under certain conditions, such as the oxidative environment of the acute-phase response, the HDL particles may lose their anti-inflammatory properties and become pro-inflammatory and pro-atherogenic. Thus, future therapeutic agents targeting HDL may be required to enhance HDL functionality rather than simply increase HDL-C concentration.

DECLARATIONS

Authors' contributions

Conception and design, jointly developed the structure and arguments for the paper, made critical revisions and approved final version: Kosmas CE

Wrote the first draft of the paper: Kosmas CE, Silverio D

Contributed to the writing of the paper: Sourlas A, Montan PD, Guzman E

Data collection, analysis and interpretation, final approval of manuscript: Kosmas CE, Silverio D, Sourlas A, Montan PD, Guzman E

Availability of data and materials

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Consent for publication

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Review

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Low density lipoprotein-induced lipid accumulation is a key phenomenon of atherogenesis at the arterial cell level

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Abstract

Lipid accumulation in cells of subendothelial intima and the formation of foam cells is the earliest and the most noticeable manifestation of atherosclerosis at the cellular level. Generally, the foam cell formation is the result of interaction of cell with pro-atherogenic low-density lipoprotein providing cholesterol delivery and anti-atherogenic high-density lipoprotein providing its efflux. In this review, we discuss possible mechanisms of foam cell formation, the role of intracellular lipid deposition as a trigger of atherosclerotic lesion development, current approaches to diagnostics and strategies for preventing atherosclerosis based on recent knowledge of causes of foam cell formation.

Keywords: Atherogenesis, atherosclerosis, foam cells, diagnostics, desialylation, low-density lipoprotein, subendothelial cells, therapy

INTRODUCTION

In most cardiovascular diseases epidemiology is related to atherosclerosis. As early as atherosclerotic lesions began to be studied under a microscope, it was found that the fundamental difference of atherosclerotic cells from normal ones is their foamy cytoplasm. This foamy structure is explained by numerous lipid inclusions in the form of fatty drops and granules that often fill the whole cell body and its processes. Lipid accumulation in cells of subendothelial intima and the formation of foam cells is the earliest and the most noticeable manifestation of atherosclerosis at the cellular level. At any stage, from the earliest microscopic



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changes to the advanced plaque, atherosclerotic lesion is characterized by the deposition of intracellular lipids (mainly cholesteryl esters) in arterial sub-endothelial cells^[1,2].

Generally, the foam cell formation is the result of interaction of cells with pro-atherogenic low-density lipoprotein (LDL) providing cholesterol delivery and anti-atherogenic high-density lipoprotein (HDL) providing its efflux. Foam cell formation through the very LDL receptor by remnant lipoprotein and lipoprotein(a)^[3] and through other ways has also been described. However, the main source of fat in the foam cells is plasma LDL^[4]. In this review, we discuss possible mechanisms of foam cell formation and the role of intracellular lipid deposition as a trigger of atherosclerotic lesion development. In addition, the objective of the review is to discuss current approaches to preventing the accumulation of lipids in arterial cells because there is a general consensus that inhibition of foam cell formation is an important strategy for preventing atherosclerosis.

MECHANISMS OF FOAM CELL FORMATION

The obvious close connection of atherosclerosis with foam cells led to the idea that lipid accumulation is a necessary condition or fundamental even of atherogenesis. The origin of foam cells is still a matter of controversy. Currently, the dominant view is that foam cells have an exclusively macrophage origin. However, in the sub-endothelial intima of human arteries, macrophages represent a minority in comparison with smooth muscle α -actin-positive cells (SMA+) that are typical smooth muscle cells and pericytes, however, even in the initial lesions, almost all cells contain lipid inclusions^[5]. Consequently, not all cells loaded with lipids are macrophages. According to the most recent data, in human coronary lesions, the proportion of smooth muscle cells is higher than 50% of total number of foam cells^[6]. Nevertheless, the culture of macrophages is widely used to study the mechanisms of foam cell formation, since the macrophage is a simple and convenient model. Taking into account the possible role of LDL in the accumulation of intracellular cholesterol, many research groups have attempted to induce the increase of cholesterol level in cultured cells by incubation with lipoprotein.

Modified LDL

In hyperlipidemia at high LDL levels, fluid-phase pinocytosis induces foam cell formation^[7,8]. However, generally native LDL does not cause lipid storage in cell cultures^[5,9,10]. On the other hand, considerable lipid accumulation was detected in the case of chemically modified LDL (acetylated, maleylated, succinylated, oxidized, etc.) or treatment with formaldehyde, malondialdehyde, phospholipase A2, C and D, etc.^[10,11]. Therefore, chemically modified LDL is able to trigger foam cell formation, hence it is atherogenic. This discovery initiated the search for a naturally occurring atherogenic modified LDL. Proceeding from the hypothesis of oxidative modification, it was the oxidized LDL that was searched for but it was not found in the blood, although some indirect signs of oxidation of circulating LDL were detected by detecting a marker for evaluating the LDL oxidation *in vivo*^[9,10]. A more reasonable way to detect the *in vivo* modified LDL, i.e., LDL particles capable of triggering lipid accumulation in cultured cells, was to search for such an atherogenic lipoprotein circulating in patients with atherosclerosis. In addition to oxidation, other forms of atherogenic modified LDL were discovered in the blood. Different groups at different times have found small dense LDL (sdLDL), electronegative or LDL(-), and desialylated lipoprotein particles circulating in the blood^[9,10].

It was found that LDL(-), sdLDL and desialylated LDL particles have many common characteristics and can all be oxidized^[5,9,10] [Table 1]. This led to the idea that all known forms of atherogenic modification of LDL can be present in the same lipoprotein particle, that is, there is multiple modification of LDL. In the blood, the same multiply modified LDL particles possess the properties of small dense, electro-negative, oxidized and desialylated lipoproteins^[5,9,10].

A mechanism of LDL multiple modification was proposed [Figure 1]. The hypothesis was tested under conditions imitating the situation *in vivo*. Native (unmodified) LDL particles were isolated from circulation

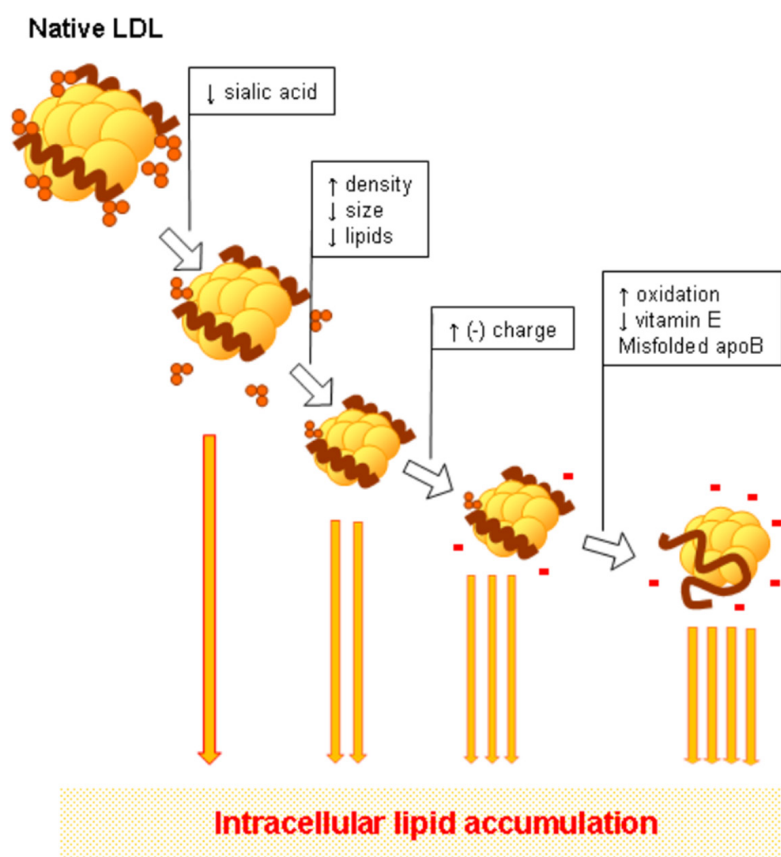


Figure 1. Cascade of multiple atherogenic modifications of low-density lipoprotein (LDL) particles. Adopted from^[9], with permission

of healthy subjects; and the serum was prepared from the blood of patient with atherosclerosis. Then the mixture of LDL and serum was incubated at 37 °C during 72 h.

After 1 h of incubation, sialic acid content of LDL decreased, and desialylated LDL particles appeared. LDL acquired the ability to induce accumulation of intracellular cholesterol; hence the lipoprotein particles became atherogenic. After 6 h, lipid content and LDL size were decreased. After 36 h, LDL became more electronegative. The incubation for 48-72 h caused a decrease in the content of antioxidants; in parallel, susceptibility of LDL to oxidation was increased, leading to the oxidation of the lipoprotein particles. Prolonged incubation led to degradation of apoproteins. The experiments described above allow us to conclude that desialylation is the first known modification that leads to the acquisition of atherogenic properties by LDL particles. Subsequent modifications, such as decrease of the lipid content and particle size, increase of the particle density and electronegativity further enhanced the LDL atherogenicity. Oxidative modification of lipoprotein particles occurs at the last stages of a cascade of events of sequential multiple modifications.

Despite the fact that LDL modification involves changes in different physical and chemical properties in lipoprotein particle, oxidation is still considered to be the main or even the only atherogenic modification. Currently, PubMed lists 9,765 publications indexed under “oxidized LDL”, and 4,728 under “oxidized LDL and atherosclerosis”. More than a thousand review articles on oxidized LDL have been published. It is generally accepted that oxidized LDL accumulating in the vascular wall triggers atherogenesis^[5,9,10]. Although oxidized LDL was detected in the vascular wall^[3], for a long time it could not be detected in the bloodstream. This gave rise to the idea that LDL oxidation does not occur in the blood but takes place in the vascular wall. There is no need for such an assumption since, as mentioned above, a marker for evaluation

Table 1. Characteristics of naturally occurring modified low-density lipoprotein discovered in circulation of atherosclerotic patients

Parameter	sdLDL	LDL(-)	Desialylated LDL
Intracellular cholesterol accumulation (atherogenicity)	↑	↑	↑
Size	↓	↓	↓
Density	↑	↑	↑
(-) Charge	↑	↑	↑
Sialic acid	↓	↓	↓
Cholesteryl esters	↓	↓	↓
Phospholipids	↓	↓	↓
Protein/lipids	↑	↑	↑
Oxidizability	↑	↑	↑
Oxidation	↑	↑	↑
Antioxidants	↑	↑	↑
Amino group modification	?	↑	↑
Self-association	↑	↑	↑

Adopted from^[9], with permission. LDL: low-density lipoprotein; sdLDL: small dense LDL; ↑: increased; ↓: decreased; ?: not known

of LDL oxidation was detected in the blood of atherosclerotic patients^[5,9,10]. Earlier, some signs of oxidation were found in LDL isolated from the blood^[5,9,10].

The following arguments are usually made in favor of the fact that oxidized LDL plays a key role in atherogenesis: (1) in the blood of patients, antibodies to malondialdehyde-LDL (MDA-LDL) were discovered^[5,9,10]; (2) antibodies obtained to *in vitro* oxidized LDL co-localized with LDL in histological sections of atherosclerotic lesions^[5,9,10]; and (3) LDL isolated from the artery wall possessed some characteristic properties of oxidized LDL^[12]. However, all these arguments can be questioned. First, MDA-LDL cannot be regarded as an adequate model of oxidized LDL. Usually, antibodies are produced against LDL modified *in vitro* with MDA or copper-induced oxidation. This modification leads to random and non-specific exposure of immunogenic sites different from those present in LDL oxidized *in vivo*. Second, antibodies isolated from the blood show cross-reactivity with MDA-LDL, naturally occurring multiply modified LDL and *in vitro* desialylated LDL. Anti-LDL antibodies possess the maximal affinity for desialylated LDL; the affinity for oxidized LDL is an order of magnitude lower^[5,9,10]. Thus, antibodies are produced primarily to the desialylated LDL. Third, although oxidized LDL is not detected in the blood, other forms of atherogenic modification (sdLDL, LDL- and desialylated LDL) are clearly detected in the circulating lipoprotein. Multiply modified atherogenic LDL circulating in the blood of atherosclerotic patients was detected, isolated and intensively studied^[5,9,10]. The presence of sdLDL in the blood is currently known and well-studied providing some impact on the anti-atherosclerotic therapy^[5,9,10]. The results of intensive study of LDL- have found implications in both diagnostics and therapy^[5,9,10]. Despite numerous attempts of clinical implementation of methods for determining MDA-LDL, they are still not widely used. Fourth, as mentioned above, modification of LDL in the plasma of atherosclerotic patients begins with desialylation followed by changes in lipid content, decrease in size and increase in density. At later stages, LDL becomes more electronegative. Only at the very end of this cascade of transformations, the lipoprotein acquires the properties of oxidized particle [Figure 1]. Thus, oxidation is neither the primary nor the only atherogenic modification of LDL.

Taking into account the key role of the multiply modified atherogenic LDL in the initiation and progression of atherosclerosis, it is reasonable to assume that atherosclerotic risks are dependent not as much on the total content of LDL in the blood as on the level of multiply modified LDL. In this regard, the level of multiply modified LDL should be a better biomarker of atherosclerosis than the total LDL level. Such a conclusion will be true for HDL because dysfunctional HDL has been detected in the blood of atherosclerotic patients.

To understand which modification plays the key role conveying atherogenic properties of a lipoprotein particle, we studied correlations between the changes in chemical and physical parameters of the multiply

modified LDL and its ability to induce intracellular cholesterol accumulation. The only statistically significant correlation was found between the atherogenic potential of LDL and sialic acid content of the particles. This correlation was reversed: the lower was the sialic acid content of LDL, the more cholesterol was accumulated by the cells. LDL parameters such as size, charge, lysine-free amino groups, phospholipids and neutral lipids content, fat-soluble antioxidants, lipid peroxidation products, oxidation and oxidation rates did not correlate significantly with LDL atherogenicity^[5,9,10]. Thus, desialylation appeared to be the most important modification inducing LDL atherogenicity. Presumably, the necessary and sufficient condition for the appearance of atherogenic properties in LDL particles is desialylation.

Studying of the mechanisms of LDL desialylation led to the discovery of trans-sialidase activity in the blood of atherosclerotic patients^[5,9,10]. It was present both in association with lipoproteins and in free form in blood plasma. The exact identity of the sialidase activity is not known, yet, however, LDL treated with isolated trans-sialidase lost sialic acid and became atherogenic causing the accumulation of cholesteryl esters in cultured cells^[5,9,10]. Thus, trans-sialidase found in the blood causes LDL-lipid desialylation, which leads to foam cells formation. Circulating trans-sialidase is a protein of about 65 kDa, whose content ranges from 20-200 µg/mL. Three optimum pH values were found: 3.0, 5.0 and 7.0. The enzyme activity was found to be stimulated by calcium and magnesium ions and dependent on the sulfhydryl groups. Donors of sialic acid for trans-sialidase are LDL, intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL), and HDL. Preferable substrate is LDL, but VLDL, IDL and the least HDL may also serve as substrate. It is likely that trans-sialidase plays a key role in the *in vivo* atherogenic modification of lipoprotein particles.

LDL association

The formation of different associates containing modified LDL considerably increase lipoprotein atherogenicity. There were found at least three ways of potentiating LDL atherogenicity: self-association of LDL particles, LDL association with the extracellular matrix components and formation of LDL-containing immune complexes.

Unlike native LDL, desialylated LDL particles are associated with spontaneously *in vitro* in cell culture conditions^[5,9,10]. A significant direct correlation was found between the atherogenicity of desialylated LDL and the degree of lipoprotein particles' association^[5,9,10]. If the LDL associates formed in culture are removed from the medium by filtration, this completely prevents the intracellular cholesterol accumulation. It was shown that increased atherogenicity of desialylated LDL self-associates is a result of increased uptake of lipoprotein particles by phagocytosis and a decrease in the rate of intracellular degradation^[5,9,10]. If the self-associates of LDL circulate in the blood of atherosclerotic patients or are formed in the arterial intima, then self-association is the actual mechanism of increasing the atherogenic potential of desialylated LDL.

Another mechanism of enhancing atherogenicity of desialylated LDL, namely, the formation of lipoprotein associates with the connective tissue matrix components has been found^[11]. It was reported that LDL forms associates with collagenase-resistant arterial debris, collagen, elastin and proteoglycans isolated from human aortic intima^[5,9,10]. All these associates caused cholesterol accumulation in cultured cells. On the other hand, LDL(-) does not induce the formation of foam cells but high susceptibility of LDL(-) to association is a factor that favors the formation of foam cells^[13].

Desialylation of LDL particles provokes the production of autoantibodies that form circulating immune complexes (CIC) containing LDL. Such CIC were detected in the blood of atherosclerotic patients^[5,9,10]. A correlation was found between the levels of LDL containing immune complexes in the blood serum and the severity of atherosclerosis in patients^[5,9,10]. It was found that LDL isolated from CIC is not only desialylated, but is also small, dense, more electronegative, and has decreased the content of neutral lipids, phospholipids and neutral saccharides, and is also characterized by conformational changes in the tertiary apoB structure. These findings allow considering LDL from CIC to be identical to multiply-modified LDL. LDL-containing

CIC potentiate the atherogenicity of desialylated LDL causing higher cholesterol accumulation in cultured cells^[5,9,10]. The addition of the complement component and fibronectin to the LDL autoantibody complex C1q resulted in a more pronounced accumulation of intracellular cholesterol. Thus, the formation of CIC is the actual mechanism for enhancing the atherogenic capacity of desialylated LDL. In addition to the accumulation of lipids, the CIC induces the secretion of pro-inflammatory cytokines and apoptosis of macrophages^[12] which can also be considered as pro-atherogenic effect. Recent studies of LDL containing CIC focus mainly on implementation into clinical practice for diagnostics (see below).

FOAM CELLS AND ATHEROGENESIS

Intracellular lipid accumulation leading to foam cell formation is the most noticeable and earliest manifestation of atherosclerosis. However, other manifestations are also generally recognized: (1) an increase in the proliferative activity of the leader leading to hypercellularity; (2) the enhancement of synthesis of the extracellular matrix, leading to the growth of connective tissue; and (3) the loss of intercellular contacts leading to rupture of the cellular network in the intima and detachment of intimal cells^[5,14]. Examined was the effect of circulating desialylated LDL on proliferative activity and synthesis of total protein, collagen and glycosaminoglycans by SMA+ cells cultured from uninvolved human aortic intima.

A 24 h incubation of SMA+ cells with native LDL had no effect on proliferative activity detected as [³H] thymidine incorporation into cultured cells^[5]. By contrast, addition of desialylated LDL subfraction leads to a 1.5- to 2-fold increase of proliferative activity.

The rate of synthesis of proteins secreted by cultured cells was evaluated by incorporation of [¹⁴C]leucine in the acid-insoluble fraction of culture medium. Native LDL had no effect on the synthesis of secreted proteins. Desialylated LDL stimulated protein syntheses by 1.5- to 2-fold^[5]. Moreover, desialylated LDL induced a 2-fold increase of collagen production, as estimated by incorporation of [¹⁴C]proline in the collagenase-released fraction of culture medium^[5]. It was also demonstrated that desialylated LDL, but not native LDL, stimulates the incorporation of [¹⁴C]glucosamine in the total glycosaminoglycan fraction of human SMA+ cells^[5]. So, in contrast to native LDL, desialylated LDL enhanced synthesis of connective tissue matrix components.

It was shown that: (1) desialylated LDL-induced lipid accumulation is sufficient to enhance proliferative activity and stimulates the incorporation of precursors of the extracellular matrix components; (2) insoluble complexes of native LDL with naturally occurring (collagen, elastin, fibronectin) and artificial (latex particles, dextran sulfate) compounds induce intracellular lipid accumulation and stimulate proliferative and synthetic activities; and (3) the increase in proliferative and synthetic activities correlates with the amount of accumulated intracellular cholesterol^[5]. In addition, after incubation of SMA+ cells with desialylated LDL, intercellular communication through gap junctions was dropped considerably^[14]. These findings indicate that intracellular lipid accumulation might be a reason for the disintegration of cellular network observed in atherosclerotic lesions.

Thus, foam cell formation induced by desialylated LDL causes enhanced proliferative activity, the synthesis of the connective tissue matrix components and also breaks intercellular communication. Therefore, desialylated LDL can induce all known atherosclerotic manifestations at the cellular level.

The interaction of atherogenic modified LDL with macrophages causes a pro-inflammatory response which, if unfavorable, develops into a local chronic inflammation characteristic of atherosclerotic lesions. In primary human monocyte-derived macrophages, modified LDL-induced cholesterol accumulation is accompanied by upregulation of genes encoding pro-inflammatory molecules^[15]. It is unclear whether foam cell formation induces a pro-inflammatory response or pro-inflammatory response promotes cholesterol accumulation. It was demonstrated that local cellular responses to oxidized LDL may stimulate pro-

inflammatory or anti-inflammatory pathways^[16]. Oxidized LDL loading of macrophages negatively regulates pro-inflammatory gene expression and implicates epigenetic mechanisms such as histone deacetylase activity^[17]. On the other hand, an increase in circulating levels of IL-17 has been demonstrated in patients with cardiovascular disease, as well as its high expression in atherosclerotic lesions, suggesting that IL-17 could affect cell targets such as macrophages in atherosclerotic lesion. In this sense, it has been shown that IL-17 alone induces few foam cells^[18].

FACTORS AFFECTING THE FORMATION OF FOAM CELLS

Once the ability of atherogenic modified LDL circulating in the blood of atherosclerotic patients to cause lipid accumulation in cultured cells (atherogenicity or atherogenic potential) has been found, a natural desire to check agents that potentially could influence the foam cell formation arose. Interest was motivated by the fact that the detection of an agent preventing the accumulation of intracellular lipids could be considered as the discovery of a potential anti-atherogenic drug. Hundreds of different substances were tested. Among them, those that suppressed the accumulation of lipids in cultured cells, and those that increased the atherogenic potential of LDL were detected; there were also such agents that had no effect^[19].

An important part of the most recent works devoted to foam cell formation is associated precisely with the search for modulators of intracellular lipid accumulation. Ceramide generated as a result of aggregated LDL catabolism in atherosclerotic plaques activates macrophage RhoA^[20]. RhoA activation plays a significant role in macrophage RhoA/Rho kinase signaling that decreases aggregated LDL degradation and foam cell formation by reducing local actin polymerization required for catabolism. This may be regarded as a possible anti-atherosclerotic effect. Proline/serine-rich coiled-coil 1 overexpression reduced foam cell formation through decrease of intracellular cholesterol and increase of cholesterol efflux by upregulating the expression of peroxisome proliferator-activated receptor γ and liver X receptor α ^[21].

A flavonoid from the *Morus alba* L., Kuwanon G, inhibits both cholesterol accumulation and inflammation reaction in macrophages stimulated by oxidized LDL^[22]. Observed effects are realized through enhancing LXR α -ABCA1/ABCG1 pathway and inhibiting NF κ B activation. Anti-atherosclerotic effect of Kuwanon G was confirmed *in vivo*. In the plaque of high-fat diet fed ApoE^{-/-} mice, Kuwanon G significantly reduces the atherosclerotic areas and macrophage content. Nuclear factor erythroid 2-related factor 2 (Nrf2) prevents foam cells formation^[23]. On the other hand, the loss of Nrf2 in macrophages enhances foam cell formation and promotes early atherogenesis. Isolated from the root of *Salvia miltiorrhiza* Bge., Tanshindiol C, an activator of Nrf2 in macrophages, markedly suppresses foam cell formation induced by oxidized LDL^[24]. Liver kinase B1 suppresses foam cell formation and atherosclerosis development. On the contrary, down-regulation of liver kinase B1 in macrophages results in such atherogenic manifestations as increased uptake of modified lipoproteins, increased foam cell formation, and, finally, increased atherosclerosis^[25].

Antibodies to specific epitopes of oxidized LDL suppress both inflammatory cytokine production and foam cell formation^[26,27].

Endogenous human vascular endothelial growth factor (VEGF) inhibits foam cell formation. VEGF-treated macrophages significantly decreased lipid accumulation caused by oxidized through down-regulation of CD36 expression^[28].

The studies of agents that promote foam cell formation provide novel insights into their pro-atherogenic effects under pathological conditions and suggests that their inhibiting may represent a new approach for treating atherosclerosis. The following is a couple of examples of such studies. Hypoxia-inducible lipid droplet-associated protein was shown to be highly expressed in atherosclerotic foam cells in human and

murine plaques. It was established that lipid droplet proteins can promote atherogenesis being critical to the foam cell formation and lipid deposition^[29]. Lysophosphatidic acid (LPA) is a bioactive phospholipid produced by activated platelets that is formed during the oxidation of LDL. The formation of foam cells is significantly enhanced by LPA alone through upsetting the imbalance between lipid uptake and efflux^[30].

CLINICAL APPLICATIONS

The results obtained in clinical studies did not always correspond to the theoretical understanding of the role of LDL in atherogenesis. As follows from the concept of evidence-based medicine, the mechanistic role of biomarkers should be confirmed by clinical studies (trials and surveys)^[31]. In other words, the modulation of the biomarker should affect the established endpoints. In the case of atherosclerotic diseases, such endpoints are: fatal and non-fatal cardiovascular events, angina pectoris, revascularization, fatal and non-fatal myocardial infarction, stroke, etc. When talking about the direct relationship with atherosclerosis, endpoints often use surrogate instrumental methods, namely angiography of coronary arteries, ultrasound imaging of the carotid intima-media thickness and, less commonly, calcification of the coronary arteries.

Diagnostics

Unfortunately, modified LDL is not used in American and European recommendations and guidelines for reducing the risk of developing atherosclerotic cardiovascular diseases^[32-34]. This is the result of the fact that the available data from clinical studies do not allow us to justify the use of modified LDL and HDL as mechanistic biomarkers of atherosclerotic disease. Apparently, the results of such studies would be more encouraging if atherogenic multiply modified LDL and dysfunctional HDL were considered as pharmacological targets and biomarkers.

The difficulties in verifying new biomarkers are related to the complexity of interaction of different markers and risk factors. In addition, against the background of usual risk factors, new biomarkers are often not supported by evidence of their ability to contribute to the assessment of atherosclerotic risk and possess no significant diagnostic or prognostic role^[35-38]. Currently, numerous traditional and experimental biomarkers are considered to assess atherosclerotic risk. Along with lipid markers, which include modified LDL, dysfunctional HDL and apolipoproteins, non-lipid markers are considered. Non-lipid biomarkers include inflammatory molecules, namely fibrinogen and a highly sensitive C-reactive protein. Lipoprotein-associated phospholipase A2 and homocysteine are considered as thrombotic markers. Other indexes are glucose metabolism markers and organ-specific markers.

Clinical studies of modified LDL as a biomarker of atherosclerosis have certain limitations. In particular, the use of the term “oxidized LDL” causes confusion. To measure the allegedly “oxidized” LDL in clinical trials, antibodies against MDA-LDL are used. Such an indicator cannot be called oxidized LDL. It is clear that in this case it is possible to evaluate the clinical significance of some indicator whose physical meaning is difficult to interpret. Autoantibodies against LDL found in the blood of atherosclerotic patients cross-react with MDA-LDL, multistage modified LDL and desialylated LDL^[39]. Moreover, these autoantibodies possess the highest affinity with desialylated LDL while they did not distinguish between native and Cu²⁺-oxidized LDL. Therefore, MDA-LDL to some extent evaluate the desialylated and not oxidized LDL.

Since it was impossible to directly demonstrate the presence of oxidized LDL in the bloodstream, the term “minimally oxidized LDL” has been introduced. This should imply some hypothetical particle that cannot be isolated or characterized by existing methods, but which actually exists penetrating the artery wall, undergoing further modification, and triggering atherosclerosis^[40]. Unfortunately, such “pure faith” is widespread and is not subject to revision.

As a result of experimental or clinical studies of oxidized LDL, both indirect and direct oxidation indices can be obtained. Indirect indices are based on the measurements using anti-MDA-LDL or anti-copper-

oxidized LDL-antibodies. The limitations of these approaches were discussed above. Direct markers of oxidation are based on evaluating oxidized lipids including phospholipids. A non-pathological retard in LDL catabolism which is reflected in the appearance of senescent LDL in the blood may be a limitation of this approach. It should be noted that the clinical studies did not examine the mechanistic significance of the modified LDL in atherogenesis but tried to position it as a biomarker. Admittedly, these attempts were unsuccessful and did not lead to definitive conclusions about the sensitivity and specificity of modified LDL.

LDL(-) as a diagnostic parameter attracts the attention of several groups^[41]. There are certain successes in the implementation of this indicator in clinical practice^[42].

The ability of atherogenic modified LDL to cause the accumulation of cholesterol in cultured cells could be used to create a diagnostic test system for assessing the atherogenicity of lipoproteins. However, the cellular test is not a useful biomarker for implementation in clinical practice. A search for more adequate tests was conducted. It was found that the LDL level in the CIC very closely correlates with the value of LDL atherogenicity estimated in the cellular test^[43-45]. The most successful were attempts to introduce into the clinic such a parameter as the content of LDL in the CIC. The association between progression of cardiovascular diseases and high levels of cholesterol in precipitated CIC has been found^[46,47]. The high diagnostic and prognostic significance of this parameter was also demonstrated for carotid and coronary atherosclerosis^[43-45,48,49].

Therapy

The mechanistic role of oxidized LDL as an effector of atherosclerosis could be confirmed by clinical trials using antioxidants. To the complete disappointment of supporters of the oxidative theory, randomized trials have shown a lack of risk reduction or even an increased risk of atherosclerosis in patients receiving antioxidants^[50]. Despite attempts to explain the negative results, the topic of oxidized LDL in atherosclerosis was closed. Unfortunately, along with this, the interest to general problems of modified LDL was lost at all. As a result, the American Heart Association did not recommend antioxidants for the prevention of atherosclerotic diseases^[51] although the question on modified LDL as a mechanistic factor or biomarker of atherosclerosis remains open.

Over the past two decades, data demonstrating the non-linear dependence of the reduction in the risk of atherosclerosis from the therapeutic reduction in total LDL level are widely discussed^[52-61]. Figure 2 clearly shows that the clinical manifestations of atherosclerosis are due not only to the total level of LDL in the blood. It is necessary to conduct similar studies with measuring the level of modified LDL in order to evaluate its value as a mechanistic effector.

In contrast to the outdated concepts of the total level of circulating LDL as a cause for the formation of atherosclerotic lesion, it seems more reasonable the idea that the key initiating event in atherogenesis is the retention, or trapping, of LDL within the arterial wall^[62,63]. The paradigm of the key role of LDL and foam cells in atherogenesis gave rise to the concept of cellular cholesterol retention^[64,65]. Cell models have been developed to evaluate the effect of various agents on the retention of cellular cholesterol caused by atherogenic modified LDL^[66].

In the *in vitro* model, primary cultures of human aortic cells or human monocyte-derived macrophages are used for the screening of potential drugs, the investigation of their mechanisms of action, and the optimization of anti-atherosclerotic drug therapy. Cells of the subendothelial intima isolated from atherosclerotic lesions retain all major characteristics of atherosclerotic cells when cultured. Many cells cultured from atherosclerotic lesions are so-called foam cells, which contain numerous inclusions, likely lipid droplets, that fill the entirety of the cytoplasm^[67]. The bulk of excess lipids in foam cells consists of free

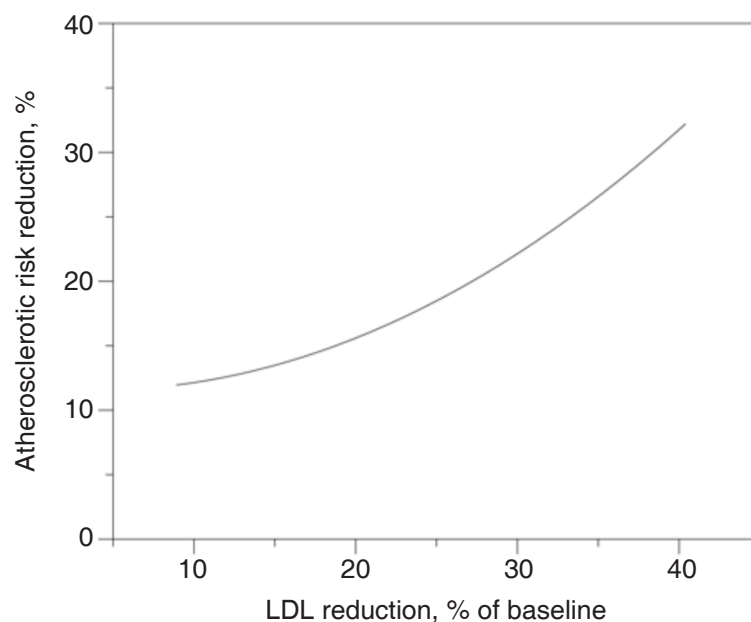


Figure 2. Consensus relationship between treatment effect on the low-density lipoprotein (LDL) level and the reduction in the risk of atherosclerosis-related cardiovascular events. Adopted from^[73], with permission

cholesterol and cholesteryl esters^[67]. It should be noted that the content and composition of lipids in cultured cells within the first 10-12 days in culture remain unchanged and correspond to the respective indices of freshly isolated cells^[67,68].

In the *ex vivo* model, instead of drugs, blood sera taken from patients after oral drug administration is added to cultured cells. Cell cultures can be employed in an *ex vivo* model to examine an indirect anti-atherogenic action of a drug and to optimize anti-atherosclerotic (anti-atherogenic) drug therapies. The cellular models can be used not only to test drugs but can also be used to test foodstuffs as well. Anti-atherosclerotic (therapeutic, causing regression of atherosclerosis) and anti-atherogenic (preventive) activities of many agents were investigated. This approach will be useful in the development and optimization of anti-atherosclerotic and anti-atherogenic dietary therapies. The anti-atherogenic effects of dietary products promote the development of anti-atherosclerotic therapies based on natural products. Atherosclerosis develops over many years, so anti-atherosclerotic therapies should be long-term or even lifelong. For such long-term therapies, conventional medicine will not work. Drugs based on natural products can be a good alternative. Among the anti-atherogenic natural products, the most effective was garlic. Two-year treatment with Allicor (garlic powder) has a direct anti-atherosclerotic effect on carotid atherosclerosis in asymptomatic men^[69]. Inflaminat (calendula, elder and violet), which possesses anti-cytokine activity, caused the regression of carotid atherosclerosis in a 1-year treatment of asymptomatic men^[70]. The phytoestrogen-rich drug Karinat (garlic powder, extract of grape seeds, green tea leaves, hop cones, β -carotene, α -tocopherol and ascorbic acid) prevented the development of carotid atherosclerosis in postmenopausal women^[71].

Although some herbal preparations are recommended by the FDA; unfortunately, natural products that possess anti-atherosclerotic therapeutic potential are not prescribed by medical practitioners as anti-atherosclerotic agents. However, the potential of these substances allows us to consider them as mainline additional supplements or prescriptions^[72].

CONCLUSION

The accumulation of intracellular lipids leading to foam cell formation is induced by modified LDL. This is a fundamental event in the genesis of atherosclerotic lesions. It is the accumulation of intracellular lipids that

triggers atherogenesis at the cellular level. This knowledge should lead to the development of fundamentally new approaches to the diagnosis of atherosclerotic diseases, but unfortunately extensive research in this direction is not carried out. On the other hand, new developments based on foam cells and LDL were translated into clinics as therapeutic tools. Using cell models and natural products, an approach has been developed to prevent the formation of foam cells. This is an example of how the results of basic research have been successfully translated into clinical practice. Drugs with direct anti-atherosclerotic activity have been developed. These drugs cause regression of atherosclerosis and/or prevention of its progression in patients.

DECLARATIONS

Authors' contributions

All authors contributed equally to the article.

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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Review

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Bridging aortic valve surgery to 21st century: what can a surgeon do?

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Abstract

Aortic valve stenosis is the most clinically relevant valvular heart disease in the elderly. Surgical aortic valve replacement (SAVR) represented, for decades, the standard of care for the treatment of severe aortic stenosis. Although SAVR still represents a valid option in this clinical scenario, transcatheter aortic valve implantation proved to be superior to medical therapy and comparable to SAVR in several randomized trials in patients at high or intermediate operative risk. At the same time, the growing aging population carrying on greater morbidities and high risk profiles has led to the development of minimally invasive technologies, as rapid deployment aortic valve replacement or Sutureless, to minimize surgical impact on patients. The Heart Team is nowadays tasked to determine the best option tailored for each patient considering patient-related factors and mastering all the surgical options in terms of both different techniques and types of available valves. Nevertheless, some open issues need to be already answered as: which has the longest durability, which the lower complication rate and the lower overall mortality. The aim of this review is to briefly resume



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the main features of these different options and explore what kind of open questions these newer-generation prosthetic valves and delivery devices carry.

Keywords: Aortic valve surgery, aortic valve stenosis, cardiac surgery, sutureless, transcatheter aortic valve implantation, new-generation devices, minimally invasive technologies

INTRODUCTION

Aortic valve stenosis is the most clinically relevant valvular heart disease in the elderly people with a prevalence of 21%-26% in the elderly above 65 years of age and it increases with age, determining prognosis worsening after symptoms occurrence^[1]. Surgical aortic valve replacement (SAVR) has represented, for decades, the standard treatment for patients with symptomatic and severe aortic stenosis, resulting in relief of symptoms, in a significant improvement of clinical outcome and in an improved survival. Although SAVR still represents a valid option in the setting of aortic valve stenosis, transcatheter aortic valve implantation (TAVI) proved to be superior to medical therapy and comparable-non inferior to SAVR in several randomized trials as well as in registries^[2-4]. Following its introduction in 2002, with the first case performed in Rouen by Cribier *et al.*^[2], TAVI has become an established treatment for patients with severe symptomatic aortic stenosis deemed inoperable or at high risk for conventional surgery. About 300,000 procedures have been performed worldwide with the first and second-generation CE-marked devices: Medtronic CoreValve® (Medtronic, Minneapolis, MN, USA) and Edwards SAPIENTM/SAPIEN XTTM (Edwards Lifesciences, Irvine, CA, USA) with an annual compound growth rate of 40%^[5]. Due to an overall increased experience and the progressing technology in transcatheter valve systems^[6], TAVI has been proposed and used in patients who are at intermediate and even low risk. The analysis of the Cohort A of the randomized trial PARTNER 2 showed that TAVR was non-inferior to surgical aortic-valve replacement in terms of primary end-point of death or disabling stroke^[7]. Therefore, recently published European guidelines^[8] has reinforced TAVI recommendation in intermediate risk patients (class Ib, level of evidence B). Moreover the growing aging population, characterized by greater co-morbidities and risk profiles has led to the development of minimally invasive technologies^[9] to reduce surgical impact on patients. An increasing number of surgeons are now endorsing minimally invasive aortic valve replacement through the sutureless valve technology (or rapid deployment valve). With this new emerging technology, TAVI, reasonable issues arise in comparison with surgical techniques and need to be answered: (1) which has the longest durability; (2) which encompasses the lower complication rate; and (3) the lower overall mortality.

SURGICAL AND INTERVENTIONAL APPROACHES

Patients usually can be scheduled to undergo a SAVR through conventional full midline sternotomy or mini-access according to the surgeon's discretion and/or patients characteristics. In these two settings, patients can receive either a conventional stented or sutureless prosthesis. Compared with conventional surgery, minimally invasive access can provide shorter hospital stay, improve postoperative respiratory function and reduces postoperative pain, blood loss and blood transfusions thanks to the lower invasiveness. Commons minimally invasive approaches are: the partial upper ministernotomy, the right anterior minithoracotomy, the right parasternal approach from the second to the fourth costal space and the transverse sternotomy. Surgical approach, minimally invasive or not, still represents the standard of care for several reasons: it has the longest follow up, it can be performed in younger patients, in patients with intermediate-low risk profile, in patients requiring combined cardiac procedures or a redo operation. Open-heart surgery allows controlled and accurate decalcification of aortic annulus and consequently a safe valve positioning under a direct visualization and with a major leaks control. In specific condition, also an aortic root enlargement can be performed with this approach. On the other hand, standard surgical intervention is a time-consuming procedure in term of cardio-pulmonary bypass (CPB), cross-clamp and myocardial ischemia times. Patients

at higher risk can easily experience the so-called CPB side effects embracing a sequelae of side effects involving several organs and apparatus^[10]. Alternative minimally invasive options has led to “sutureless” or rapid deployment aortic valves (RD-AV), which avoid the placement and tying of sutures finally leading to shorter CPB time. Sutureless valves are biological pericardial prostheses that can be anchored to the aortic annulus with only three sutures. Three different prostheses has to date, been developed: 3F Enable (Medtronic, Minneapolis, USA - CE approval withdrawn), Perceval S (Sorin, Saluggia, Italy), and Intuity Elite (Edward Lifesciences, Irvine, USA). The 3F Enable and Perceval S sutureless prostheses have a nitinol metal frame and can be deployed and positioned with no sutures in the case of Perceval S valves and only one suture for the Enable 3F valves. The Intuity rapid deployment aortic valve prosthesis has a cloth-covered frame and it is implanted through a balloon catheter delivery system that expands the frame once it achieved the appropriate annular position. Three sutures are required in the case of the Edwards Intuity valve. This technique may lead to shorter cross-clamp and CPB times, with a smaller amount of related adverse side effects, shorter in-hospital stay and similar survival rates as compared with conventional AVR. Actually, the procedure time is operator-dependent and relates to the specific operator learning curve. The prosthesis is usually landed in an intranular position and this may lead to better hemodynamic performance. RD-AV need careful patient selection since they need symmetric sinus configuration for adequate fitting in order to avoid paravalvular leakage and they are not recommended in bicuspid aortic valves. On one hand, it is not recommended to entirely decalcify the aortic annulus since it can be useful for the anchorage and likewise it avoids paravalvular leaks; on the other hand, heavily calcified sinutubular junctions may require special caution in sizing and deployment stages. Risk of stroke still represents an open issue since there are no specific recommendations regarding anticoagulation regimen after RD-AV implantation and no data are available on the risk of thrombus formation because of the stent frame and leaflet designs.

Greater morbidities and high-risk profiles on the contemporary patient population have driven the development of percutaneous TAVI, delivered in a micro-invasive fashion, with no need of CPB neither cardioplegic induced cardiac arrest. TAVI procedures have dramatically increased worldwide even if this data did not result in a decrease of overall SAVR performed compared with prior years^[11]. This means that a consistent part of patients currently treated with TAVI could not benefit any surgical options in the previous years, the so called “pre-TAVI era”. Although it proves to be a good and safe technique in patients at intermediate - high risk, there are several factors to consider for eligibility as: (1) native annulus size, since < 18 mm or > 29 mm precludes this procedure; (2) cardiac anatomy, necessary in the choice of implant and method of delivery; (3) left ventricular outflow size and shape; (4) porcelain aorta or horizontally placed aortic root, that may complicate a transfemoral delivery; (5) height of coronary artery ostia, to prevent incidental occlusion of the coronaries; (6) assessment of the peripheral arterial vessels, in terms of diameter, tortuosities, kinks, preexisting stents, aneurysms or thrombi.

In this setting, it is clear how an appropriate patient selection is the key of success for a good outcome. Nevertheless, there are still open issues in terms of possible complications that deserve to be considered: (1) risk of malpositioning, since some models can not be repositioned, retrieved or resheathed; (2) risk of annular rupture because of oversizing or over dilatation, even if this is an experience-related complication more than a limit of the technique itself; (3) atrioventricular conduction abnormalities requiring a postoperative permanent pacemaker (PPM), this complication ranges between 3%-8%^[11]; (4) paravalvular leaks; (5) risk of stroke; (6) vascular and bleeding complications; (7) limited durability.

Due to their multifaceted aspects, requiring several different evaluations by several different professionals, in the last years a new concept has emerged and gained increasing attention in the context of several procedures like complex coronary interventions and TAVR: the Heart Team.

The “Heart Team” should play a pivotal role in decision making, since most procedures entail a complex interplay of multispeciality & multi modality skills such as in the case of TAVI. The Heart Team is thus

Table 1. Types of prosthesis available and main features

	TAVI							RD-AVR			
Models	SAPIEN XT Edwards	SAPIEN 3 Edwards	Corevalve Medtronic	Evolut R Medtronic	Evolut PRO Medtronic	Allegra NVT	Jena Valve JenaValveTech	Portico St Jude Med	Lotus Boston Sc.	Perceval LivaNova	Intuity Edwards
Approach	Femoral Trans apical Trans aortic	Femoral Trans apical Trans aortic	Femoral Suxlavian/ axillar Trans aortic	Femoral Suxlavian/ axillar Trans aortic	Femoral Suxlavian/ axillar Trans aortic	Femoral	Transapical	Femoral	Femoral	Trans aortic (Full sternotomy or mini-invasive fashion)	Trans aortic (Full sternotomy or mini-invasive fashion)
Introducer	16, 18, 20	14, 16 Fr	18, 20 Fr	14, 16 Fr	16 Fr	18 Fr	32 Fr	18 Fr	18, 20 Fr	--	--
Deployment	Balloon	Balloon	Self Exp	Self Exp	Self Exp	Self Exp	Self Exp	Self Exp	Balloon	Self Exp	Balloon
Valve sizes	23, 26, 29	23, 26, 29	26, 29, 31	23, 26, 29, 34	23, 26, 29	23, 27, 31	23, 25, 27	23, 25	23, 25, 27	S, M, L, XL (19-27 mm)	19-27
Recaptable	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes No Recommend	No
Valve in valve	Yes	Yes	Yes	Yes	Yes	No data	No data	Yes	Yes	--	--

TAVI: transcatheter aortic valve implantation; RD-AVR: rapid deployment aortic valve replacement

tasked to determine the best option for each patient considering many patient related factors and mastering all the surgical options both in terms of different techniques and types of valves available. Hereafter we offer a summing up table of all the current options as listed below [Table 1].

DISCUSSION

Conventional surgical treatment of aortic valve stenosis still represents the standard of care that can be performed with excellent outcomes. The growing aging population, the increasing number of comorbidities and the higher score risk of a consistent part of patients have led to the introduction of novel opportunities both as interventional and as surgical approaches [TAVI vs. rapid deployment aortic valve replacement (RD-AVR)]. The decision to schedule one of these options should be based on a multilevel evaluation that includes the assessment of patients' frailty, anamnesis, anatomy and degree of atherosclerosis of the aorta and peripheral vessels. Data available on prostheses' hemodynamic performance and patients' clinical outcomes play a crucial role in the decision process of both, type of procedure and device selection. Over the latest years, many comparative studies and meta-analysis are emerging on this argument.

Thanks to the analysis of the Cohort A of the randomized trial PARTNER 2^[7], it is now clear that TAVI procedure is non-inferior to surgical standard aortic valve replacement in terms of primary end-point of death or disabling stroke in intermediate risk patients. In particular, transfemoral TAVI procedure showed less mortality rate than SAVR, compared to transapical TAVI that showed the same mortality rate and the same kind of complications^[7,12]. Also a previous work of our group showed a similar survival after transcatheter or SAVR^[13]. Takagi *et al.*^[14] analyzed 8 studies comparing SAVR and TAVI in 4244 patients from 2010 and 2015 and compared results in terms of gained left ventricular ejection fraction (LVEF) and left ventricular mass (LVM). This meta-analysis suggests greater LVM improvement after AVR, which may be due to higher incidence moderate aortic regurgitation after TAVI. LVEF improvement seems to be the same between TAVI and SAVR; but in patients with low baseline LVEF (< 40%) the improvement may be grater after TAVI than after SAVR. Witberg *et al.*^[15] made a systematic review and meta-analysis on the relative risks and benefits of TAVR vs. SAVR in patients who are at low surgical risk. TAVI and SAVR resulted equivalent in short-term mortality but, in intermediate term mortality, TAVI showed increasing mortality rates compared to SAVR suggesting that TAVI should not be performed in this population. The analysis of TAVI in the low surgical risk population (Shot Term Risk Calculator - STS < 4%) is currently under evaluation by three randomised controlled trials (RCTs): the PARTNER 3 (NCT02675114), Medtronic

transcatheter aortic valve replacement in low risk patients (NCT02701283), and NOTION 2 (NCT02825134) trials^[16]. Tam *et al.*^[17] conducted a cost utility analysis comparing TAVI and SAVR in intermediate risk patients with severe aortic stenosis and TAVI appeared to be also a cost - effective treatment not only in terms of absolute value but also in terms of perspective quality of life and re-hospitalization. Villablanca *et al.*^[18] published a meta-analysis on long-term outcomes of TAVR vs. SAVR. Their analysis confirms the findings from RCTs about similar longterm mortality between SAVR and TAVR. TAVR showed higher incidence of PPM implantation, residual aortic regurgitation, and vascular complications; SAVR showed higher incidence of myocardial infarction. Incidence of stroke, atrial fibrillation and acute kidney injury were lower with TAVR, especially in the high-risk population. Lastly the risk of PPM implantation is similar in intermediate-risk patients between both TAVR and SAVR.

Data published on the comparison between RD-AVR and SAVR underline the benefits of RD-AVR in terms of operative time reduction, CBP duration and increased effective orifice area and consequent lower post-operative transvalvular gradient^[19]. The most common complications reported in the case of RD-AVR are higher incidence of pacemaker implantation, postoperative stroke and residual aortic regurgitation^[20], while the most common complications reported in the case of standard procedure tend to be exclusively surgery related as major bleeding or acute renal failure^[21]. Totally in contrast to these previous studies, the German Aortic Valve Registry (GARY) recently analyzed a total of 22,062 patients who underwent isolated SAVR using SAVR or RD-AVR between 2011 and 2015^[22]. GARY analysis demonstrated that the advantages carried by RD-AVR may not translate into effective benefits. Patients currently undergoing SAVR are at low - intermediate surgical risk, with consequent low expected complication rates, so low pacemaker rate is still a strong argument in favor of SAVR. According to the authors, there are no reasons for choosing RD-AVR and increasing the risk of a post-operative PPM implantation. In contrast to the previous studies, this analysis also showed significantly elevated post-operative transvalvular gradients in sutureless valves, independently of the implanted valve sizes. At last, many data exist regarding comparison between RD-AVR and TAVI even if they do not always stratify patients for surgical scoring risk, in consequence TAVI patients tend to have higher scores. Meco *et al.*^[23] made a metanalysis of 6 studies including 1,462 patients (RD-AVR 731 vs. TAVI 731) with similar operative risk (Euroscore1: 15.45 ± 9 RD-AVR vs. 15.58 ± 8.1 TAVI). Thirty days all cause mortality and complications as stroke, paravalvular regurgitation, vascular complications were significantly lower in RD-AVR. The rate of acute kidney injury and pacemaker implantation were similar. RD-AVR group required more transfusion. Mid term survival rates (at 1 or 2 years) were significantly better in RD-AVR. SAVR using sutureless valves may be associated with better early and mid-term outcomes compared with TAVI in high- or intermediate-risk patients; the authors found a 50% risk reduction in early all causes of death and a 65% and 62% risk reduction in 1- and 2-year mortality for TAVI^[23]. Recently Shinn *et al.*^[24] provided a meta analysis including 7 observational studies comprising 617 RD-AVR and 621 TAVI patients: early mortality was 2.5% and 5% respectively, post procedural paravalvular leak was lower in RD-AVR and post procedural stroke and need for pacemaker implantation were comparable between the two cohorts.

From several studies, it appears that post operative need for pacemaker implantation is similar in both techniques, TAVI seems to have lower transvalvular gradients but more common peri-prosthetic leaks and, in the end, RD-AVR seems to have lower mortality rates^[25]. Finally, a large study from D'onofrio *et al.*^[26] observed 2,177 patients (1,885 TAVI vs. 292 RD-AVR): they found similar incidence of 30-day and 1 year mortality rates, stroke, bleeding and myocardial infarction. Patients treated with TAVI showed less device success and more postoperative perivalvular leak, even if this was less evident in trans apical procedures. RD-AVR resulted in higher transaortic gradients, longer post operative length and similar pacemaker implantation rate^[26].

CONCLUSION

The Heart Team is nowadays tasked to determine the best option for each patient considering patient related factors and cost effectiveness. The choice between surgical AVR vs. TAVR is based on multiple factors

including the surgical risk, patient frailty, comorbidities and also patient preferences. Since 2012, TAVR indications have been extended into groups of patients who are at intermediate to high risk; TAVR has also become the alternative to reoperation for those with bioprosthetic aortic valve degeneration. There are still few data on TAVI for patients < 75 years of age and for surgical low-risk patients, in whom SAVR remains the preferred approach since long-term durability data for TAVI prosthetic valves are still lacking. Moreover, younger patients often carry a bicuspid valve disease and this anatomic pattern may affect the results of TAVI. The past above cited trials excluded bicuspid valve patients. Surgical approach still plays a crucial role in all the combined procedures as concomitant severe coronary artery disease, concomitant ascending aorta disease or concomitant mitral and tricuspid valve disease. On the other side, longer follow up for both TAVI and RD-AVR are going to be needed, not only for the effective durability, but to clear risk of paravalvular leaks and pacemaker requirement, since they are likely to have greatest impact on low-risk and younger populations on life expectancy and they are currently being investigated in randomized trials. Another open issue is represented by anticoagulation regimen, since no specific recommendations exist on it and no data are available on the risk of thrombus formation because of the stent frame and leaflet designs.

In the end, the approval of a TAVR valve for lower STS SAVR risk population does not mean, by now, that TAVR is going to be the chosen procedure for any patient but this would certainly promote and encourage the scientific debate and future researches.

DECLARATIONS

Authors' contributions

All authors contributed equally.

Availability of data and materials

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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.

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Original Article

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MtDNA mutations linked with left ventricular hypertrophy

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Abstract

Aim: In left ventricular hypertrophy (LVH), the heart muscle thickens. One third of individuals with LVH never complain of heart problems. However, such patients have a high risk of sudden death. LVH can be caused by arterial atherosclerotic lesions. The linkage of mtDNA mutations 652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A with atherosclerosis was described earlier by our laboratory. The aim of the study was to analyze the linkage of these mtDNA mutations with LVH.

Methods: DNA from white blood cells was isolated using a phenol-chloroform method. PCR-fragments of DNA contained the region of the investigated mutations. The heteroplasmy level of mtDNA mutations was analyzed using a pyrosequencing-based method developed by our laboratory.

Results: We investigated two groups of individuals. One hundred and ninety-four patients with LVH. Two hundred and ten were conventionally healthy. It was found that mtDNA mutation m.5178C>A was significantly associated with LVH. Single nucleotide replacement m.1555A>G was associated with LVH at the level of significance $P \leq 0.1$. At the same time m.12315G>A and m.3336T>C were significantly associated with the absence of this pathology. Single nucleotide replacement m.14459G>A was associated with the absence of LVH at the significance level $P \leq 0.1$.

Conclusion: MtDNA mutations m.5178C>A and m.1555A>G can be used for molecular genetic assessment of the predisposition of individuals to the occurrence of left ventricular hypertrophy. They can also be used for the family analysis of this pathology. Mutations m.12315G>A, m.3336T>C and m.14459G>A can be used in the development of LVH gene therapy methods.



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Keywords: Left ventricular hypertrophy, heteroplasmy level, mutation, mitochondrial genome, mtDNA

INTRODUCTION

In case of left ventricular hypertrophy (LVH), the heart muscle thickens. Often septum between the left and right ventricles mutates in this disease^[1]. In LVH muscle fibers in the myocardium are arranged randomly. The main criterion for LVH is considered to be an increase in myocardial thickness larger than or equal to 1.5 cm in the presence of left ventricular diastolic dysfunction^[1-3]. The third part of individuals with LVH never complain of heart problems. However, such patients have a high risk of sudden death, which reaches 4% per year^[2,3]. Echocardiography helps to identify such patients. It can be used to identify a left ventricle and left atrium enlargements. It can also detect heart rhythm disorders. This helps to assess the risk of sudden death. About 50% of deaths from left ventricular hypertrophy per year happen precisely because of ventricular arrhythmias. The second cause of death of patients with LVH is congestive heart failure. It is most common in patients older than 40 years^[4,5].

The onset and development of left ventricular hypertrophy can be caused by atherosclerotic lesions of the arteries, in particular, atherosclerotic plaques and thickening of the intima-medial layer of these vessels^[6-10]. Risk factors for LVH include diabetes mellitus, stress, smoking, hyperlipoproteinemia, hypodynamia, arterial hypertension, hyperfibrinogenemia, homocysteinemia, obesity, hypothyroidism and metabolic syndrome^[11-15].

In addition, left ventricular hypertrophy may occur due to hereditary and somatic mutations of the human genome. At present, many scientists are studying, basically, the single-nucleotide polymorphism (SNP) of the nuclear genome associated with this pathology^[16-20]. However, nuclear polymorphisms are associated only with a small number of LVH cases. Meanwhile mitochondrial genome mutations with left ventricular hypertrophy were analyzed by a very small number of research groups around the world^[21-23].

It should be noted that in human cells there are plenty of mitochondria. Each mitochondria contains several copies of the mitochondrial genome. Therefore, during the analysis of DNA samples from the study participants it is necessary to determine the heteroplasmy level of each investigated mitochondrial genome mutation (ratio of mtDNA molecules containing the mutation to the total number of mtDNA molecules)^[24-28]. It differs significantly from SNP analysis of the nuclear genome, where it is necessary to identify homozygous and heterozygous individuals according to this SNP.

The linkage of mtDNA mutations 652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A with atherosclerosis was described earlier by our laboratory researchers^[24,28-31]. Since LVH has common risk factors with atherosclerosis, it was decided to analyze the relationship of these mutations to mtDNA with left ventricular hypertrophy.

METHODS

In this study two groups of study participants were examined. One hundred and ninety-four patients had left ventricular hypertrophy. Two hundred and ten study participants were conventionally healthy. For identifying patients with LVH among the study participants, the method of echocardiography was used. The main criterion of LVH was considered to be an increase in myocardial thickness of more than or equal to 1.5 cm in the presence of left ventricular diastolic dysfunction. Individuals with diabetes mellitus, hypercholesterolemia and patients, who used drugs, were excluded from the study. In order to compare the samples of patients with LVH and conventionally healthy study participants more correctly, the composition of the samples was changed so that they did not contain significant differences in age, sex, diastolic and systolic blood pressure.

Clinical, anthropometric and age characteristics were determined for patients with left ventricular hypertrophy and conventionally healthy individuals [Tables 1 and 2]

The study was carried out in accordance with the Declaration of Helsinki. The study protocol was inspected and approved by the Ethics Committee of the National Medical Research Center of Cardiology. Each study participant has signed a written informed consent to participate in this investigation.

DNA from white blood cells was isolated using the phenol-chloroform method^[32-34]. PCR-fragments of DNA were obtained. They contained the region of studied mutations (652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A). It should be noted that biotin was attached to one of the DNA chains of the PCR fragment using the primer “bio-” (biotinylated). This was necessary for the analysis of the biotinylated DNA chain of the investigated amplificate using the pyrosequencing^[35-37].

PCR primer sequences, taken for the present research^[24,26,28-30]:

1. For m.652insG
F: TAGACGGGCTCACATCAC (621-638)
R: bio-GGGGTATCTAATCCCAGTTTGGGT (1087-1064)
2. For m.5178C>A
F: bio-GCAGTTGAGGTGGATTAAAC (4963-4982)
R: GGAGTAGATTAGGCGTAGGTAG (5366-5345)
3. For m.3336T>C
F: bio-AGGACAAGAGAAATAAGGCC (3129-3149)
R: ACGTTGGGGCCTTTGCGTAG (3422-3403)
4. For m.14459G>A
F: CAGCTTCCTACACTATTAAAGT (14303-14334)
R: bio-GTTTTTTTAATTTATTAGGGGG (14511-14489)
5. For m.652delG
F: TAGACGGGCTCACATCAC (621-638)
R: bio-GGGGTATCTAATCCCAGTTTGGGT (1087-1064)
6. For m.14846G>A
F: bio-CATTATTCTCGCACGGACT (14671-14689)
R: GCTATAGTTGCAAGCAGGAG (15120-15100)
7. For m.1555A>G
F: TAGGTCAAGGTGTAGCCCATGAGGTGGCAA (1326-1355)
R: bio-GTAAGGTGGAGTGGGTTTGGG (1704-1684)
8. For m.15059G>A
F: bio-CATTATTCTCGCACGGACT (14671-14689)
R: GCTATAGTTGCAAGCAGGAG (15120-15100)
9. For m.3256C>T
F: bio-AGGACAAGAGAAATAAGGCC (3129-3149)
R: ACGTTGGGGCCTTTGCGTAG (3422-3403)
10. For m.12315G>A
F: bio-CTCATGCCCCCATGTCTAA (12230-12249)
R: TTACTTTTATTTGGAGTTGCAC (12337-12317)
11. For m.13513G>A
F: CCTCACAGGTTTCTACTCCAAA (13491-13512)
R: bio-AAGTCCTAGGAAAGTGACAGCGAGG (13825-13806)

Table 1. Clinical and anthropometric characteristics of the groups of individuals

Characteristic	Patients with left ventricular hypertrophy/standard deviation	Conventionally healthy individuals/standard deviation	Significance of differences
Diastolic blood pressure, mmHg	86/23	83/17	0.395
Systolic blood pressure, mmHg	128/23	122/21	0.228
Age, years	64/8.6	58/8.2	0.119
Smoking, %	35	28	0.111
Sex (man/women)	112/82	94/116	0.118
Body mass index, kg/m ²	33.8/4.5	28.9/4.1	0.149
Total cholesterol, mol/L	6.51/1.12	6.45/1.05	0.135
Triglycerides, mol/L	1.65/0.64	1.48/0.61	0.115
High-density lipoprotein, mol/L	1.55 (0.53)	1.68 (0.44)	0.104
Low-density lipoprotein, mol/L	4.36 (1.23)	4.04 (1.21)	0.142

Table 2. Age of participants in the study groups

Groups of individuals	Age minimum	Mean age	Age maximum	Standard deviation
Patients with left ventricular hypertrophy	53 years old	64 years old	75 years old	8.3
Conventionally healthy individuals	54 years old	58 years old	62 years old	7.9

PCR fragments of the following size were obtained^[24,26,28-30]:

- (1) m.652insG - 467 bp;
- (2) m.5178C>A - 383 bp;
- (3) m.3336T>C - 294 bp;
- (4) m.14459G>A - 209 bp;
- (5) m.652delG - 467 bp;
- (6) m.14846G>A - 450 bp;
- (7) m.1555A>G - 379 bp;
- (8) m.15059G>A - 450 bp;
- (9) m.3256C>T - 294 bp;
- (10) m.12315G>A - 108 bp;
- (11) m.13513G>A - 335 bp.

The reaction mixture for PCR was 30 µL. It contained:

- (1) 0.3 pM of each primer;
- (2) 67 mM tris-HCl (pH 8.8);
- (3) MgCl₂: 2.5 mM for m.652insG, m.5178C>A, m.3336T>C, m.652delG, m.1555A>G, m.3256C>T, m.12315G>A and m.13513G>A; 1.5 mM for G14846A, G15059A and G14459A;
- (4) 16.6 µM (NH₄)₂SO₄;
- (5) 3 units of Taq-polymerase;
- (6) 200 µM of each deoxyribonucleotriphosphate;
- (7) 0.4-0.6 µg^[24,26,28-30].

Annealing temperature for the PCR is shown in Table 3.

To carry out the polymerase chain reaction, we used thermocycler “PTC DNA Engine 200”.

Pyrosequencing of PCR fragments was performed on an automated pyrosequencing device PSQTMHS96MA (Biotage, Sweden).

For pyrosequencing the following primer sequences were used^[24,26,28-30]:

1. For m.652insG

Table 3. Annealing temperature for the PCR^[24,26,28-30]

Mutations	Annealing temperature for primers
m.15059G>A	
m.3336T>C	
m.13513G>A	55 °C
m.3256C>T	
m.14846G>A	
m.652insG	
m.5178C>A	60 °C
m.652delG	
m.14459G>A	
m.1555A>G	50 °C
m.12315G>A	

CCCATAAACAAATA (639-651);

2. For m.5178C>A

ATTAAGGGTGTTAGTCATGT (5200-5181);

3. For m.3336T>C

TGCGATTAGAATGGGTAC (3354-3337);

4. For m.14459G>A

GATACTCCTCAATAGCCA (14439-14456);

5. For m.652delG

CCCATAAACAAATA (639-651);

6. For m.14846G>A

GCGCCAAGGAGTGA (14861-14848);

7. For m.1555A>G

ACGCATTTATATAGAGGA (1537-1554);

8. For m.15059G>A

TTTCTGAGTAGAGAAATGAT (15080-15061);

9. For m.3256C>T

AAGAAGAGGAATTGA (3300-3286);

4. For m.12315G>A

TTTGGAGTTGCAC (12328-12316);

8. For m.13513G>A

AGGTTTCTACTCAA (13497-13511).

The heteroplasmy level of mtDNA mutations was analyzed using a quantitative method developed on the basis of pyrosequencing technology by our laboratory^[24-26,38,39]. The statistical analysis was performed using SPSS 22.0 software package^[40]. The bootstrap analysis and the Spearman correlation coefficient were used. The results were considered statistically significant at $P \leq 0.05$. In addition, the results were taken into account, the significance level of which was $P \leq 0.1$. It was supposed that such results had a tendency to have statistical significance. They may be significant if the sample is expanded.

RESULTS

According to Table 1, statistically significant differences by clinical and anthropometric characteristics between samples of patients with left ventricular hypertrophy and conventionally healthy study participants were not found.

It should be noted that the age of patients with left ventricular hypertrophy ranged from 53 to 75 years. At the same time, the age of conventionally healthy participants ranged from 54 to 62 years [Table 2]. The mean age of patients with left ventricular hypertrophy was 6 years higher than the age of conventionally healthy study participants. This age difference between samples of patients with left ventricular hypertrophy

Table 4. Spearman correlation analysis of 11 mtDNA mutations with left ventricular hypertrophy

Mutations	Spearman correlation coefficient	Significance
m.652insG	-0.036	0.357
m.5178C>A	0.114	0.038 ^a
m.3336T>C	-0.126	0.014 ^a
m.14459G>A	-0.082	0.082 ^b
m.652delG	0.065	0.214
m.14846G>A	-0.028	0.427
m.1555A>G	0.096	0.064 ^b
m.15059G>A	0.064	0.236
m.3256C>T	0.057	0.258
m.12315G>A	-0.122	0.017 ^a
m.13513G>A	-0.034	0.369

^a $P \leq 0.05$; ^b $P \leq 0.1$

and conventionally healthy participants was not statistically significant. The linkage of mtDNA mutations 652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A with atherosclerosis was described earlier by our laboratory researchers^[24,28-31]. Since LVH has common risk factors with atherosclerosis, it was decided to analyze the relationship of these mutations to mtDNA with left ventricular hypertrophy.

The results of this analysis are presented in Table 4.

The direction of the linkage of mtDNA mutations with left ventricular hypertrophy was detected using the coefficient of correlation. If the Spearman correlation coefficient was positive, the investigated mutation was associated with left ventricular hypertrophy. If the Spearman correlation coefficient was negative, the mutation was associated with the absence of left ventricular hypertrophy.

According to the obtained results, mtDNA mutation m.5178C>A was significantly associated with LVH. Single nucleotide replacement m.1555A>G was associated with left ventricular hypertrophy at the level of significance $P \leq 0.1$. It showed a tendency to a positive correlation with LVH. Meanwhile m.12315G>A and m.3336T>C were significantly associated with the absence of this pathology. Single nucleotide replacement m.14459G>A was associated with the absence of left ventricular hypertrophy at the significance level $P \leq 0.1$. Mutation m.14459G>A showed a tendency to negative correlation with LVH.

DISCUSSION

Due to the fact that several mitochondria can be found in a human cell, and several copies of the mitochondrial genome can be found in the mitochondria, in particular the level of mtDNA mutations in the mitochondrial genome was analyzed. The presence of heteroplasmy threshold level of a mitochondrial genome mutation may be associated with the occurrence of the disease. Our previous article was devoted to the detection of threshold heteroplasmy level of mitochondrial genome mutations 652insG, m.5178C>A, m.3336T>C, m.14459G>A, 652delG, m.14846G>A, m.1555A>G, m.15059G>A, m.3256C>T, m.12315G>A and m.13513G>A, which are associated with atherosclerosis and its risk factors^[25].

Mutations m.5178C>A and m.1555A>G can be used to assess the molecular genetic predisposition of individuals to occurrence of left ventricular hypertrophy. They can also be used for family analysis of this pathology. Mutations m.12315G>A, m.3336T>C and m.14459G>A could be used in the development of LVH gene therapy methods.

It is noteworthy that one of the mutations of the mitochondrial genome (m.5178C>A) can lead to a defect in the respiratory chain enzyme (NADH dehydrogenase), leading to a decrease in ATP synthesis and an energy

deficit in the mitochondria and cells of humans. At the same time, two other mutations (m.3336T>C and m.14459G>A) of this enzyme seem to have a protective, stabilizing effect and positively affect mitochondria and cardiac muscle cells. In our preliminary studies, it was found that mutation m.12315G>A, localized in the transfer RNA-Leucine gene (recognition codon CUN), was associated with atherosclerosis^[29]. However, in the present study it was found that this mutation has a protective effect on left ventricular hypertrophy. A possible reason for this may be a difference in the mechanisms of the occurrence and development of these pathologies.

It should be noted that single nucleotide substitutions which have a protective (antipathological) effect on diseases are called “protective mutations”, but not polymorphisms. Since polymorphisms are neutral, they exist in populations without influencing the occurrence and development of diseases. In addition, polymorphisms do not have a protective effect in various pathologies. Therefore, the name “protective (antipathological) mutations” seems to us more correct.

It is necessary to say that in literary sources there are very few studies that have investigated the linkage of mitochondrial genome mutations with LVH. In particular, in the article of Zhu *et al.*^[21] the association of mutation m.4401A>G with left ventricular hypertrophy was found. In a research work by Govindaraj *et al.*^[22] heteroplasmic mutations m.4797C>M and m.8728T>Y MT-tRNA, were found to be associated with hypertrophic cardiomyopathy. In the article by Bates *et al.*^[23] the association of mtDNA mutation m.3243A>G with concentric hypertrophic remodelling and subendocardial dysfunction was studied. In none of such studies the association of the heteroplasmy level of the detected by us mtDNA mutations with left ventricular hypertrophy was analyzed.

In conclusion, five mutations of the mitochondrial genome associated with left ventricular hypertrophy were found in the present study. They can be used for molecular genetic assessment of the predisposition of individuals to the occurrence of LVH, family analysis and gene therapy of this pathology.

DECLARATIONS

Authors' contributions

Conception, design and statistical analysis: Sazonova MA
Pyrosequencing of PCR fragments: Sazonova MA, Sinyov VV
PCR: Ryzhkova AI, Khasanova ZB
DNA extraction: Sazonova MD
Administrative and material support: Sobenin IA

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study was carried out in accordance with the Declaration of Helsinki. The study protocol was inspected and approved by the Ethics Committee of the National Medical Research Center of Cardiology. Each study

participant has signed a written informed consent to participate in this investigation.

Consent for publication

Not applicable.

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Case Report

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Successful plasmapheresis treatment of severe hypertriglyceridemia during late pregnancy

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Abstract

During pregnancy, physiologic hormonal changes provoke a significant increase in triglyceride levels. Genetic abnormalities of triglyceride metabolism and secondary factors may multiply the risk of severe lipid abnormalities. Although severe gestational hypertriglyceridemia can be a life-threatening condition for both mother and fetus, its optimal treatment has not been fully clarified. A 33-year-old woman at 37 weeks of her second pregnancy was admitted to our clinic. Her triglyceride level was 57.8 mmol/L. Abdominal pain, nausea, vomiting or any other complaints were not reported. She kept a fat-restricted diet, however her triglyceride level remained 41 mmol/L. Therefore we decided to perform plasmapheresis with a replacement of human albumin as a colloidal solution. Complications did not occur during the treatment. Plasmapheresis reduced her triglyceride level by 54.1% (to 18.8 mmol/L), and the patient delivered a healthy female neonate at 40 weeks. In case of significantly increased values, plasmapheresis is a fast, effective and safe method for decreasing triglyceride level even in the third trimester.

Keywords: Hypertriglyceridemia, pregnancy, plasmapheresis

INTRODUCTION

Maternal lipids are extremely important for developing a fetus. While cholesterol is a crucial molecule in placental steroid synthesis, fatty acids participate in placental oxidation processes and membrane development^[1]. In pregnant women, significant alterations in lipid levels are in connection with estrogen, progesterone and human placental lactogen synthesis^[2]. During normal pregnancy, increased lipolytic activity raises the release of free fatty acid and the production of very low-density lipoprotein (VLDL).



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Besides, the elimination of VLDL is decreased due to the reduced activity of lipoprotein lipase in fatty tissue^[3]. Eventually, there is a 2-4 fold increase in triglyceride level by third trimester^[4].

Genetic abnormalities of triglyceride metabolism can provoke severe hypertriglyceridemia in pregnancy. In familial combined hyperlipidemia and familial hypertriglyceridemia, we see an increased formation of triglyceride-rich lipoproteins (chylomicrons and VLDLs). Ineffective lipolysis of these particles can cause familial chylomicronemia due to the mutations in lipoprotein lipase, apolipoprotein CII or apolipoprotein AV^[5]. In familial dysbetalipoproteinemia, apolipoprotein E2/E2 genotype reduces hepatic clearance of chylomicron remnants^[5]. In addition, secondary factors such as poorly controlled diabetes mellitus (via decreasing the activity of lipoprotein lipase), hypothyroidism, nephrotic syndrome and some medications (glucocorticoids) may aggravate gestational hypertriglyceridemia^[6].

Hypertriglyceridemia during pregnancy increases the risk of acute pancreatitis (especially if triglyceride level is above 11.4 mmol/L), hyperviscosity syndrome and pre-eclampsia in mother, macrosomia, premature birth or fetal death in fetus^[6-9].

At first, treatment of the underlying disease and fat-restricted diet are recommended^[6]. During pregnancy, lipid-lowering medications (fibrate, nicotinic acid, statin, and ezetimibe) should be stopped due to their possible teratogenic effects^[10]. Novel lipid-lowering agents including microsomal transfer protein and proprotein convertase subtilisin/kexin type 9 inhibitors are also contraindicated. Therefore, in the case of non-responding severe hypertriglyceridemia plasmapheresis should be considered. This is an extracorporeal procedure, when plasma is separated from the blood and processed to eliminate selective components including triglyceride-rich lipoprotein particles. The treated plasma is then reinfused although, on occasion, it is completely eliminated and replaced by an isovolumetric solution. Most of the studies use plasmapheresis as a treatment of hypertriglyceridemia associated pancreatitis. In some cases, they perform plasmapheresis to prevent pancreatitis. It must be noted that the timing and frequency of plasmapheresis procedures were not uniform in these studies^[11-13]. During plasmapheresis serum triglyceride level can be decreased by 66%-70%^[12,13].

CASE REPORT

A 33-year-old woman at 37 weeks of her second pregnancy was admitted to our clinic. Her gynecologist indicated routine laboratory examinations, which remained unsuccessful because of lipemic blood sample. Therefore, we checked her triglyceride level and found that it was significantly elevated (57.8 mmol/L). Abdominal pain, nausea, vomiting or any other complaints were not reported. Based on physical examination, there were no eruptive xanthomas, palmar crease xanthomas or lipaemia retinalis. Her body mass index was within the normal range before pregnancy (53 kg), and her total body weight gain was 9 kg during pregnancy. In her history, there was no diabetes mellitus or any other secondary causes of hypertriglyceridemia. The diagnosis of gestational diabetes mellitus was evaluated according to the Hungarian national recommendation. We used the modified 1999 WHO recommendation (gestational diabetes mellitus: 75 g CH oral glucose tolerance test (OGTT) at 24-28 gestation weeks: fasting plasma glucose \geq 6.1 mmol/L, 120 min plasma glucose \geq 7.8 mmol/L)^[14]. Based on the fasting plasma glucose (5.2 mmol/L) and the OGTT (plasma glucose: 120 min: 7.1 mmol/L) the diagnosis of gestational diabetes mellitus was excluded. In her family history, her mother and grandmother had mixed hyperlipidemia. Her first pregnancy had terminated without any complication however, lipid parameter measurement was not performed. Before her pregnancy, she had not taken any lipid-lowering medications. She had not followed a fat-restricted diet during her pregnancy. Table 1 shows the laboratory parameters before treatment. Markedly elevated triglyceride and total cholesterol levels were detected. Apolipoprotein B100 and lipoprotein (a) levels were also above the upper border of the normal range. Based on the result of lipid electrophoresis, a significant increase in the level of VLDL may cause hypertriglyceridemia.

Table 1. Laboratory parameters before treatment

Parameter	Unit	Value	Reference range
Total cholesterol	mmol/L	24.7 (H)	< 5.2
Triglyceride	mmol/L	41.00 (H)	< 1.7
Apolipoprotein A	g/L	1.86	> 1.15
Apolipoprotein B100	g/L	2.25 (H)	< 1
Lipoprotein(a)	mg/L	114 (H)	< 30
sTSH	mU/L	0.978	0.300-4.200
fT3	pmol/L	1.8 (L)	2.4-6.3
fT4	pmol/L	8.2 (L)	12.0-22.0
CRP	mg/L	8.2 (H)	< 5.2
Lipid electrophoresis			
AlfaLP	%	7 (L)	15-40
PreβLP	%	53 (H)	2-31
BetaLP	%	34 (L)	42-70
Chylomicron	%	6	-

Her levels of lipase, amylase, transaminases, and inflammatory markers were in the normal range. Abdominal ultrasound showed neither cholelithiasis nor any symptoms of pancreatitis. The development of fetus was appropriate. The patient case was followed by obstetrician, endocrinologist, neonatologist, and dietitian.

She started on a fat-restricted diet (< 20% of total calories from fat daily) and consumed 15 g medium-chain triglycerides and 3 g omega-3-fatty esters daily, but two days later her triglyceride level remained 41 mmol/L. Therefore, she was immediately admitted to our ICU and we decided to perform plasmapheresis using central veins with human albumin infusion as a colloidal solution with one plasma volume exchange and FRESenius Com.tec Kabi Therapeutic Plasma Exchange machine.

There were not any complications during plasmapheresis. The patient delivered a healthy female neonate weighing 3,150 g at 40 weeks. One day after delivery her triglyceride level was decreased to 15.68 mmol/L, two more days later it was 7.3 mmol/L without any further plasmapheresis procedures. The alterations of lipid parameters are shown in [Figures 1 and 2](#).

During lactation, we suggested fat-restricted diet and regular control of triglyceride level. Gene polymorphisms of lipoprotein lipase and Apolipoprotein E were determined. The patient has ApoE 3/3 genotype, therefore, we could exclude the familial dysbetalipoproteinemia as a possible cause. The patient is wild type for two common polymorphisms of LPL (LPL-D9N and LPL-N291S) causing hypertriglyceridemia. Although, there was some chylomicron increment, a significant increase in the level of VLDL determined by lipid electrophoresis excludes the possibility of severe LPL deficiency.

DISCUSSION

In the last decades, women had increasing access to graduation in professional schools, building careers, and using contraceptive methods worldwide^[15]. This has led to the delay of first marriage and first child birth. In fact, the average age at first birth rose from 22.7 years in 1980 to 28.2 years in 2013^[16]. Indeed, many women are choosing to delay attempts to conceive to their thirties and forties, when they have a significantly higher risk of type 2 diabetes, metabolic syndrome and other non-communicable diseases causing lipid abnormalities. Furthermore, the proportion of women of reproductive age who are overweight or obese is also increasing at an alarming rate^[17]. The incidence of severe hypertriglyceridemia in pregnant women might be higher than we believe.

Although during pregnancy measurement of lipid parameters is not performed routinely, in case of a lipemic blood sample, suggestive patient history or previous pancreatitis, determination of triglyceride level is definitely required.

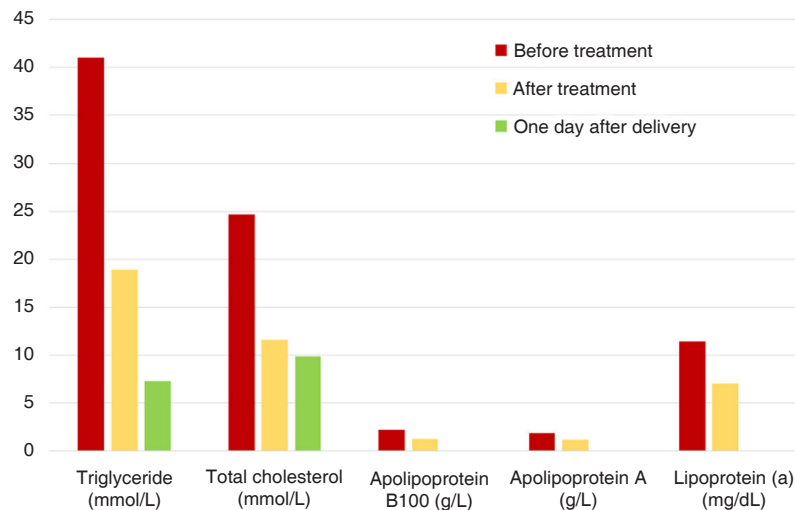


Figure 1. Lipid parameters before and immediately after treatment plasmapheresis, and one day after delivery

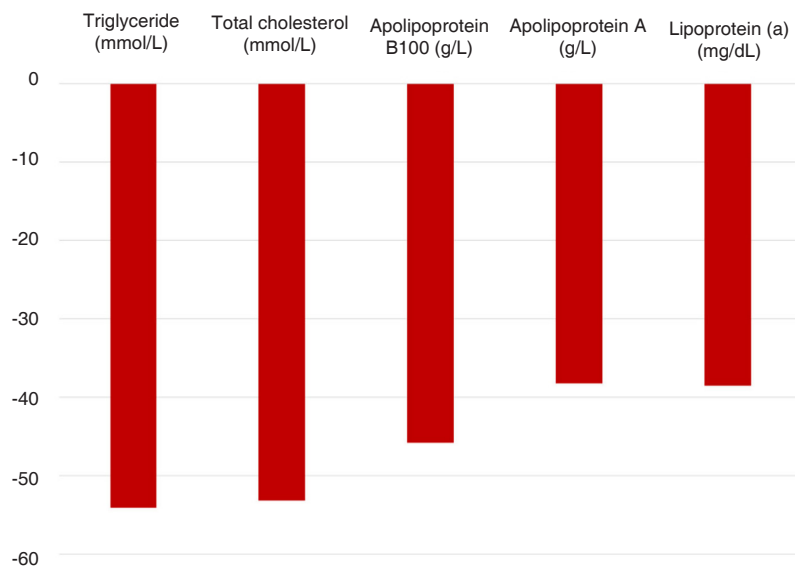


Figure 2. Changes (%) in lipid parameters before and immediately after plasmapheresis treatment

While treatment of gestational hypertriglyceridemia-induced pancreatitis with plasmapheresis is well-defined^[6], the timing and frequency of plasmapheresis procedures in gestational hypertriglyceridemia without pancreatitis should be determined individually, depending on patient's history, symptoms, duration of pregnancy and the lowest triglyceride level available with diet. To date, there is no available guideline for the management of severe hypertriglyceridemia that develops during pregnancy. Although some case reports and series were published^[12], including treatment methods such as intravenous insulin and glucose, heparin and apheresis, they are not comparable, therefore, the clinician has to come to the optimal decision. The timing of the extracorporeal treatment can be an especially tender spot, since all of these procedures may provoke adverse events such as hemodynamical instability, which can be especially critical in late pregnancy. Early plasmapheresis as a successful treatment in hypertriglyceridemia-induced acute pancreatitis in first-trimester pregnancy following in vitro fertilization was reported previously^[18]. Here, we present a successful treatment in late pregnancy without adverse events.

Regular check-up is highly recommended after delivery, during lactation and especially during subsequent pregnancies. Close cooperation between endocrinologist or lipid specialist and gynaecologist is also necessary. With the exception of lipoprotein lipase deficiency, primary hypertriglyceridemia disorders usually present in adulthood. Indeed, dozens of causative genetic abnormalities were described in severe hypertriglyceridemia. Genetic testing is available for suspected cases of familial chylomicronemia syndrome and dysbetalipo-proteinemia, but is not necessary for treatment^[5]. Still, sequencing of candidate genes may help to guide future individualized therapeutic strategies in order to prevent further complications and to identify affected relatives using cascade screening.

In case of significantly increased values, plasmapheresis is a fast, effective and safe method for decreasing triglyceride level even in late pregnancy. However, multicentre, prospective studies including a larger number of participants are required to support the observations and to define novel therapeutic guidelines.

DECLARATIONS

Authors' contributions

Collection of data: Zsíros N, Harangi M

Analysis and/or interpretation of data: Zsíros N, Harangi M

Writing (not revising) all or sections of the manuscript: Zsíros N

Manuscript review: Kovács B, Harangi M

Supervision: Paragh G, Balla J

Study design: Paragh G, Harangi M

Availability of data and materials

The clinical and laboratory data used to support the findings of this work are included within the article.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Informed consent was obtained from the patient after the approval of the local ethics committee.

Consent for publication

Informed consent was obtained from the patient.

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Review

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Trigger mechanisms in insulin resistance and diabetes mellitus development

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Abstract

Type 2 diabetes mellitus characterized by chronic hyperglycaemia is caused by insulin resistance and β -cell dysfunction. Glycogen accumulation, due to impaired metabolism, contributes to this "glucotoxicity" via dysregulated biochemical pathways promoting β -cell dysfunction. Thus, long-term exposition of insulin-secreting cells or isolated islets together with increased free fatty acids (FFA) and glucose levels can cause insulin-induced glucose secretion depression, damage to insulin gene expression and apoptotic death of cells. It is known that, the main regulator of pancreatic β -cells functioning and regulator of insulin gene expression, synthesis and secretion of insulin is glucose. Glucose enters cells and progressively metabolizes, in particular, to pyruvate in a cycle of tricarboxylic acids, subjected to oxidative phosphorylation, during which formed adenosine triphosphate and reactive oxygen radicals (ROS). Although, when more glucose enters the cell, there are other ways in which extra glucose can be transferred to reserve and of the glucose molecules can form ROS. The release of excessive amounts of FFA leads to lipotoxicity, as lipids and metabolites produce ROS in the endoplasmic reticulum and mitochondria. This affects both adipose and non-fat tissue, making up its pathophysiology in many organs. This overview demonstrates that the insulin gene is expressed in pancreatic β -cells. Glucose is the main physiological regulator of insulin gene expression. It controls the effect of transcription factors, insulin mRNA stability, and transcription rate. Glucolipotoxicity mechanisms affect the transcription factors MafA and PDX-1. Important is the β -cells damaging, which is connected with the oxidative stress and the synthesis of ceramides.

Keywords: Diabetes mellitus, insulin resistance, glucotoxicity, lipotoxicity



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INTRODUCTION

Our narrative review summarizes the results of studies of trigger mechanisms (in particularly, glucotoxicity) in insulin resistance (IR) and type 2 diabetes mellitus (DM) development. When searching PubMed for the next keyword combination “insulin resistance and type 2 diabetes and mechanisms”, we received 4,307 results (2,976 of them in the last 10 years). When narrowing the search with the keyword combination “insulin resistance and type 2 diabetes and glucotoxicity”, we received 104 results (55 of them in the last 10 years). Mostly, the article presented literary sources limited to the search for the last 10 years (until now). Nevertheless, we included older publications (preferring high-ranking journals) in case of seminal studies or when few studies were available for a specific topic. Of the articles retained, we included 57 that were most specific to trigger mechanisms in IR and DM development.

Type 2 DM is a progressive chronic disease characterized by chronic hyperglycemia caused by IR and β -cell dysfunction^[1]. Pancreatic β -cells play a fundamental role in the maintenance of glucose homeostasis in mammals^[2]. It is proved that by the time of type 2 DM development, sensitivity of peripheral tissues to insulin is reduced by 70%, and insulin secretion - by 50%^[3]. The loss of insulin sensitivity in muscle, fat and liver tissues has the greatest clinical significance. Understanding of IR development mechanisms, search for genes responsible for its development is extremely important for the working out of new approaches to the treatment of type 2 DM. A study in the field of molecular biology has shown that patients with type 2 DM have genetic defects that are responsible for transmission of the signal after joining of insulin with the receptor (post-receptor defects)^[4].

Insulin realizes the metabolic effect through the activation of phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB, Akt). Serine/threonine kinase Akt phosphorylates GSK3 β and FOXOs transcription factors that directly or indirectly mediate the effect of insulin on transcription of genes involved in carbohydrate metabolism. The deletion or deactivation of the genes Akt1 and Akt2 blocks the effect of insulin on glucose metabolism^[5,6].

Through PI3K and phosphorylation of proto-oncogene Cbl, activates glucose transporter 4 (Glut4)^[7]. Thus, the biological effect of insulin is associated with the activation of glucose uptake by adipocytes and myocytes (Glut4), activation of glycogen synthesis (glycogen synthase) and protein (S6-kinase). Insulin regulates transcription of more than 150 genes. There are 7 groups of insulin-regulated sequences or elements (IRS/IRE)^[8], including sterol-regulated element-binding protein 1c (SREBP-1c). SREBPs are transcription factors of the helix-loop-helix (bHLH) family in the liver. Forms SREBP-1a, SREBP-1c and SREBP-2 affect the homeostasis of cholesterol and lipids. Srebp-1c expression is regulated by insulin regardless of glucose level [Figure 1]^[9].

Hyperexpression of dominant negative forms of SREBP-1c prevents induction of hepatic pyruvate kinase, spot 14 and fatty acid synthase mediated by insulin^[10]. The expression of glucokinase (GK) in the liver is regulated by insulin, regardless of the level of glycemia.

Insulin also causes repression through removal from the core and acceleration of the degradation of a FOXO transcription positive regulator. In β -cells, the FOXO1 nuclear factor is a repressor of the positive activity of the nuclear transcription factor Hnf3 β (FOXO2) in the PDX1 promoter, while insulin increases repression through deletion of FOXO1 from the nucleus.

The highly conservative area, located 340 bp upstream of the start of transcription initiation, subsequently renamed as a promoter of insulin, controls the production of insulin by tissues and the regulation of the insulin gene itself. Most factors act here, which determine high transcription, these include cis-regulatory

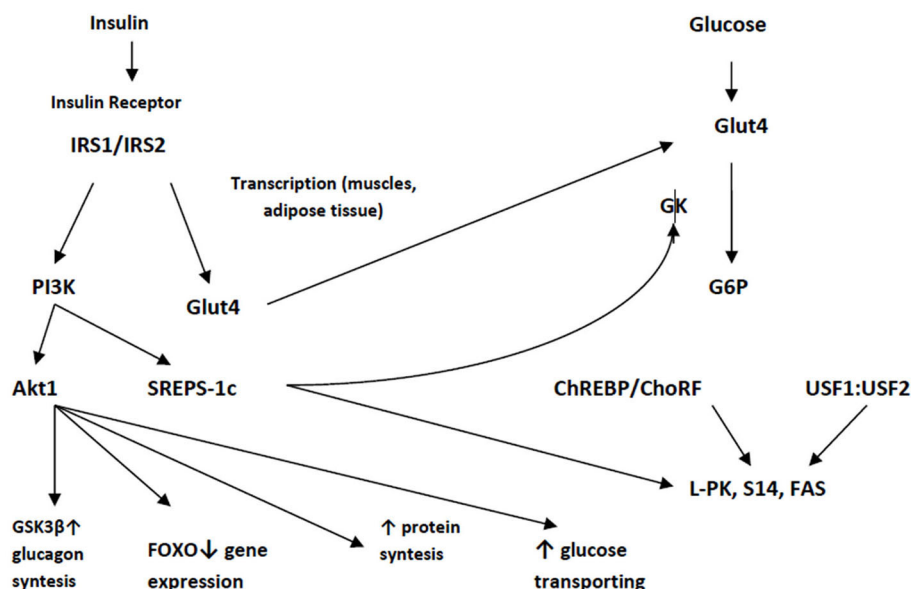


Figure 1. The effect of insulin and glucose on the regulation of gene expression^[9]. IRS1/IRS2: insulin receptor substrates 1 and 2; Akt1: proteinase B; PI3K: phosphatidylinositol-3-kinase; SREBP-1c: sterol-regulated element-binding protein 1C; FAS: fatty acid synthase; FOXO: transcription factors; Glut4: glucose transporter; GK: glucokinase; L-PK: liver pyruvate kinase; S14: spot 14; G6P: glucose-6-phosphate

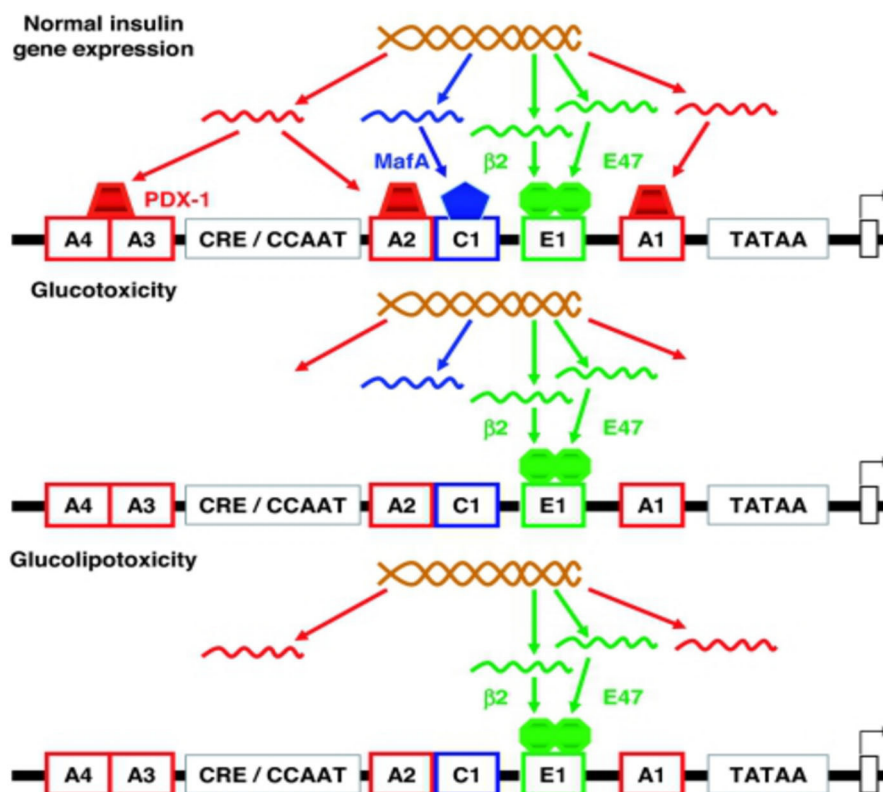


Figure 2. Schematic representation of the proximal area of the insulin 2 promoter in rats^[10]. C1 (RIRE3b1): promoter node; BETA2 (NeuroD): transcription factor; PDX-1 (ID1-1/STF-1/IPF1): pancreatic/duodenal homeobox-1

elements, which are involved into activation *in vitro* [Figure 2].

Insulin promoter factor (PDX-1) also works with proteins of the basic dimerization transcription factors

(bHLH), bind with box E1^[12]. Activator E1 is a dimer consisting of two polypeptide chains. The interaction between the dimerization transcription factors (bHLH) and the insulin promoter factor (PDX-1), includes other coactivators and DNA-binding proteins, for example, MafA, co-promoter C1^[13]. MafA controls the expression of β -cells, a specific expression of the insulin gene through a cis-regulatory element called RIPE3b1, and function as a powerful transactivator of the insulin gene^[13,14]. While PDX-1 and NeuroD are expressed in different types of islet cells, MafA is a specific transactivator of the insulin gene only in β -cells. Therefore, the power of MafA as an activator of the insulin gene, together with the unique expression in β -cells, increases the probability that MafA is the main factor in the formation and function of these cells^[15].

The deterioration of β -cells may result from a combination of genetic and environmental factors. Hyperglycemia, even moderate, observed before the development of diabetes, can lead to damage to β -cells through a process known as “glucotoxicity”. The second phenomenon, lipotoxicity, also leads to β -cell damage^[12]. Both of these processes can be considered as abnormal, because glucose and lipid levels are not toxic, but very important for normal functioning of β -cells. Therefore, there is a range of variances from the normoglycemic and normolipidemia conditions to disturbed hyperglycemic and hyperlipidemia conditions. Unger and colleagues were the first to introduce the concept of “glucotoxicity” and “lipotoxicity”^[16]. In their first article, glucose toxicity means continuous super-stimulation of β -cell glucose, which ultimately leads to insulin stores exhaustion, aggravation of hyperglycemia and to final worsening of β -cells functioning. The hypothesis about the cause of β -cell functioning aggravation was based on the exposure of β -cells in conditions of excessive lipid levels. The recognition of glucolipotoxicity presence is based on changes in intracellular lipids, which form the basis of lipotoxicity mechanism and depend on elevated glucose levels. Thus, glucotoxicity and lipotoxicity are correlated and have the same mechanisms^[17].

GLUCOTOXICITY

Glycogen accumulation, due to impaired metabolism, contributes to this “glucotoxicity” via dysregulated biochemical pathways promoting β -cell dysfunction^[18].

In general, some studies *in vitro*, tried to find glucolipotoxicity mechanisms formation, using isolated Langerhans islets and β -cells in pancreatic tissue. Long-term exposition of insulin-secreting cells or isolated islets together with increased free fatty acids (FFA) levels and glucose can cause insulin-induced glucose secretion depression, damage to insulin gene expression and apoptotic death of cells^[11].

The increase of IR and defeat of β -cells are the basis of type 2 DM progression and these two factors have different values and degrees of change. IR provokes changes that occur before the onset of hyperglycemia, and reaches its maximum values at relatively early stages of disease progression. Data extrapolated from UKPDS and Belfast Diet Study suggests that β -cell dysfunction precedes the development of diabetes for up to 15 years. In addition, the UKPDS showed a correlation between long-term, gradual deterioration of glycemic control and progressive dysfunction of β -cells, confirming the view that the defeat of these cells may be the leading factor in the disease^[19,20].

Studies, which are currently being conducted, show that disorders on the part of β -cells are not only in an insulin secretion depression. They are multi-factorial and involve a lot of defects, in particular: alternation in the ability of β -cells to respond to the stimulation of glucose (change I phase of insulin secretion), violation of insulin formation (proinsulin/insulin ratio), changes in β -cell mass.

The main regulator of pancreatic β -cells functioning and regulator of insulin gene expression, synthesis and secretion of insulin is glucose. Due to glucose action, all the stages of insulin release (transcription, slicing of pre-RNA, stability of mRNA) are made. In this case, the main elements that regulate the transcription of the insulin gene are C1, E1, A3 [Figure 2]. In addition, the peripheral glucose sensitive element binds

glucose-sensitive cells that take place in the islets of Langerhans. Glucose increases the connecting of PDX-1 with node A3^[21] and affects the transactivating of PDX-1 ability. Furthermore, insulin gene transcription stimulation of PDX-1 involves co-activators, like p300, which can change the structure of chromatin through posttranslational histones modification (methylation and/or acetylation)^[22].

At the moment, it's been examined in detail but still controversy regarding the mechanisms by which glucose transduction contributes to enhancing the binding of the insulin promoter and PDX-1. PDX-1 probably experiences multiple posttranslational modifications, possibly by O-binding N-acetylglucosamine^[23], or a small modifier 1^[24]. A number of kinases have been suggested for phosphorylation of intermediate PDX-1, including mitogen-activated protein kinase (MARK) and PI3K^[25].

Regardless of insulin, glucose regulates the expression of genes responsible for carbohydrate metabolism. G6P, xylitol or hexosamine synthesis intermediates can be signal molecules in these processes. The study of the role of glucose is hampered by the influence of insulin [Figure 1]. First, the entry of glucose into the myocytes and adipocytes is carried out as a result of translocation Glut4. Secondly, GK activity is transcriptionally regulated by glucose and insulin in the liver and pancreas.

Most of the earliest works showed paradoxical glucose ability to reduce the function of β -cells. Using the HIT-T15 cell line and levels of glucose (0.8 or 11.1 mmol/L), long-term cell cultivation in RPMI 1640 medium was observed to lead to low glucose concentrations, levels of insulin mRNA, which proved the fact that high glucose concentrations cause glucotoxicity action on pancreatic β -cells. When incubation lasted for 5 or 10 weeks after the occurrence of glucotoxic effects, the function of β -cells was reversible^[26]. Hit-T15 cells have been cultivated for a long period in an environment with a glucose concentration of 0.8 or 11.1 mmol/L with and without somatostatin, an inhibitor of insulin secretion. Cells cultured with somatostatin had critically low insulin levels in culture media, demonstrating the rest of β -cells. Cells exposed to high glucose and somatostatin showed glucotoxic effect on insulin gene expression, insulin secretion content and stimulation, depletion of β -cells.

Experiments on the line of β -cells, β -TC-6, also associated with HIT-T15 cells that are produced at high and low glucose concentrations, have demonstrated that it is the reduction of MafA that leads to a decrease in insulin gene expression in the presence of glucotoxicity, rather than the binding of insulin promoter factor 1 (PDX-1) and DNA.

In this case, it is important to note that these factors (PDX-1, MafA, *etc.*) do not work independently, but with each other, while activating transcription of insulin^[13]. The effect of cells that secrete insulin on the background of an elevated glucose levels for less than a month reduces the expression of the insulin gene is associated with a decrease in MafA and PDX-1 binding activity^[3]. The decrease in PDX-1 binding activity involves post-transcriptional control^[21], although the exact mechanisms remain unclear. *In vivo* PDX-1 expression was also reduced in partially pancreatectomized hyperglycemic rats^[27] and in diabetic gerbils Psammomysobesus^[28] and its binding activity was decreased in islets in Zucker rats with obesity and diabetes^[29]. With glucotoxicity, the binding activity of MafA in insulin-producing cells decreases^[21].

It is obvious that glucotoxicity may be associated with oxidative stress (OS). In the early studies it was reported that antioxidants N-acetylcysteine and aminoguanidine protect HIT-T15 and isolated islets from toxic action of long-term cultivation in environments with high concentrations of glucose^[30]. It is known that β -cells, in contrast to other sources, contain a sufficiently low concentration of antioxidant enzymes. This confirms that β -cells are at risk for oxidative stress. In diabetic rats that received antioxidants, normalization of glucose concentrations occurs, insulin production is restored, and insulin mRNA levels are reduced^[29].

Glucose enters cells and progressively metabolizes, in particular, to pyruvate in a cycle of tricarboxylic acids, subjected to oxidative phosphorylation, during which formed ATP and reactive oxygen radicals (ROS). There are other ways in which elevated glucose concentrations can be moved to the reserve, while ROS is formed from glucose^[31].

In an animal with diabetes, the binding of PDX-1 to DNA in HIT-T15 cells decreases, while this effect decreases with the use of antioxidants. Studies were conducted to clarify the place where the loss of PDX-1 occurs in the OS^[13]. These results show that the OS induces the loss of PDX-1 post-transcriptional mRNA.

In the 1990s, separate groups measured OS markers at type 2 DM using various methods (high-performance liquid chromatography, mass spectrometry, immunocytochemistry). It has been shown that markers of OS are increased in subjects with type 2 DM, namely: the levels of 8-OH-guanine, 8-epi-PGF₂ (prostaglandin F₂), 4-OH-2-nonenal, hydroperoxides^[13,32]. Yoshida *et al.*^[33] reported that the appointment of antidiabetic drug at the half-year review led to a normalization of the activity of glutamylcysteine synthetase, glutathione and thiol transport.

LIPOTOXICITY

In case of dyslipidemia and obesity, the oversupply of fat to tissues not suited for lipid storage induces cellular dysfunction that underlies 2 type DM and coronary artery disease^[34]. Disorders of lipid metabolism lead to the dysfunction of β -cells in patients with type 2 DM^[12]. It was shown that the influence on islets and β -cells with high concentrations of FFAs *in vitro* weakens the expression of the insulin gene at the same time with increased glucose concentration. Damaging action of FFAs on expression of insulin gene in isolated pancreatic islets is associated with high levels of triglycerides (TG)^[35,36].

Since both palmitate and oleate inhibit insulin secretion, but only palmitate reduces the expression of the insulin gene^[37], it is accepted without proof that these two functional effects are based on similar mechanisms. Scientists suggest that the inhibition of the expression of the insulin gene by palmitate may be due to the formation of ceramides. Ceramide - an intermediate in the biosynthesis of sphingomyelin - is formed by the interaction of sphingosine with acyl-coenzyme-A. The most important role of ceramide is participation as a messenger in the signal path of sphingomyelin and in the regulation of cellular processes such as differentiation, proliferation and apoptosis.

Excess quantities of ceramide inhibit the path of insulin signal transduction by inhibiting phosphorylation of protein kinase Akt/PKB and inhibits the translocation of Akt/PKB from the cytoplasm to the plasma membrane, which then inactivates the path of transduction of the insulin signal. Indeed, in obese people suffering from IR, levels of ceramide in skeletal muscles are increased by 2 times. This increase is due to the high concentrations of free fatty acids in plasma and a decrease in the intensity of phosphorylation of protein kinase Akt^[38].

In connection with the decrease in the binding of MafA and PDX-1 is the inhibition of transcription of the insulin gene by palmitate [Figure 3]^[13,39]. Interestingly, in contrast to glucotoxicity mechanisms that include various types of MafA^[39], the ways by which the formation of ceramide from palmitate decrease PDX-1 concentration and the expression of MafA are unknown. Two main ways of signaling are modulated by ceramides, MAPK and PI3K regulate the transcription of the insulin gene with impact on the activity of the transcription factor. The form of C-jun N-terminal kinase (JNK) is a direct target for ceramide in many cell systems. In β -cells, JNK inhibits transcription of insulin gene and via C-jun-dependent transcription inhibition of E1^[40] and C-jun-independent binding inhibition PDX-1 [Figure 3]^[41].

To prove that increased levels of ceramide lead to glucose homeostasis disorders, experiments with animals and pharmacological agents were carried out. Indeed, the reduction of ceramide levels (by inhibiting its

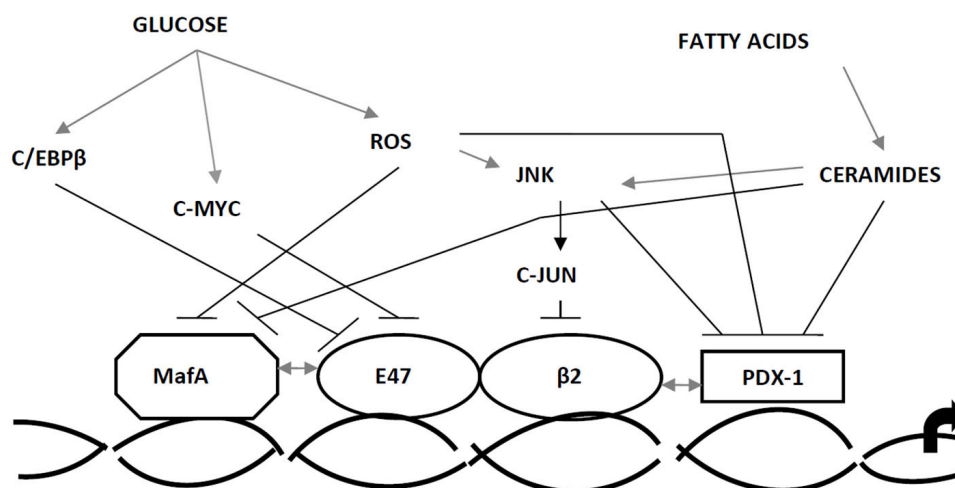


Figure 3. Intracellular mechanisms of insulin gene transcription damage by high concentrations of glucose and fatty acids[11]. ROS: reactive oxygen radicals; PDX-1: pancreatic/duodenal homeobox-1; C/EBP β : CCAAT/enhancer binding protein β ; JNK: C-jun N-terminal kinase

synthesis) improves glucose homeostasis in insulin-resistant transgenic mice with obesity and diabetes^[42].

Thus, PDX-1 and MafA are main targets of gluco- and lipotoxic processes, they operate in other ways. Glucotoxicity is connected with a decrease in the expression of MafA and PDX-1 proteins, whereas in the conditions of PDX-1 lipotoxicity it is expressed, but retained in the cytosol, the levels of MafA mRNA are reduced.

At IR, the level of FFA in hepatocytes increases, because in these cells: (1) lipogenesis de novo increases; (2) esterification of free fatty acids exceeds their oxidation; (3) esterified free fatty acids are stored in the form of triglycerides or sent to the synthesis of cholesterol lipoproteins of very low density (VLDL); (4) insulin-regulated mobilization of triglycerides decreases.

Insulin-resistant adipocytes intensely break down the triglycerides contained in them and release the formed of them into the bloodstream. The flow of FFA from fat cells increases and FFA also comes out of VLDL and chylomicrons from plasma and blood partially go to other organs, and partially - back to the liver where they are again transformed into TG. A certain “pumping” of the liver by FFA and triglycerides takes place. This has the most serious consequences.

Prolonged exposure of β -cells with fatty acids in vitro increases the main insulin release and inhibits glucose-stimulated insulin secretion^[43], a phenomenon observed *in vivo* in rats^[44,45]. These two effects have different time intervals and are likely to have separate mechanisms. Citrate-synthase activity decreases in the presence of fatty acids in the islets cultivated within 24 hours, causing a decrease in citrate levels and an increase in phosphofructokinase activity^[46]. This, in turn, reduces the levels of G6P, disinhibits GK and increases the utilization of glucose at low concentrations of glucose. *In vivo*, FFA also reduces the response of the sympathetic nervous system, so insulin secretion increases^[47].

Particular attention should be paid to the separating protein-2 (UCP-2), a mitochondrial transporter that splits the respiratory chain at the ATP synthesis stage^[48], although its biological functions are still unclear. UCP-2 is assumed to modulate secretion of hormone insulin and to enact in glucolipotoxicity formation. On one hand, the increasing expression of UCP-2 - in β -cells weakens the secretion of insulin. On the other, UCP-2 elevates insulin concentrations in animals and protects against diabetes^[48,49]. Third, UCP-2 expression

is increased in islets after high-calorie feeding in rodents or after exposure with fatty acids *in vitro*^[50]. Fourthly, oleic acid activates UCP-2 promoter in INS-1 cells, the effect mediating directly SREBP1c^[51] and indirectly by peroxisome proliferation activator receptor^[52]. These observations confirm that the FFA stimulates the UCP-2 expressions in β -cells, possibly causing by dissociation of mitochondria.

Thus, the expression of UCP-2 levels in return to FFA can be a cellular ways of protection from overabundance of energy-containing components and OS. Harmful FFA action on the function of β -cells occurs if only glucose levels are increased. Prentki et al.^[17] assumed that glucose was the main determinant of FFA in β -cells. When the glucose concentration is not elevated, the FFA are transported via carnitine-palmitoyl-transferase-1 (CPT-1) to the mitochondria. When the concentrations of both glucose and FFA increase, the cycle of tricarboxylic acids generates signals that promote the formation of malonyl-CoA in the cytosol. Its role is to inhibit CPT-1, thus blocking the oxidation of fatty acids and, as a consequence, the accumulation of acetyl-CoA in the cytosol^[11,17]. In addition, the accumulation of long-chain acyl-CoA in the cytosol, possibly directly, or by generating lipid-stimulating signals, either directly or through the generation of lipid-forming signals, can affect pancreatic β -cells^[11]. Among the metabolic effects that guide the splitting of FFA to formation, glucose stimulates the gene expression which is taken partly in lipogenesis^[53]. There is an enzyme - AMP-activated protein kinase (AMPK), which works as a metabolic sensor, and during an over-nutrition feeds signal to the β -cells to go into the “storage state”^[54]. AMPK activity inversely correlates with levels of glucose and is expressed with the help of palmitate^[55] in pancreatic β -cells. The SREBP1c transcription factor acts as an stimulator, transmitting the signal received by AMPK about changes in the expression of gene, which leads to an increase of lipids formation. It is known that glucose is able to stimulate the production of the X liver receptor, which is able to stimulate the production of SREBP1c and hyperlipidemia^[5].

The release of excessive amounts of FFA leads to lipotoxicity, as lipids and their metabolites produce OS in the endoplasmic network and mitochondria. This affects both adipose and non-fat tissue, making up its pathophysiology in such organs like liver and pancreas^[12]. FFA released from excessive triglycerides deposits also inhibit lipogenesis, breaking a sufficient clearance triglyceride levels. The release of FFA under the action of endothelial lipoprotein lipase from increased serological triglycerides within the limits of increased β -lipoproteins causes lipotoxicity, which leads to insulin receptor dysfunction. Long-term insulin-resistant state creates hyperglycemia with compensated hepatic gluconeogenesis. The latter increases hepatic glucose production, further exacerbating hyperglycemia caused by insulin resistance. FFA also reduce the utilization of insulin-stimulated glucose in the muscles. Lipotoxicity from excessive amounts of FFA also reduces insulin secretion by pancreatic β -cells, which ultimately leads to the depletion of these cells^[56].

Therefore, the constant high glucose directs the fatty acids to cellular lipid synthesis. More recently, studies have shown that the metabolism of lipids and their transport are also involved in the mechanism of glucolipotoxicity.

In *in vitro* studies, important information regarding the molecular and cellular bases of glucolipotoxicity has been provided. Various functional results of chronically elevated levels of FFA are mediated by clear mechanisms and some of *in vitro* observations have been reproduced *in vivo* on rodent models.

CONCLUSION

Thus, the insulin gene is expressed in pancreatic β -cells. The main physiological regulator of the expression of insulin gene is glucose. It controls the effect of transcription factors, insulin mRNA stability, and transcription rate. Deterioration of the function of β -cells causes increased levels of glucose and lipids (glucolipotoxicity) at type 2 DM. Glucolipotoxicity mechanisms affect the transcription factors MafA and PDX-1. The OS and the synthesis of ceramides have important damages to the value of the β -cells. Reducing glucose levels is a key factor in the treatment of type 2 DM, preventing macro- and microvascular

complications, while taking into account existing pathological effects that can affect pancreatic β -cells^[57].

DECLARATIONS

Authors' contributions

Study design, manuscript review: Zlatkina V, Karaya O, Yarmish N, Shalimova A

Development of methodology: Zlatkina V

Collection of data, analysis and/or interpretation of data, writing (not revising) all or sections of the manuscript: Zlatkina V, Karaya O, Yarmish N, Shalimova A

Supervision: Shalimova A

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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.

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Original Article

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Mitochondrial mutations associated with cardiac angina

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Abstract

Aim: Cardiac angina is a disease in which discomfort or retrosternal pain may occur. Atherosclerosis of coronary arteries is one of the main risk factors for cardiac angina. The aim of the investigation was to analyze the association of 11 mitochondrial genome mutations with cardiac angina. In our preliminary studies an association of these mutations with atherosclerosis, a risk factor for cardiac angina, was found.

Methods: We used samples of white blood cells collected from 192 patients with cardiac angina and 201 conventionally healthy study participants. DNA from blood leukocyte samples was isolated using a phenol-chloroform method. DNA amplicons containing the investigated regions of 11 mitochondrial genome mutations (m.12315G>A, m.652delG, m.5178C>A, m.14459G>A, m.3336T>C, 652insG, m.3256C>T, m.1555A>G, m.15059G>A, m.13513G>A, m.14846G>A) were pyrosequenced. The heteroplasmy level of mitochondrial DNA (mtDNA) mutations was analyzed using a method developed by our laboratory on the basis of pyrosequencing technology.

Results: According to the obtained data, three mitochondrial mutations of human genome correlated with cardiac angina. A positive correlation was observed for mutation m.14459G>A ($P \leq 0.05$). One single nucleotide substitution m.5178C>A ($P \leq 0.1$) had a trend for positive correlation. A negative correlation for mutation m.15059G>A with cardiac angina ($P \leq 0.05$) was found.



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Conclusion: MtDNA mutations m.14459G>A and m.5178C>A can be used for evaluation the predisposition of individuals to atherosclerotic lesions. At the same time, mitochondrial genome mutation m.15059G>A may be used for gene therapy of atherosclerosis.

Keywords: Cardiac angina, gene, mutation, heteroplasmy level, mitochondrial genome, molecular cellular models

INTRODUCTION

Cardiac angina is a disease in which discomfort or retrosternal pain may occur. Discomfort is felt by patients as pressure or retrosternal burning. Pain often occurs during physical exertion, excessive food ingestion, stress, being in cold air or with a sharp increase in blood pressure^[1-5]. Cardiac angina is supposed to be caused by narrowing of the arterial lumen up to 50%-75%. As a result, there is a discrepancy between the blood flow to the heart and its need for blood. In this case, acute insufficiency of blood supply to the heart happens. The redox processes in the heart muscle become disrupted^[2-6]. An excessive accumulation of insufficiently oxidized metabolic products (lactic, pyruvic, carbonic and phosphoric acids) and other metabolites occurs. Cardiac angina occurs most often in men over 40, and in women over 50 years. The prevalence of cardiac angina increases with age. For example, in patients who were older than 65 years, the frequency of occurrence of cardiac angina reached 10%-20%. One of the main risk factors for cardiac angina is atherosclerosis of coronary arteries^[2-6]. Other risk factors for cardiac angina include hypertension, diabetes mellitus, obesity, smoking; stress, hypodynamia, infectious diseases, allergic lesions and genetic mutations^[7-12].

Molecular genetic markers for cardiac angina could help identification of predisposition to the disease much earlier than clinical methods for examining patients. At the present time, such studies are mainly devoted to polymorphisms of the genes in nuclear genome.

Our research group found a number of mitochondrial genome mutations associated with cardiac angina. It should be noted that in the study we investigated those mitochondrial mutations for which, in our preliminary studies, we detected an association with atherosclerosis^[13-17]. Since atherosclerosis is a risk factor for cardiac angina, we decided to investigate whether these mutations are linked with cardiac angina.

It should be noted that during the investigation of the mitochondrial genome mutations, the level of heteroplasmy is determined. The ratio of the number of mutant mitochondrial DNA (mtDNA) copies in a sample to the total number of mtDNA copies is estimated^[13-17]. This is the difference between quantitative analysis of mutations in the mitochondrial genome and the analysis of nuclear mutations. In the quantitative analysis of nuclear genome mutations, the number of homozygotes in which both alleles are either mutant or normal. The number of heterozygotes is detected too. Afterwards the mutation frequency in the investigated sample is estimated^[18,19].

The level of heteroplasmy in mitochondrial genome mutations was measured using a quantitative method developed in our laboratory^[14,17,20]. This method is based on the pyrosequencing technology^[21,22]. Short DNA fragments (6-10 bp), containing the area of mutation were investigated. Such a small length of the studied DNA fragments significantly reduces the number of errors during sequencing.

METHODS

We used samples of white blood cells collected from 192 patients with cardiac angina and 201 conventionally healthy study participants. These individuals were examined in Moscow State University clinic. In order to

Table 1. The size of DNA amplicons and primers for PCR

Mutation	Primers	Size of DNA amplicons
m.12315G>A	F: bio-CTCATGCCCCCATGTCTAA(12230-12249) R: TTACTTTTATTGGAGTTGCAC(12337-12317)	108 bp
m.652delG	F: TAGACGGGCTCACATCAC(621-638) R: bio-GGGGTATCTAATCCCAGTTTGGGT(1087-1064)	467 bp
m.3336T>C	F: bio-AGGACAAGAGAAATAAGGCC(3129-3149) R: ACGTTGGGGCCTTTGCGTAG(3422-3403)	294 bp
m.14459G>A	F: CAGCTTCCTACACTATTAAAGT(14303-14334) R: bio-GTTTTTTTAAATTTATTTAGGGGG(14511-14489)	209 bp
m.5178C>A	F: bio-GCAGTTGAGGTGGATTAAAC(4963-4982) R: GGAGTAGATTAGGCGTAGTAG(5366-5345)	383 bp
m.13513G>A	F: CCTCACAGGTTTCTACTCCAAA(13491-13512) R: bio-AAGTCCTAGGAAAGTGACAGCGAGG(13825-13806)	335 bp
m.652insG	F: TAGACGGGCTCACATCAC(621-638) R: bio-GGGGTATCTAATCCCAGTTTGGGT(1087-1064)	467 bp
m.3256C>T	F: bio-AGGACAAGAGAAATAAGGCC(3129-3149) R: ACGTTGGGGCCTTTGCGTAG(3422-3403)	294 bp
m.15059G>A	F: bio-CATTATTCTCGACGGACT(14671-14689) R: GCTATAGTTGCAAGCAGGAG(15120-15100)	450 bp
m.1555A>G	F: TAGGTCAAGGTGTAGCCCATGAGGTGGCAA(1326-1355) R: bio-GTAAGGTGGAGTGGGTTTGGG(1704-1684)	379 bp
m.14846G>A	F: bio-CATTATTCTCGACGGACT(14671-14689) R: GCTATAGTTGCAAGCAGGAG(15120-15100)	450 bp

bp: base pairs

compare the samples of patients with cardiac angina and conventionally healthy study participants more correctly, the samples were composed so that they did not have significant differences in age and sex.

The work was conducted in compliance with the Declaration of Helsinki. The study protocol has been accepted by Ethics Community of National Medical Research Center of Cardiology, and all subjects signed an informed consent for inclusion in the research.

DNA from blood leukocyte samples was isolated using a phenol-chloroform method^[13,14,23-25]. DNA amplicons containing the investigated regions of 11 mitochondrial genome mutations (m.12315G>A, m.652delG, m.5178C>A, m.14459G>A, m.3336T>C, 652insG, m.3256C>T, m.1555A>G, m.15059G>A, m.13513G>A, m.14846G>A) were pyrosequenced. The heteroplasmy level of mtDNA mutations was analyzed using a method developed by our laboratory.

The size of DNA amplicons and primers for PCR are listed in [Table 1](#)^[13-16,20].

In order to be able to perform pyrosequencing of DNA amplicons, one of the primers for PCR was biotinylated.

The total volume of PCR reaction mixtures for each sample was 30 mL. The composition of the reaction mixture for PCR^[13-16,20]: 0.4-0.6 mg mitochondrial DNA, 0.3 pmol/L of each primer, 200 mmol/L of each deoxyribonucleotriphosphate, 16.6 mmol/L (NH₄)₂SO₄, MgCl₂ (1.5 mmol/L for mutations m.14846G>A, m.15059G>A and m.14459G>A; 2.5 mmol/L for the rest of investigated mutations), 67 mmol/L tris-HCl (pH 8.8), and 3 units of Taq-polymerase.

In PCR, the following annealing temperature was used for the primers^[13-16,20]:

1. For mutations m.3336T>C, m.14846G>A, m.13513G>A, m.15059G>A and m.3256C>T - 55 °C;
2. For mutations m.5178C>A, m.652delG and m.652insG - 60 °C;
3. For mutations m.12315G>A, m.14459G>A and m.1555A>G - 50 °C.

Table 2. Primers for pyrosequencing

Mutation	Primer
m.12315G>A	TTTGGAGTTGCAC(12328-12316)
m.652delG	CCCATAAACAAATA(639-651)
m.3336T>C	TGCGATTAGAATGGGTAC(3354-3337)
m.14459G>A	GATACTCTCAATAGCCA(14439-14456)
m.5178C>A	ATTAAGGGTGTTAGTCATGT(5200-5181)
m.13513G>A	AGGTTTCTACTCCAA(13497-13511)
m.652insG	CCCATAAACAAATA(639-651)
m.3256C>T	AAGAAGAGGAATTGA(3300-3286)
m.15059G>A	TTTCTGAGTAGAGAAATGAT(15080-15061)
m.1555A>G	ACGCATTATATAGAGGA(1537-1554)
m.14846G>A	GCGCCAAGGAGTGA(14861-14848)

PCR was conducted using “PTC DNA Engine 200”^[13-16,20].

The DNA amplicons were analyzed on automated pyrosequencing device PSQTMHS96MA (Biotage, Sweden)^[10,11]. Primers for pyrosequencing are listed in Table 2^[13-16,20].

For statistical analysis of the obtained results software package SPSS 22.0 was used^[26]. Bootstrap analysis was also conducted. Correlation was considered statistically significant at the level of $P \leq 0.05$. The results at the significance level of $P \leq 0.1$ were considered to show a tendency to statistical significance.

RESULTS

The age characteristics for study participants are presented in Table 3. The age of conventionally healthy participants ranged from 51 to 73 years. In the meantime, the age of patients with cardiac angina ranged from 52 to 76 years. The average age of conventionally healthy study participants was 2 years less than the age of patients with cardiac angina. This age difference between samples of patients with cardiac angina and conventionally healthy participants was not statistically significant.

Demographic characteristics for study participants are presented in Table 4. The data in Table 4 is presented as an average value with indicating the standard deviation (in parentheses).

According to Table 4, statistically significant differences by clinical and anthropometric characteristics between samples of patients with cardiac angina and conventionally healthy study participants were not found.

The aim of the investigation was to analyze the association of 11 mitochondrial genome mutations with cardiac angina: m.12315G>A, m.652delG, m.5178C>A, m.14459G>A, m.3336T>C, 652insG, m.3256C>T, m.1555A>G, m.15059G>A, m.13513G>A, m.14846G>A. In our preliminary studies, an association of these mutations with atherosclerosis, a risk factor for cardiac angina, was identified. Therefore, we decided to investigate whether these mutations have a link with cardiac angina.

Statistical analysis of the link of these mitochondrial genome mutations with cardiac angina is presented in Table 5.

As illustrated in Table 5, three mitochondrial mutations of human genome correlated with cardiac angina. A positive correlation was observed for mutation m.14459G>A ($P \leq 0.05$). One single nucleotide substitution m.5178C>A ($P \leq 0.1$) had a trend for positive correlation with this disease. We suppose that in case of

Table 3. Age characteristics of the study participants

Investigated individuals	Age			Standard deviation
	Minimum, (years)	Mean, (years)	Maximum, (years)	
Conventionally healthy study participants	51	62	73	8.3
Patients with cardiac angina	52	64	76	8.1

Table 4. Demographic characteristics of the study participants

Parameter	Conventionally healthy study participants	Patients with cardiac angina	Significance of differences
Sex, M/F	91:101	103:98	0.146
Age, years	62 (8.3)	64 (8.1)	0.111
Body mass index, kg/m ²	24.8 (5.9)	26.5 (6.3)	0.152
Systolic blood pressure, mmHg	123 (16)	147 (26)	0.214
Diastolic blood pressure, mmHg	82 (18)	91 (23)	0.319
Smoking, %	29	38	0.167

expansion of the sample, positive correlation m.5178C>A with cardiac angina will become significant. For mutation m.15059G>A a significant negative correlation with this disease was found ($P \leq 0.05$).

DISCUSSION

From the data obtained in this study, it can be concluded that mitochondrial genome mutations m.14459G>A and m.5178C>A are risk factors for the occurrence and development of cardiac angina. Meanwhile, the mutation m.15059G>A had a protective effect in this disease.

The detected mutations were localised in the coding region of mtDNA. Single nucleotide replacements m.14459G>A and m.5178C>A were localised in the genes of the second and sixth subunits of NADH dehydrogenase. We assume that the defects of this mitochondrial respiratory chain enzyme is a trigger of pathological mechanisms in the human body, as a result of which ATP deficiency occurs. Energy deficit, in turn, leads to the emergence and development of cardiac angina.

At the same time, mtDNA mutation m.15059G>A is localised in the cytochrome B gene. Perhaps this mutation is involved in molecular cell processes which protect a person from the occurrence of cardiac angina.

Mitochondrial genome mutations m.14459G>A and m.5178C>A may be candidates for the creation of molecular cell models in the development of drug therapy for patients with cardiac angina. Mutation m.15059G>A can be used for creating gene therapy approaches to this disease.

Molecular genetic markers for cardiac angina could help the identification of predisposition to the disease much earlier than clinical methods for examining patients. At the present time, such studies are mainly devoted to polymorphisms of nuclear genome genes. Studies of mitochondrial genome mutations in cardiac angina are practically absent. Therefore, analysis of the association of mtDNA mutations with cardiac angina, conducted by our research group, is very relevant.

In conclusion, according to the obtained data, three mitochondrial mutations of human genome correlated with cardiac angina. A positive correlation was observed for mutation m.14459G>A ($P \leq 0.05$). One single nucleotide substitution m.5178C>A ($P \leq 0.1$) had a trend for positive correlation. A negative correlation for mutation m.15059G>A with cardiac angina ($P \leq 0.05$) was found.

Table 5. Spearman correlation analysis of 11 mitochondrial genome mutations with cardiac angina

Mutation	Correlation coefficient	Significance
m.12315G>A	0.079	0.121
m.652delG	0.061	0.224
m.3336T>C	0.047	0.342
m.14459G>A	0.116	0.034**
m.5178C>A	0.092	0.068*
m.13513G>A	-0.075	0.129
m.652insG	-0.078	0.124
m.3256C>T	0.069	0.218
m.15059G>A	-0.122	0.036**
m.1555A>G	-0.065	0.217
m.14846G>A	-0.068	0.222

** $P \leq 0.05$; * $P \leq 0.1$

MtDNA mutations m.14459G>A and m.5178C>A can be used for evaluation the predisposition of individuals to atherosclerotic lesions. At the same time, mitochondrial genome mutation m.15059G>A may be used for gene therapy of atherosclerosis.

DECLARATIONS

Authors' contributions

Conception, design and statistical analysis: Sazonova MA
 Pyrosequencing of PCR fragments: Sazonova MA, Sinyov VV
 PCR: Ryzhkova AI, Shkurat TP
 DNA extraction: Sazonova MD, Nikitina NN
 Administrative and material support: Sobenin IA, Orekhov AN

Availability of data and materials

The data were strictly obtained from medical records according to the privacy policy and ethics code of our institute.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study was carried out in accordance with the Declaration of Helsinki. The study protocol was inspected and approved by the Ethics Committee of the Institute of General Pathology and Pathophysiology. Each study participant has signed a written informed consent to participate in this investigation.

Consent for publication

Not applicable.

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Original Article

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The use of lipoprotein apheresis for the treatment of high-risk patients with elevated lipoprotein(a) and hypercholesterolemia

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Abstract

Aim: To assess the safety and efficiency of H.E.L.P.-apheresis and cascade lipid-filtration in the treatment of severe lipid disorders in high-risk patients.

Methods: From 2016 to 2018 we observed 6 patients hyperLDLemia and high Lp(a)emia (> 60 mg/dL). The first group with H.E.L.P.-apheresis ($n = 74$ sessions) included 3 patients who underwent revascularization (coronary, femoral arteries). In the second group with cascade lipid-filtration ($n = 92$ sessions) - one patients underwent revascularization, two patients received drug therapy. Despite the lipid-lowering conventional therapy, no targeted low density lipoprotein (LDL) was obtained.

Results: The patients of the 1st group had threefold decrease of LDL, in patients of the 2nd group LDL decreased by 68%. At the same time, in both groups, we noted a decrease in Lp(a) after the procedure by 65%-68%. Despite a decrease in high density lipoprotein (by 22%-29%) after lipid apheresis procedures, there was a positive trend in apoB100/apoA index (a decrease of 33% after HELP-apheresis procedures and 60% after cascade lipid-filtration) and a decrease in atherogenic index (38% and 53%, respectively). The changes in hematological and haemostatic parameters remained within physiological intervals.

Conclusion: We noticed the successful application of lipid apheresis in patients with multifocal atherosclerosis and its complications.



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Keywords: Lipid disorders, hyperLDLemia, high Lp(a)emia, multifocal atherosclerosis and its complications, atherogenic index, H.E.L.P.-apheresis, cascade lipid-filtration

INTRODUCTION

Despite the progress in diagnostics and therapy of cardiovascular diseases, atherosclerosis and related events (myocardial infarction, stroke, and peripheral vessels damage) are still the leading causes of morbidity and mortality. As assessed by World Health Organization, cardiovascular mortality ranges from 48% to 56% all over the world^[1,2].

The pathogenesis of atherosclerosis includes disorders of lipid and carbohydrate metabolism, hemostasis and immune systems. Dyslipidemia (mainly presented as hypercholesterolemia type IIa and IIb) is the risk factor of atherosclerosis and related events^[3-6]. The process of atherosclerosis can be triggered even in childhood and can evolve throughout life. It is so important to timely evaluate the existing hemostatic disorders, screen risk factors, make the right choice of the treatment, and take early preventive measures^[7].

Treatment of lipid metabolism disorders mainly involves conventional tactics (diet, statins, fibrates, cholesterol absorption inhibitors and, *etc.*). In most cases hyperlipidemia can be quite adequately corrected with the conventional therapy. It is known that statins had beneficial effects on cardiovascular pathology and mortality. However, there are some cases noted for lack of efficiency and resistance to lipid-lowering therapy (monotherapy or combination with medications of multiple effects), intolerance to the pharmacological therapy and development of side effects.

On the other hand, there are combinations of different types of dyslipidemia [hyperLDLemia, hyperLp(a)emia]. Low density lipoprotein (LDL) is well-established risk factor for atherosclerosis that can be treated by lipid-lowering drugs. Lipoprotein(a) [Lp(a)] is independent risk factor for atherosclerotic cardiovascular diseases, that cannot be corrected by dietary changes or medication^[8]. Lp(a) occurs in isolation or in combination with other types of dyslipidemia, that increases atherogenic properties of them^[9-12]. In such cases extracorporeal therapy is an additional and/or alternative approach with proved efficiency. In nowadays apheresis techniques are used for patients with incurable dyslipidemia, hyperLp(a)emia, hyperviscosity syndrome with high fibrinogenemia, high risk of cardiovascular events with damaged vessels^[13-16]. An alternative therapeutic option of hypercholesterolemia can be plasmapheresis.

The first extracorporeal treatment of hypercholesterolemia was performed in 1967 by plasma exchange in patients with familial hypercholesterolemia^[17]. In the late '70s - early '80s in Great Britain, Thompson (1980) managed to reach regression of coronary artery atherosclerosis when lipids' level was lowered aggressively by plasmapheresis^[18,19].

Since then methods, equipment and understanding of extracorporeal therapy have changed significantly. Our goal is to eliminate a large amount of atherogenic substances from the circulation and to change the ratio of lipid in the direction of antiatherogenic. After all, the removal of atherogenic lipids in a large amount (up to 60%-80% per one session) can create conditions for the "release" of cholesterol from plaques. This can be considered as one of the specific mechanisms of influence on the development of atherosclerosis^[20,21]. This problem is managed by lipoprotein apheresis^[22-24].

Current lipoprotein apheresis methods are based on different technologies (filtration, adsorption, precipitation), and their main aim is to remove atherogenic lipoproteins from the circulation^[14,25-27]. They are cascade lipid-filtration, heparin-extracorporeal LDL-precipitation, direct adsorption of lipoprotein,

immunoabsorption of lipoproteins, dextran sulfate adsorption, and all these procedures alter the physicochemical and biochemical properties of lipoproteins.

Greater efficiency and selectivity have been gained with the implementation of new synthetic membranes for rheofilters [Cascadeflow-EC50, Lipidfilter EC-50, Evaflux 4A, 5A (Japan)] in the treatment of lipid metabolism disorders. This type of blood purification procedures was named cascade lipid-filtration^[28]. Klingel *et al.*^[28] (2004) observed the decrease of total cholesterol, LDL, Lp(a) and fibrinogen after treatment of more than 3300mL plasma; no significant changes of levels of high density lipoprotein (HDL), proteins, immunoglobulin were detected, so it is a safe and effective method.

Another LDL-apheresis method based on the precipitation of atherogenic lipids in the acid buffer and with high doses of heparin is called H.E.L.P.-apheresis (Heparin-induced extracorporeal LDL-precipitation). This method aimed at lipids reduction and correction of rheological parameters, hemostasis, immunological homeostasis^[29,30].

The aim of this study is to evaluate safety and efficiency of H.E.L.P.-apheresis and cascade lipid-filtration in the treatment with severe disorder of lipid metabolism in high-risk patients.

METHODS

From 2016 to 2018 we observed 6 patients with multifocal atherosclerosis before and after sessions of myocardial revascularization, arteries of lower limbs (CABG, angioplasty and stenting). The study was approved by the Local Ethical Committee of the Center. Patients included in the study signed an informed consent for extracorporeal therapy. Patients were chosen by the decision of doctors' consilium (cardiologists, cardiac surgeons, specialists of blood purification).

The patients had severe dyslipidemia (type IIa), heart and vessels diseases. All the patients showed hyperLDLemia combined with high Lp(a)emia (> 60 mg/dL), and the level of Lp(a) of 5 patients was higher than 90 mg/dL. The conventional therapy included antiplatelet medications (Clopidogrel, acetylsalicylic acid), lipid-lowering drugs (statins, Ezetrol), if necessary according to the indications - calcium antagonists, ACE/ARA inhibitors, β -blockers [Tables 1 and 2].

The decision to initiate the selective lipid apheresis - treatment was made considering the anamnesis and laboratory data. The first group with H.E.L.P.-apheresis ($n = 74$ sessions) included 3 patients with multifocal atherosclerosis, who had undergone revascularization (coronary arteries, femoral artery) [Table 1]. In the second group with cascade lipid-filtration ($n = 92$ sessions) one patients underwent revascularization surgery, two patients received conventional therapy [Table 2]. Despite the lipid-lowering conventional therapy, no targeted LDL was obtained. Atherogenic indexes remained moderate: in the 1st group on average 3 (2.35-4.5) and in the second group 3.8 (3-6.15).

The main effect of H.E.L.P.-apheresis is the elimination of atherogenic lipoproteins due to precipitation. During H.E.L.P.-apheresis, atherogenic lipoproteins and plasma fibrinogen precipitate on-line in the presence of high heparin doses and acetate buffer. Primarily, blood passes through the plasma filter (surface area $0.3-0.5$ m², rate 60-80 mL/min). Red blood cells are returned to the patient, and plasma is mixed with acetate buffer (pH = 4.85) in the ratio 1:1 and with heparin solution (100 U/mL). This acidic mixture (pH = 5.12) reaches the precipitating filter with the rate 20-30 mL/min (25%-30% of blood flow) and precipitates there with further deposition of insoluble sediments of LDL, Lp(a), triglycerides and fibrinogen. Heparin excess is eliminated from plasma on heparin adsorber (DEAE of cellulose). Bicarbonate dialysis is used for restoration of plasma pH. After that plasma is returned to the patient in combination with red blood cells. If necessary,

Table 1. Clinical characteristics of patients before H.E.L.P.-apheresis therapy

Patients	The disorders of lipid metabolism (with drug therapy)	The manifestations of atherosclerosis (vessels)	Revascularization procedure	Drug therapy	Comorbidity
No.1	Hypercholesterolemia (LDL > 4 mmol/L, atherogenic index > 4) hypertriglyceridemia (> 4 mmol/L), hyperLp(a)emia (> 60 mg/dL)	Coronary	Coronary artery stenting (<i>n</i> = 2)	Statin (atorvastatin 10) antiplatelet agents (clopidogrel, acetylsalicylic acid), angiotensin receptor inhibitors, β -blockers, L-thyroxine	Hypertensive disease Type 1 diabetes, angiopathy, retinopathy, nephropathy. Hypothyroidism
No.2	Hypercholesterolemia (LDL > 4 mmol/L, atherogenic index > 5.5) hypofibrinogenemia (> 4 g/L), hyperLp(a)emia (> 200 mg/dL)	Coronary Brachiocephalic	Coronary artery stenting (<i>n</i> = 6)	Statin (rosuvastatin 10) antiplatelet agents (clopidogrel, acetylsalicylic acid) β -blockers	Iron deficiency anemia Vascular calcification
No.3	Hypercholesterolemia (LDL > 3.5 mmol/L, atherogenic index > 5.5), hypofibrinogenemia (> 4.5 g/L), hyperLp(a)emia (> 60 mg/dL)	Coronary Brachiocephalic Femoral	1. Coronary artery stenting (<i>n</i> = 2); 2. Femoral-popliteal bypass	Statins (rosuvastatin 10), antiplatelets (aspirin, xarelto, clopidogrel), ACE inhibitors, CA antagonists, cytostatics	Chronic kidney disease, after renal transplantation Hypertensive disease Iron deficiency anemia Vascular calcification

LDL: low density lipoprotein

ultrafiltration can be applied (up to 600 mL per session). Up to 4000 mL of plasma can be treated during one session; it corresponds approximately to one plasma volume circulating in an adult. H.E.L.P.-therapy was performed on Plasmate Futura (B|Braun, Germany), which is easy to use and safe to apply. Circuit preparation and reinfusion are automated.

Cascade lipid-filtration is based on the separation (by filtration) on membrane plasma filters with different permeability capacities. It is a consecutive cascade technique affecting specific range of substances with the principle of double-filtration plasmapheresis. First, blood is separated from red blood cells when passing through the plasma filter, and then rheofilter is used for targeted specific elimination of substances. Rheofilters with different permeability are chosen depending on the aim of the treatment. For lipid apheresis techniques, extracorporeal circuit should contain the rheofilter with permeability for substances, whose molecular weight is less than the weight of IgG (< 150000D). After passing through the rheofilter, plasma filtrate containing IgG, HDL and other substations of plasma with lower weight molecules, is returned to the patient with the red blood cells. High weight molecules (LDL, Lp(a), VLDL, triglycerides, chylomicrons, fibrinogen) remain in the rheofilter. Treated plasma volume was 3,500-4,500 mL per session. Cascade lipid-filtration was performed on Plasauto (Asahi, Japan).

In our study we chose cubital veins as vascular access, and no problems with the satisfactory blood flow were noted. We evaluated the clinical and laboratory indications before and after the session.

Statistical analyses were performed with IBM SPSS statistics for Windows (Mann-Whitney *U* test, *P* values less than 0.05). The data are expressed as the median and 25th-75th percentiles.

RESULTS

We performed 166 sessions of H.E.L.P.-apheresis and cascade lipid-filtration for 6 patients with cardiovascular diseases. The procedure frequency was once per 3-4 weeks. No side effects were detected in the patients during the study (allergic reactions, bleeding, *etc.*). No circuit thrombosis was observed. The interviewed patients observed significant improvement of the clinical state. New acute cardiovascular events

Table 2. Clinical characteristics of patients before lipid-filtration therapy

Patients	The disorders of lipid metabolism (with drug therapy)	The manifestations of atherosclerosis (vessels)	Revascularization procedure	Drug therapy	Comorbidity
No.1	Hypercholesterolemia (LDL > 4.5 mmol/L, atherogenic index > 4.5) hypefibrinogenemia (> 4 g/L) hyperLp(a)emia (> 150 mg/dL)	Coronary Brachiocephalic Femoral	Coronary artery bypass graft ($n = 3$), Mitral valve repair with Carpentier techniques	Statin (rosuvastatin 20), Ezetrol antiplatelet agents (clopidogrel, acetylsalicylic acid) ACE inhibitors β -blockers Calcium antagonists	Hypertensive disease Hyperuricemia
No.2	Hypercholesterolemia (LDL > 6.5 mmol/L, atherogenic index > 7) hypefibrinogenemia (> 4 g/L) hyperLp(a)emia (> 60 mg/dL)	Coronary Brachiocephalic	No	Statin (rosuvastatin 40), Ezetrol antiplatelet agents (acetylsalicylic acid) β -blockers	Left ventricular aneurysm Hyperuricemia
No.3	Hypercholesterolemia (LDL > 4 mmol/L, atherogenic index > 3) hypefibrinogenemia (> 4 g/L) hyperLp(a)emia (> 300 mg/dL)	Brachiocephalic	No	Statin (rosuvastatin 40) antiplatelet agents (acetylsalicylic acid)	Iron deficiency anemia Vascular calcification

LDL: low density lipoprotein; ACE: angiotensin converting enzyme

didn't occur, but in one case. The patient with high Lp(a) (more than 180 mg/dL) had dyspnea on exertion (fast walking) and needed coronarography. Subtotal stenosis of the right coronary artery was found out, and was exposed to stenting. In our opinion, it was caused by the extensive posttraumatic bruising of the lower limb in the context of inflammation. During this period according to the laboratory data the patient had high fibrinogenemia (6.5-7.4 g/L), high level of C-reactive protein 3.4-12.6 mg/dL, erythrocyte sedimentation rate (ESR) - 25-32 mm/min, Lp(a) 185-171 mg/dL, LDL - 2.5-2.7 mmol/L, atherogenic index - 1.9-2. The high level of CRP is responsible for atherosclerotic process progression and development of acute complications (even in the presence of normal levels of LDL).

As anticoagulation we used the heparin (15-30 U/kg/h). The level of circuit anticoagulation was estimated according to the activated clotting time, which was maintained within 180-200 s. The heparin supply was stopped before the last 10-15 min of the session.

We noted statistically significant dynamics of almost the studied indications after the procedures [Tables 3-6]. The patients of the 1st group had twofold decrease of total cholesterol and threefold decrease of LDL. The patients of the 2nd group had similar changes: threefold decrease of the total cholesterol and 68% decrease of LDL. Both types of lipid apheresis treatment proved to be effective for Lp(a)emia. We noted significant decrease (more than 65%) of this atherogenic indication following these therapies [Tables 3 and 4]. Hematological parameters, ESR, hemoglobin concentration, fibrinogen, coagulation factors and activity of antithrombin had statistical significance immediately after the procedures [Tables 5 and 6].

After H.E.L.P.-apheresis HDL decreased by 29%, and after cascade lipid-filtration - by 22%. It was confirmed by the dynamics of apoprotein index ApoB100/apoA before and after the therapy (decrease by 33% and almost by 60% while H.E.L.P.-apheresis and cascade lipid-filtration, respectively) and by the atherogenic index (38% and 53%, respectively) [Tables 3 and 4]. Significant changes were also found in the decrease of total protein and albumin levels following both the techniques. The total level of protein decreased by 24% and albumin - by 22% during H.E.L.P.-apheresis, and by 22% and 14%, respectively, during cascade lipid-

Table 3. Changes of laboratory data in the H.E.L.P.-apheresis group

Indices	Before procedures	After procedures	P value
Lp(a), mg/dL	151.5 (80.8-185)	47.6 (33-68.6)	0.001
Total cholesterol, mmol/L	5.0 (4.6-5.5)	2.38 (2.2-2.7)	0.001
Triglyceride, mmol/L	1.5 (1.1-1.9)	0.9 (0.5-1.2)	0.001
LDL, mmol/L	2.9 (2.6-3.2)	1.0 (0.9-1.3)	0.001
HDL, mmol/L	1.28 (1.0-1.5)	0.91 (0.7-1.1)	0.001
Atherogenic index	2.9 (2.3-4.2)	1.8 (1.3-2.8)	0.001
ApoA, mg/dL	142 (116-151)	93 (78-114)	0.001
ApoB100, mg/dL	89 (81-99)	41 (32-49)	0.001
Index ApoB100/ApoA	0.7 (0.6-0.8)	0.47 (0.3-0.6)	0.001
C-RP, mg/dL	0.13 (0.9-0.3)	0.06 (0.04-0.4)	0.001
Total protein, g/L	66 (62-69)	50 (46-53)	0.001
Albumin, g/L	41 (39-43)	32 (29-34)	0.001

LDL: low density lipoprotein; HDL: high density lipoprotein; C-RP: C-reactive protein

Table 4. Changes of laboratory data in the lipid-filtration group

Indices	Before procedures	After procedures	P value
Lp(a), mg/dL	124 (93-169)	42.7 (32.4-54.2)	0.001
Total cholesterol, mmol/L	5.7 (4.5-7.4)	1.9 (1.7-2.8)	0.001
Triglyceride, mmol/L	2.2 (1.7-2.6)	0.7 (0.5-0.8)	0.001
LDL, mmol/L	3.8 (2.5-5.7)	1.2 (0.8-2.1)	0.001
HDL, mmol/L	0.9 (0.9-1.0)	0.7 (0.6-0.8)	0.001
Atherogenic index	4.5 (3.8-6.9)	2.1 (1.5-3.5)	0.001
ApoA, mg/dL	119 (105-140)	95 (83-107)	0.001
ApoB100, mg/dL	111.5 (97.75-147.75)	33 (21.75-53)	0.001
Index ApoB100/ApoA	0.8 (0.75-1.28)	0.34 (0.2-0.65)	0.001
C-RP, mg/dL	0.1 (0.7-0.18)	0.06 (0.04-0.1)	0.001
Total protein, g/L	70 (67-72)	55 (52-57)	0.001
Albumin, g/L	43 (42-46)	37 (35-38)	0.001

LDL: low density lipoprotein; HDL: high density lipoprotein; C-RP: C-reactive protein

filtration. However, these changes were within the physiological intervals and didn't influence the clinical state of patients.

DISCUSSION

Our study presented 2 techniques of selective lipid apheresis for corrected lipid metabolism disorders in the high-risk patients with cardiovascular diseases. This group of patients needed more aggressive lipid-lowering therapy with selective methods of lipid apheresis as the conventional therapy was insufficient and the level of Lp(a) was high. In this regard, we adhered to the clinical guidelines of MH of RF for treatment of Familial hypercholesterolemia, to the recommendations of associations for atherosclerosis treatment and recommendations of apheresis societies^[1,31-34]. According to them, the program lipid apheresis is recommended for the patients with cardiovascular diseases due to atherosclerosis with hypercholesterolemia combined with high levels of Lp(a) (more than 60 mg/dL).

Lipid apheresis therapies (regardless of the type of technique) are mainly aimed at dyslipidemia correction, atherogenic lipids elimination with preservation in antiatherogenic fractions circulation.

The decrease of lipid levels [LDL, Lp(a)] was statistically significant in both groups, and our results correlate with other studies. According to a number of other trials, decrease of atherogenic lipoproteins levels during the treatment is approximately within 60%-80%^[35,36]. Selective elimination of a great amount of lipid substances modifies the ratio of their fractions expectedly during the treatment. Although we

Table 5. Changes of hematological and hemostatic parameters in the H.E.L.P.-apheresis group

Indices	Before procedures	After procedures	P value
Hemoglobin, g/L	131 (128-137)	116 (112-127)	0.001
Hematocrit, %	39 (38-42)	35 (34-38)	0.001
Platelets, 10 ⁹ /L	189 (172-218)	164 (149-178)	0.001
WBC, 10 ⁹ /L	5.9 (4.8-8.4)	5.6 (4.3-7.1)	0.002
ESR, mm/h	15 (12-20)	3 (2-4)	0.001
Fibrinogen, g/L	4.5 (4.2-5.2)	1.92 (1.57- 2.31)	0.001
International Normalized Ratio INR	1.02 (0.97-1.12)	1.53 (1.33-1.76)	0.001
Antithrombin, %	114 (104-118)	78 (72-79)	0.001

WBC: white blood cells; ESR: erythrocyte sedimentation rate; INR: International Normalised Ratio

Table 6. Changes of hematological and hemostatic parameters in the lipid-filtration group

Indices	Before procedures	After procedures	P value
Hemoglobin, g/L	148 (145-153)	138 (134-144)	0.001
Hematocrit, %	45 (43-46)	41 (39-43)	0.001
Platelets, 10 ⁹ /L	209 (199-221)	184 (167-202)	0.001
WBC, 10 ⁹ /L	8.4 (7.4-9.3)	10.2 (9.7-10.3)	0.002
ESR, mm/h	5 (4-6)	2 (2-3)	0.012
Fibrinogen, g/L	3.9 (3.7-4.0)	2.0 (1.8- 2.2)	0.001
INR	1.00 (0.93-1.05)	1.26 (1.17-1.37)	0.001
Antithrombin, %	99 (95-109)	83 (73-94)	0.043

WBC: white blood cells; ESR: erythrocyte sedimentation rate; INR: International Normalised Ratio

observed decrease of LDL level, we pointed out positive correlation changings of atherogenic (LDL) and antiatherogenic (HDL) lipid fractions. This was approved by the change of apoprotein index apo B100/apo A-I and by the dynamics of atherogenic index^[15,30].

One explanation for the beneficial effect on vascular endothelium (and as a consequence, decrease of acute cardiovascular events frequency) is shock pulse decrease of atherogenic lipoproteins after the treatment^[20]. The solution of this problem is lipid apheresis^[33-35].

Some researchers observe that simultaneous decrease of prothrombotic factors and atherogenic lipoproteins during lipid apheresis also can favour endothelium dysfunction improvement, inhibiting the progression of atherosclerotic damage and stabilizing the existing plaque^[21,37].

It is known that the elimination of fibrinogen (more than 60%) and large-molecular substances according to the lipid apheresis techniques affects the blood and plasma viscosity, rheological characteristics and aggregation properties of cells (erythrocytes, platelets)^[37,38]. Terai *et al.*^[39] (2010) noted that changes of retina vessels' diameter are connected with systemic effect of LDL-apheresis, making basis for ocular perfusion improvement in the patients with hypercholesterolemia. In our study, positive feedback from the patients about subjective state improvement, particularly, increase of tolerance to the exertion, no drowsiness, productivity improvement, no dizziness, decrease of heart attacks was explained by objective findings, concerning decrease of large lipid molecular and fibrinogen levels, and as a consequence rheological blood characteristics improvement and microcirculation.

It should be noted a number of important pleiotropic and non-lipid, anti-inflammatory and rheological effects during the selective lipid apheresis^[37,40-42]. Hibino *et al.*^[43] (2009) demonstrated anti-inflammatory and homeostasis-correcting effects of cascade rheofiltration. Hovland *et al.*^[44] (2010) studied the influence of different lipid apheresis therapies (DALI-hemoperfusion, plasma sorption LA-15 and cascade rheofiltration

EC-50W) on hematological and rheological indications (hemoglobin, leukocytes, platelets, fibrinogen, thrombin-antithrombin complex, PAI-1, homocysteine) in the patients with familial hypercholesterolemia. It was shown, that regardless of the technology used apheresis therapy hematological and hemostatic parameters are affected differently, but still remain within the physiological intervals^[44]. This was also proved by our observations, as we didn't note cases with bleedings or thrombosis.

Probably, changes in hematological parameters, decrease of fibrinogen, coagulation factors and antithrombin levels can be also partially associated with some moderate dilution. Taking into account differences of treatment techniques at the stage of the extracorporeal circuit volume return, we noted more expressed changes of these indications immediately after H.E.L.P.-apheresis. The explanation is that approximately, 1.2-1.5 L of saline solution is necessary for returning maximum blood components from the circuit after H.E.L.P.-apheresis. On the other hand, decrease of fibrinogen concentration, INR and antithrombin level can be associated with consumption as a result of procoagulant activation of the blood in contact with the artificial circuit surface. Procoagulant activity is less advanced during cascade lipid-filtration.

Decrease of ESR is associated with reduction of lipid and fibrinogen concentration. The wide variation of ESR in the context of H.E.L.P.-apheresis can be associated with higher residual activity of heparin in the blood of the patient, the indirect evidence of which is elevated ESR.

The sets for measure the level of prothrombin time (INR) (HemosIL, RecombiPlastin 2G, ACL-TOP) include calcium chloride polybrene, which has the capacity to inhibit not more than 1 U/mL dose of heparin. For further studies we plan to estimate the level of heparin anti-Xa activity at different treatment stages. Multidirectional changes of the WBC amount are more likely to be associated with the small randomization and with individual body reactivity of the patients. This reaction is a physiological response to the procedure. Statistically significant changes of hematological parameters after procedures were within or on the border of reference intervals. Taking into account the slight dynamics differences of the analytes measured between the sessions, the treatment is recommended to be individually choice to each patient's condition^[38,45].

Lipid apheresis sessions frequency depends on the response to the therapy and lipidemia level (LDL, Lp(a)). The decreased level of lipids begins to increase gradually after the apheresis treatment. The "growth" degree is defined by catabolism rate and eliminated particles volume, as well as synthesis rate of these molecules. Given the cholesterol synthesis pool (10-14 days), a question occurs, whether it is necessary to perform program extracorporeal therapies, i.e., repeated sessions once per 2-3 weeks for a long period of time^[32,46]. To complete one of the tasks in our study - provide gradual decrease of atherogenic cholesterol baseline to the target level - the sessions were performed once per 3-4 weeks along with pharmacological lipid-lowering therapies and diets. Though the researchers mainly point out the advisable interval of 2 weeks between the sessions, a number of authors show 3-4 weeks interval efficiency, and it correlates with our findings^[25,30,37].

The lipid apheresis therapy was safe and effective. In general, patients had a high appreciation, the sessions proved to be safe and well-tolerated. Types and rates of side effects of lipid apheresis treatment are described by different authors and in the registry of World Apheresis Association^[47-50]. Heigl *et al.*^[51] (2015) studied safety during 6 years and noted good tolerance to different techniques of lipid apheresis. As a whole, according to the results of Heigl *et al.*^[51] (2015), side effects were not more than 1.1% (vascular issues, technical issues). The study of Borberg *et al.*^[48] (2009) on lipid apheresis safety evaluation with more than 2,500,000 sessions registered in the world, confirm the general assessment of a small number of slight and moderately expressed side effects - 3.3%. The issues, described by him, are problems of vascular access, hypotension at the connection stage, allergic reactions, bruising after puncture and technical issues.

Even in the '90s Geiss *et al.*^[52] found out, that membrane cascade filtration is an effective method for decrease of elevated concentrations of atherogenic lipoproteins. And the concomitant loss of other macromolecules

improves blood rheology temporarily, but careful monitoring of proteins and immunoglobulin levels changes is needed to provide the safety of the treatment. Development of new membranes with different cut-off points (permeability) and improvement of equipment will enable safe and effective double or triple filtration in the near future. These hollow fibers will be manufactured with pores of certain size. They will allow performing selective and quite accurate filtration. The main clinical benefit will be the possibility to preserve important physiological proteins, coagulation factors, hormones and enzymes. Thus, cascade rheofiltration methodology can be attributed to the selective lipid apheresis sessions group.

As a result of our study and based on the data of the other researchers, we can notice about successful application of therapeutic apheresis in the complex treatment of patients with multifocal atherosclerosis and its complications. This type of treatment is safe and highly effective for dyslipidemia corrections, elimination of atherogenic lipoproteins, stabilization of atherosclerotic process and endothelium state, hemostasis correction and immunological indications, metabolism disorders, *etc.*

Extracorporeal therapy for lipid metabolism disorders should be tailored to the individual patient condition, depending on clinical and laboratory parameters of the patient (type of dyslipidemia, hemostasis state, comorbidities). Besides, the priority attention while choosing the apheresis method is given to staff expertise and technical capabilities of the clinic.

Lipid apheresis techniques should be applied for primary and secondary prevention of atherosclerosis events in the context of severe disorders of lipid metabolism, refractory and conventional therapy (diet, medications) to improve the treatment prognosis and the quality of life of the high-risk group of patients. Blood purification techniques are known to have high cost, and not all the medical centers are able to apply them for complex therapy of cardiovascular diseases. But this tendency is evolving and penetrating the clinical practice.

DECLARATIONS

Author's contributions

Contributed to the conception, design and methodology of the study, analysed the results and wrote the manuscript: Yaroustovsky M, Abramyan M

Collected samples and provided clinical data: Rogalskaya E, Komardina E

Read and approved the final manuscript: All authors

Availability of data and materials

The relevant data in this study can be obtained from corresponding author.

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None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The related study was approved by the Local Ethics Committee of the A.N.Bakulev NMRCCVS. Patients included in the study signed an informed consent for extracorporeal therapy.

Consent for publication

Not applicable.

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Review

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Monocyte differentiation and macrophage polarization

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Abstract

Circulating monocytes are recruited to tissues, where they differentiate to macrophages and take part in the inflammation process or tissue remodeling. According to the traditional concept, macrophages are classified into pro-inflammatory (M1), non-activated (M0) or anti-inflammatory (M2) subsets that play distinct roles in the initiation and resolution of inflammation. This heterogeneity exists already at the monocyte level since monocytes can also belong to pro- or anti-inflammatory phenotypes. Growth factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and M-CSF play a principal role in their activation: GM-CSF drives the differentiation of “pro-inflammatory” monocytes to M1 macrophages, while M-CSF regulates differentiation of the “anti-inflammatory” subset of monocytes to M0 macrophages that have M2-like phenotypic and functional properties. More recent experimental findings led to a substantial update of monocyte-macrophage nomenclature to include the nature of the polarizing signal. In response to pro-inflammatory stimuli, monocytes can be directly polarized into 3 subsets of macrophages with the pro-inflammatory M1-like phenotype; with macrophages induced by interferon- γ having the strongest pro-inflammatory properties. When exposed to various anti-inflammatory stimuli, monocytes can differentiate to at least 5 subsets of M2-like macrophages. Of those, a subset generated under exposure to IL-4 (IL-13) has the most typical M2-like characteristics. Both in humans and in mice,



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monocyte-to-macrophage differentiation involves global transcriptome changes that are tightly controlled by various transcriptional regulators and signaling mechanisms. In this review, we discuss monocyte-macrophage heterogeneity and signaling pathways regulating the differentiation at transcription level.

Keywords: Monocyte, macrophage, differentiation, polarization, inflammatory M1 phenotype, anti-inflammatory M2 phenotype, transcriptional regulation

INTRODUCTION

Being a key component of the innate immunity, macrophages are involved in phagocytosis and clearance of cell debris, invading microorganisms, foreign bodies, modified or damaged cells, and other objects and substances that do not express on their surface markers specific for normal body cells^[1]. Initially, 2 major phenotypes of macrophages have been distinguished: pro-inflammatory M1 phenotype, and anti-inflammatory M2 phenotype. This simplified classification reflected a similar distinction between Th1 and Th2 lymphocytes. M1 macrophages release cytokines and chemokines essential for activation and recruitment of lymphocytes to the inflamed sites. Macrophages also perform antigen-presenting function essential for the induction of the humoral immune response^[2]. Alternatively-activated M2 macrophages control and resolve inflammation through releasing anti-inflammatory cytokines. These macrophages participate in wound healing, post-inflammatory tissue repair and remodeling^[2]. While M1 activity suppresses cell proliferation and promotes tissue damage, M2 activity induces tissue regeneration and stimulates cells to proliferate. The differences in functional properties of the M1 and M2 macrophage subsets are reflected by differences in arginine metabolism. M1 macrophages possess a unique capacity to generate a “killer” molecule nitric oxide (NO) from arginine, which is widely used to damage and kill pathogens through production of peroxynitrite. By contrast, M2 macrophages transform arginine to the “repair” amino acid molecule ornithine, which is further involved in the synthesis of proline and polyamines^[3].

In macrophages, polarization and phenotype switching are accompanied by global changes in cell transcriptome and proteome that are strictly regulated by exogenous and intrinsic stimuli. Failure to control macrophage plasticity may result in maladaptive response leading either to inflammatory diseases and tissue damage (in a case of excessive M1-polarized response) or to tissue fibrosis and cancer (in case of extensive M2-polarized response).

MONOCYTE HETEROGENEITY

Monocytes that give rise to the tissue macrophage population are also characterized by substantial heterogeneity, which may underlie that of macrophages. Early studies showed the presence of two main monocyte subsets in mice^[4]. Pro-inflammatory monocytes (characterized as Gr1⁺/Ly6C^{high}CCR2⁺CX3CR1^{low}) can give rise to inflammatory macrophages and dendritic cells, while anti-inflammatory monocytes (Gr1⁻/Ly6C^{low}CCR2⁻CX3CR1^{high}) perform patrolling functions and differentiate to M2 macrophages^[5]. It was hypothesized that the occurrence of such subsets that serve as precursors of either pro-inflammatory or anti-inflammatory macrophages can suggest for the presence of two distinct or overlapping mechanisms in monocyte differentiation. However, this hypothesis remains to be confirmed experimentally. Recently, Ly6C^{high} monocytes were shown to serve as precursors of Ly6C^{low} cells in homeostatic conditions when transplanted to the control mice being able to spontaneously differentiate to Ly6C^{low} cells both in the blood and in bone marrow^[6]. In the absence of inflammation, Ly6C^{high} monocytes migrate and accumulate in the bone marrow where they transform to the Ly6C^{low} sub-population^[6,7]. Functionally, the Ly6C^{high} subset is involved in restoration of tissue-specific resident macrophages and replenishment of Ly6C^{low} monocytes (in steady-state conditions) or induction of inflammation and antigen processing (in inflammatory conditions). Apart from patrolling, Ly6C^{low} monocytes participate in anti-viral response thanks to their ability to recognize viral nucleic acids due to high expression of toll-like receptor (TLR)-7^[8]. This subset can also be

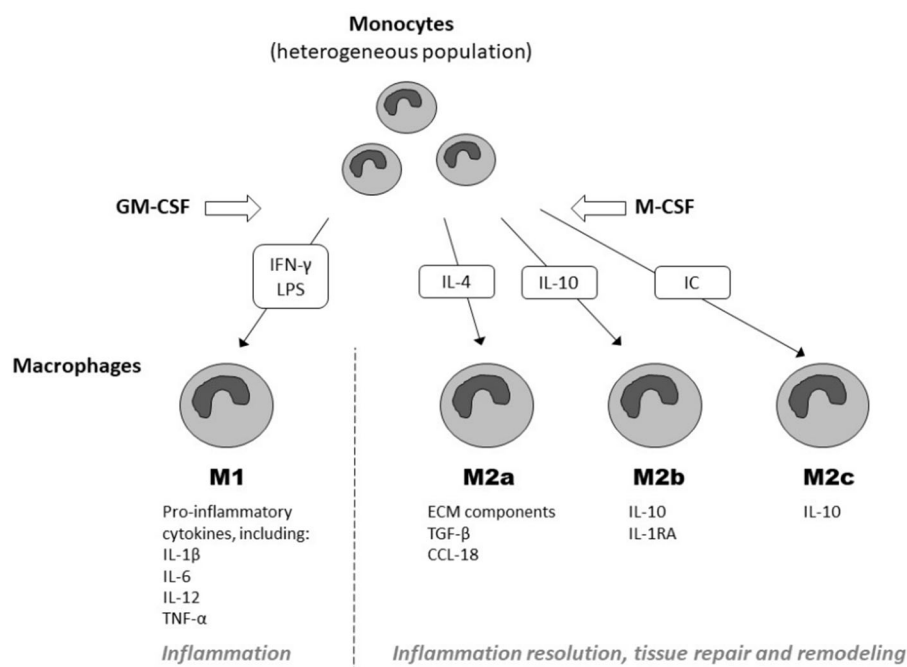


Figure 1. Principal transcriptional regulators of M1 and M2 activation of macrophages and mechanisms of their stimulation/inhibition. GM-CSF: granulocyte-macrophage colony-stimulating factor; M-CSF: macrophage colony-stimulating factor; IFN-γ: interferon γ; LPS: lipopolysaccharide; IL: interleukin; IC: interferon consensus; TNF-α: tumor necrosis factor α; TGF-β: transforming growth factor β; CCL18: chemokine CC-motif ligand 18

involved in post-inflammatory tissue repair^[9]. These observations suggest that the plasticity of monocytes may precede the plasticity of macrophages.

The transcription factor NR4A1 (also known as the nerve growth factor IB) is essential for the commitment of Ly6C^{low} monocytes^[8]. Deficiency of this factor reduces the cell count of the Ly6C^{low} cells in the bone marrow, but does not affect their numbers in the blood and spleen^[10,11]. These observations suggest for the possibility of Ly6C^{low} cells to develop from myeloid precursors in the bone marrow or define the Ly6C^{low} subset as terminally differentiated population of tissue macrophages. The latter notion is supported by the observation that Ly6C^{low} cells have a significantly (10 to 20-fold) longer half-life than Ly6C^{high} cells^[6].

In humans, the population of monocytes is also heterogeneous^[12]. The majority of human monocytes (85%-90%) is represented by so called “classical” monocytes that express CD14 but do not express CD16 (i.e., have CD14^{high}CD16⁻ or CD14⁺CD16⁻ phenotype). The remaining population (10%-15%) is divided to two monocyte subsets: “the intermediate subset” that highly expresses CD14 and has CD16 expression at low levels (CD14^{high}CD16⁺ or CD14⁺CD16⁺) and “non-classical monocytes” that have high CD16 expression and a relatively low expression of CD14 (CD14^{dim}CD16⁺ or CD14⁺CD16⁺⁺)^[13]. Classical monocytes are involved in the phagocytosis and inflammation. The “intermediate” subset also participates in the inflammatory responses while non-classical monocytes mainly perform the patrol function and contribute to the antiviral responses^[14].

THE ROLE OF GM-CSF AND M-CSF IN MACROPHAGE DIFFERENTIATION

M-CSF and GM-CSF are the primary cytokines that stimulate macrophage differentiation [Figure 1]^[15]. Macrophages induced by both factors have distinct properties and functions: cells induced by GM-CSF participate in antigen presentation and produce inflammatory factors IL-12, IL-23 and tumor necrosis factor (TNF)-α, while M-CSF-induced cells are more involved in scavenging and phagocytosis and release anti-

inflammatory cytokine IL-10^[16]. It can be postulated that GM-CSF-induced macrophages belong to M1, and M-CSF-induced - to M2-like phenotype^[17].

Moreover, M-CSF can also drive differentiation of monocytes to naïve M0 (M2-like) macrophages that can be subsequently polarized to pro- or anti-inflammatory phenotypes by the different activating stimuli^[18]. Therefore, M1 macrophages can be induced directly by GM-CSF or by stimulation of M0 cells with inflammatory factors. Human-specific pro-inflammatory M4 macrophages can be induced by chemokine C-X-C motif ligand 4 (CXCL4) and are phenotypically distinct from M1 and M2 macrophages due to the weak phagocytic capacity, increased resistance to foam cell formation, down-regulated expression of hemoglobin scavenger receptor CD163, and elevated expression of matrix metalloproteinases (MMP)-7 and MMP-12^[19,20].

Exposure of M0 to Th2 cytokines IL-4/IL-13 leads to the formation of classical alternatively activated M2a macrophages. Immune complexes, lipopolysaccharide (LPS) and IL-1 β induce M0 polarization to M2b phenotype, while anti-inflammatory agents such as IL-10, transforming growth factor β (TGF- β) or glucocorticoid hormones promote the conversion of M0 macrophages to M2c^[21]. Furthermore, murine M1 macrophages exhibit IL-4 receptor-independent phenotypic switch to vascular endothelial growth factor- and IL-10-producing M2d macrophages in the presence of adenosine^[22]. In summary, macrophages are fully differentiated cells that, however, show significant phenotypic plasticity in response to dynamically changing tissue environment^[23].

Functional and phenotypic plasticity of macrophages is essential for successful healing of tissue injury and elimination of infection. In the inflamed site, M1 macrophages are involved in fighting and killing pathogens/cancer cells and further removal of dead cells and cell debris. M1 macrophages strongly contribute to the recruitment of monocytes and lymphocytes to the site of injury. When the injured site is cleared, M1 macrophages do not disappear but undergo a phenotypic switch to M2 macrophages in response to anti-inflammatory signals that are responsible for resolving inflammation and inducing tissue repair and remodeling in a pathogen-free microenvironment^[24]. M1-M2 switching also helps to avoid excessive influx of pro-inflammatory immune cells to the inflamed site^[25].

UPDATES TO MACROPHAGE CLASSIFICATION

In the recent years, macrophage classification was updated to reflect the complexity of the identified macrophages subtypes. The proposed nomenclature takes into account the activation signal that drive monocytes differentiation^[26]. According to this classification, macrophages with best pronounced features of M1 phenotype are induced by interferon (IFN)- γ both in mice and humans. Noteworthy, human and murine IFN- γ -induced macrophage transcriptomes differ by the activation of suppressor of cytokine signaling cytokines (SOCS) in mouse cells that inhibit their polarization to the anti-inflammatory M2-like phenotype. In humans, IFN-regulatory factor (IRF)-5 is a major regulator in the inflammatory polarization to the M(IFN- γ) subset.

The exposure of naïve (M0) macrophages to LPS or a combination of LPS and IFN- γ results in the formation of the M2-like macrophage phenotypes that seem to be less pro-inflammatory than the macrophage subset generated under influence of IFN- γ alone. In the presence of LPS+IFN- γ or LPS alone, phenotypic polarization of macrophages is mediated by STAT1^[27]. In addition, in both murine LPS+IFN- γ - or LPS-induced macrophages, SOCS1 and NF- κ B inhibitor ζ (NFKBIZ) are involved in polarization of murine monocytes to these macrophage sub-populations^[26]. NDKBIZ is induced by LPS and serves as a conductor of the pro-inflammatory effect of LPS by the interaction with other NF-B proteins *via* C-terminal ankyrin-repeat domains^[28]. In humans, STAT-1, IRF-1, and IRF-5 drive the polarization towards the LPS+IFN- γ -induced phenotype, while IRF-5 alone is primarily involved in the transcriptional control of polarization to

macrophages induced by LPS^[29].

According to the modern nomenclature, at least five M2-like macrophage subsets can be distinguished. Three of those induced by IL-4 (IL-13), immune complexes or IL-10 [termed M(IL4), M(Ic), and M(IL-10) respectively] exhibit the most anti-inflammatory properties and correspond roughly to M2a, M2b, and M2c subsets of the old classification. In mice, IL-4-activated macrophages are characterized by high expression of STAT-6, but also express STAT-1, SOCS2, and IRF-4, while M(IL-10) macrophages produce STAT-3, SOCS3, nuclear factor, interleukin 3-regulated (NFIL3), and strawberry notch homolog 2 (*Drosophila*) (SBNO2) transcription factors. While SOCs are needed to block the pro-inflammatory activation of macrophages, both NFIL3 and SBNO2 contribute to the downstream anti-inflammatory effects of IL-10^[30].

In humans, IL-4-activated macrophages produce IRF-4, SOCS1, and GATA3 essential for the establishment of the IL-4-dependent anti-inflammatory transcriptional program. In humans, M(IL10) cells highly produce SOCS3 while TGF- β and glucocorticoids-induced macrophages (roughly corresponding to M2c phenotype) express SMAD2, DNA-binding protein inhibitor ID3, and regulator of G-protein signaling 1 transcriptional regulators. These proteins are required for the activation of the multistep TGF- β -specific transcription program. Glucocorticoid-dependent stimulation of the surface expression of the TGF- β receptor II is necessary for TGF- β -mediated signaling^[31]. Glucocorticoid hormones are also involved in the induction of TGF- β receptor II in human M(GC) macrophages. By contrast, expression of this receptor is absent in IL-4-induced human macrophages^[31].

IRF/STAT SIGNALING

The IRF/STAT-regulated pathways are key mediators of M1/M2 macrophage activation. In response to Th1 cytokines and inflammatory stimuli, stimulation of STAT-1/STAT-2 and IRF-5 primes differentiation to M1 cells, while STAT-3/STAT-6 and IRF-3/IRF-4 critically contribute to the formation of the M2 phenotype.

Role of IRF/STAT signaling in M1 differentiation

Generally, GM-CSF drives the commitment of the myeloid cell lineage to the bone marrow but also supports monocyte transformation to M1 macrophages. The GM-CSF receptor exists as heterodimer consisted of α - and β -subunits, which can form a homohexamer as a functional ternary complex^[32]. Activation of the GM-CSF receptor leads to Janus kinase 2 (Jak2)-mediated stimulation of STAT-5 and Erk/Akt-dependent pathway, and nuclear translocation of transcription factors IRF5 and NF- κ B^[33].

In monocytes, stimulation with GM-CSF upregulates the antigen-presenting function, phagocytosis, anti-microbial activity, and production of pro-inflammatory cytokines (IL-1 β , IL-6, IL-8, TNF- α) and growth factors (M-CSF, GM-CSF). A global transcriptome analysis of GM-CSF-induced macrophages revealed up-regulation of 340 genes responsible mainly for antigen presentation, lipid metabolism, and innate immune signaling including macrophage-specific surface markers/receptors such as CD14, CD163, C5R1, CSF3R, GDF15, and Fc γ R1A^[34].

STAT-1 and STAT-2

IFN- γ binding to its receptor leads to the recruitment of Jak1/2 and formation of the functionally active STAT-1 dimer^[35], which then binds to the interferon- γ activated sequence (GAS) in the promoter of its target genes, such as IL-12 and inducible NO-synthase (iNOS) and stimulates their expression^[36]. LPS binding to TLR4 induces the subsequent activation of NF- κ B, which drives transcription of a whole set of inflammatory genes^[37]. LPS also stimulates production of Type 1 IFN, which through autocrine binding to its receptor IFNAR leads to the Jak1/Tyk2-mediated activation of STAT-1 and STAT-2^[38]. Activated STAT-1 and STAT-2 form, in turn, a complex with IRF-9. The assembly of STAT-1/2 and IRF-9 results in the induction of

the IFN-stimulated gene factor 3 complex to drive transcription of target genes from the regulatory IFN-stimulated response elements^[39].

Experiments involving genetic deletion/inactivation of STAT-1 showed great significance of this transcriptional activator for induction of M1-mediated pro-inflammatory responses. STAT-1-deficient mice had limited capacity to remove pathogens and decreased resistance against *Listeria monocytogenes*, an infectious bacterium. In macrophages lacking STAT-1, Type I IFN- and IFN- γ -dependent responses were greatly reduced and impaired resulting in the loss of IFN- β production, Type I IFN-dependent signaling and generation of M1 phenotype^[40]. STAT-2-deficient mice exhibited defects in antiviral immune response due to the absence of Type I IFN autocrine/paracrine signaling^[41].

However, the role of STAT-2 in IFN- γ signaling is probably dispensable since could be partially compensated by STAT-1 that is able to form transcriptionally active homodimers and drive expression of IFN- γ target genes including IRF-1 and major histocompatibility complex (MHC) class I^[41]. In STAT2-deficient mice, IFN- α is able to induce MHC class II expression due to the dysfunctionality of the inhibitory feedback loop in response to Type I IFN. This feedback mechanism is mediated by SOCS1, which prevents Jak1-dependent phosphorylation of STAT-2^[42].

Role of IRF signaling in M1 differentiation

IRF family contains 9 members that perform different signaling functions. Several of them, IRF-1 to IRF-5 and IRF-8 play key roles in macrophage polarization. Pro-inflammatory macrophage polarization largely depends on IRF-5, which can play a decisive role in choosing the pro- or anti-inflammatory polarization pathways^[43]. The expression of IRF-5 is stimulated by GM-CSF and can be induced by the activation of TLR and other pattern-recognizing receptors during infection^[44]. Activation of the nucleotide-binding oligomerization domain-containing protein 2 receptor leads to the IRF-5 up-regulation due to receptor-interacting serine-threonine kinase 2 (RIPK2)-mediated phosphorylation^[45]. IRF-5 transcriptional activity can be also stimulated by inhibitor of NF- κ B kinase subunit β (IKK- β)^[46] and TANK-binding kinase 1 (TBK1)^[45].

IRF-1 is another IRF involved in pro-inflammatory polarization of macrophages. Its expression is low in resting macrophages and can be up-regulated by IFN- γ upon M1 polarization^[47]. The activation of IRF-1 is promoted by casein kinase II^[48] and inhibited by I κ B kinase- ϵ NF- κ B^[49]. The effects of IRF-1 include up-regulation of pro-inflammatory genes, which can be mediated by cooperation with NF- κ B and c-Jun, known as “enhansosome” formation NF- κ B^[50]. In murine macrophages, IRF-1 and IRF-2 were shown to regulate LPS-induced expression of TLRs^[51]. At the same time, IRF-1 and IRF-2 can transcriptionally repress the expression of anti-inflammatory (M2) genes, as was shown for IL-4^[52].

IRF-8 is characterized by a weak interaction with chromatin in macrophage nucleus, which can, however, be strengthened following interaction with PU.1, IRF-1 or IRF-2, which reduces its mobility^[53]. Such interaction can occur as a result of LPS stimulation, and results in up-regulation of a number of target genes^[54]. Among the pro-inflammatory genes induced by IRF-8 are IL-12p40, IL-12p35, IFN- β , and iNOS, that are characteristic for the M1 macrophage phenotype^[55]. The enhancement of IRF-8 function as transcription activator can occur via its ubiquitination in an E3 ubiquitin ligase tripartite-motif 21 (TRIM21)-dependent manner^[56]. By contrast, sumoylation of IRF-8 increases its activity as transcription repressor^[57].

IRF-2 recognizes the same regulatory elements in the promoters of target genes as IRF-1 and can act as its competitive inhibitor^[58]. At the same time, IRF-2 can play a role of activator for IRF8, leading to the induction of neurofibromin 1 (NF1) transcription^[54]. Like IRF-8, IRF-2 is regulated by sumoylation, which enhances its transcriptional suppressor function^[59]. The role of IRF-2 in macrophage polarization is not

straightforward, since it regulates the LPS-induced expression of pro-inflammatory cytokines differently, stimulating the production of IFN- γ , IL-1, IL-6, and IL-12, but suppressing that of TNF- α ^[60]. Experiments on knock-out mice suggested that IRF-2 can play a role in macrophage survival and inhibition of apoptosis, regulating the expression of caspase-1^[61]. Moreover, IRF-2 was shown to be important for the immune response to bacterial infection^[62]. The overexpression of IRF-2 had a protective effect in a liver ischemia-reperfusion injury model via suppression of inflammation and hepatic damage mediated by IRF-1^[63]. Taken together, these observations suggest that the role of IRF-2 in macrophage activation can be context-dependent, promoting inflammation during infection, while dampening inflammation in sterile conditions.

Role of IRF/STAT signaling in M2 differentiation

Polarization of anti-inflammatory M2 macrophages is mediated by STAT-3 and STAT-6 signaling in response to IL-4 and IL-13 with the involvement of IRF-3 and IRF-4. In monocytes, IRF-3 prevents the pro-inflammatory polarization to M1 macrophages while IRF-4 promotes the anti-inflammatory M2a phenotype. During M2 polarization, IRF-4 strongly cooperates with the epigenetic regulator histone demethylase Jumonji D3 (JMJD3), which activates the expression by demethylating repressive histone H3 lysine 27 H3K27 trimethylation (H3K27me3) epigenetic marks in the promoters of many immune-related genes^[64]. JMJD3 can activate TGF- β signaling through the SMAD3 pathway^[65] and induce M2 polarization through STAT-6 signaling^[66].

STAT-6

STAT-6 is involved in IL-4-induced expression of JMJD3 by binding to consensus sites of the *Jmjd3* promoter^[66]. Up-regulated expression of JMJD3 reduces demethylation and trimethylation of H3K27 and stimulates the expression of specific M2 marker genes. STAT6 is important for transcriptional regulation of IL-4/IL-13-induced M2 activation. The receptors of both cytokines share the common α -chain (IL4R α) that mediates signal transduction^[67]. Interaction of either IL-4 or IL-13 with the ligand-binding receptor subunit results in Jak1/Jak3- or Jak1/Tyk2-mediated activation of STAT-6 respectively. Phosphorylated STAT-6 forms a transcriptionally active dimer, which in cooperation with IRF-4 conducts transcription of target genes^[68].

Like most other STATs, STAT-6 binds to IFN- γ -activated sites, which represent a palindromic sequence TTCNNNGAA separated by a 3-bp spacer. STAT-6 binds with a greater affinity to the sites with 4-bp spacers like TTCNNNGAA^[69]. In murine macrophages, STAT-6 mediates the transcription of M2-specific marker genes such as *Fizz1* (resistin-like- α ; *Retnla*), mannose receptor 1 (CD206), *Ym1* (chitinase 3-like 3; *Chi3l3*), and arginase-1 and contributes to IL-4-dependent down-regulation of anti-inflammatory genes^[70]. STAT-6-deficient mice lack IL-4-dependent Th2 responses, M2 polarization, and increased IFN- γ -STAT-1 signaling that suggests a key role of this immunoregulator in providing IL-4-dependent stimuli and commitment of the M2 phenotype^[71].

STAT-3

STAT-3 mediates the effects of anti-inflammatory cytokine IL-10 on gene transcription^[72]. In macrophages, IL-10 binding to the receptor stimulates STAT-3 recruitment *via* phosphorylation by Jak1. Up-regulation of STAT-3 is followed by down-regulation of pro-inflammatory cytokines including IL-1, IL-12, TNF- α , and IFN- γ through IL-10-dependent signaling indicating the role of STAT-3 in M1-M2 switch. Deficiency of STAT-3 in mice is characterized by impaired antimicrobial immune responses and increased release of pro-inflammatory cytokines and IL-10 in response to stimulation by LPS^[73] suggesting an important role of STAT-3 in IL-10-mediated inhibition of inflammation. However, STAT-3 can provide either anti-inflammatory or pro-inflammatory responses in a signal-dependent manner. In severe combined immunodeficient mice, IL-10 produced by transplanted regulatory T cells induces STAT-3-mediated M2 polarization in macrophages, while activation with IL-6 and IFN- β is pro-inflammatory^[72].

STAT-3 is negatively regulated by SOCS3, which can be induced by pro-inflammatory mediators in order

to prevent M2 differentiation. SOCS3 can induce STAT-3 degradation through ubiquitination-dependent mechanisms or inhibit Jak-dependent activation of STAT-3^[74]. STAT-3 and p38 mitogen-activated protein kinases (MAPK) participate in the reciprocal control of macrophage response to activation with LPS. This includes control of SOCS3 expression and p38 MAPK-dependent stimulation of protein kinases MK2 and MK3, which mediate up-regulation of pro-inflammatory NF- κ B or anti-inflammatory IRF-3 in response to LPS^[73]. The crosstalk between STAT-3 and p38 MAPK is important for initiation of the pro-inflammatory macrophage response and regulation of the inflammation resolution, which is largely mediated by IL-10 and STAT-3.

Role of IRFs in M2 differentiation

It was suggested that IRF-3 may play an important role mediating M2 differentiation of macrophages. Dephosphorylated IRF-3 maintains self-inhibitory conformation and is inactive^[75]. TBK1 and/or IKK- ϵ phosphorylate IRF-3 lead to conformational changes, which abolish self-inhibitory structure and allow binding of coactivators CBP/p300 followed by nuclear translocation and activation of the factor^[76]. IKK- β -dependent phosphorylation of IRF-3 abrogates its transcriptional activity and stimulates further polyubiquitination and degradation mediated by E3 ubiquitin ligase cullin or RBCK1^[77,78]. M1 macrophages induced by GM-CSF are characterized by inactive IRF-3 and up-regulated MyD88 and active NF- κ B and AP-1 transcription factors^[16]. By contrast, macrophages stimulated by M-CSF are characterized by active TLR-induced IRF-3 and decreased NF- κ B activity^[16,79]. These observations were confirmed in the glial cells, which can be regarded as resident macrophages, where IRF-3 suppressed pro-inflammatory genes IL-1, IL-6, IL-8, TNF- α and CXCL1 and activated anti-inflammatory genes, such as IL-1 receptor agonist, IL-10 and IFN- β ^[80]. In summary, IRF-3 mediates M-CSF-dependent polarization of alternatively-activated macrophages. At the same time, it can also promote the expression of a number of pro-inflammatory genes, such as IFN- β and chemokine CCL5^[81].

Together with IRF-8, IRF-4 belongs to 'hematopoietic' transcription factors^[82]. The activation of IRF-4 leads to homodimerization and assembling with Pu.1, a Ets transcription factor, which can also bind IRF-8^[83,84]. Complexes of IRF-4 and IRF-8 with Pu.1 can cooperate to enhance the expression of target genes^[85].

The prominent role of IRF-4 in the alternative polarization of macrophages has been demonstrated^[86]. M2-specific macrophage genes are controlled by epigenetic regulation, and JMJD3 is able to remove the methylation marks and induce their expression^[66]. In macrophages, IL-4 activates both JMJD3 and IRF4^[87], which can, in their turn, activate each other. As a result of this activation, a set of M2-specific genes is up-regulated, including arginase 1, Fizz1, Ym1, and mannose receptor (MR). Moreover, IRF-4 induces the expression of IL-4 and IL-10 cytokines^[88]. In accordance with these data, IRF-4-deficient mice are susceptible to LPS-induced sepsis and have increased expression of IL-6 and TNF- α in response to TLR ligands^[89]. IRF-4 also prevents M1 polarization of macrophages by competing with IRF-5 for interaction with MyD88, a potent activator of pro-inflammatory factors NF- κ B^[43].

Role of NF- κ B and AP-1 in M1 differentiation

NF- κ B and AP-1 are two key transcription factors that drive expression of a bulk of inflammatory genes in macrophages.

NF- κ B

NF- κ B activates transcription of various inflammatory genes. In the absence of inflammatory stimuli, NF- κ B forms inactive complex with I κ B. Upon inflammatory activation with LPS and other ligands, I κ B phosphorylation induces its dissociation and NF- κ B transition to the nucleus^[90]. On the cell surface, LPS binding protein (LBP) serves for LPS capture and delivery to the pattern recognition receptor CD14^[91]. CD14-LPS complex then binds to TLR-4 assembled with lymphocyte antigen 96 (also known as MD2) and activates TLR-4-dependent intracellular signaling, which is mediated by MyD88 or in MyD88-independent (TRIF-dependent) manner.

MyD88-dependent mechanism activates the expression of pro-inflammatory cytokines, while TRIF-

dependent pathway controls induction of Type I IFNs and IFN-responsive genes. I κ B kinase (IKK) mediates inhibitory I κ B phosphorylation and depression of NF- κ B^[92]. LPS also induces the expression of IL-1 β and TNF- α that support and propagate NF- κ B signaling in an autocrine manner^[93]. LPS activates various MAPKs that induce AP-1. The catalytic subunit of NF- κ B RelA (p65) controls MAPK-independent IKK ϵ -mediated activation of AP-1 through phosphorylation of c-Jun and subsequent removal of the nuclear receptor corepressor (NCoR) from the target promoters^[94]. NF- κ B facilitates AP-1-mediated transcription thereby promoting the integration of NF- κ B and AP-1-dependent expression of pro-inflammatory genes including iNOS, CCL2, CCL5, and Cox-2^[90].

The homodimerization of NF- κ B1 (p50) and NF- κ B2 (p52) leads to generation of transcriptional repressors, since both molecules lack the transcription activation domain presented in the other members of the NF- κ B family: RelA, RelB, and c-Rel^[95]. The inhibitory subunit p50 binds to the promoters of NF- κ B-inducible inflammatory genes and blocks their transcription. Macrophages lacking p50 develop enhanced M1-polarized response to stimulation with LPS^[79,96]. In macrophages, p50 deficiency is associated with altered recruitment of RNA polymerase II to M2-specific promoters such as CCL17 and arginase-1 whereas transcriptional activation of M1-specific promoters including iNOS, IFN- β , and TNF- α is up-regulated^[95]. Regulation of NF- κ B family transcriptional activity plays a central role in M1-M2 switching and macrophage polarization towards either anti-inflammatory or pro-inflammatory phenotype.

AP-1

AP-1 recruitment is mediated by the Jnk-dependent mechanism in response to inflammatory stimuli. The spectrum of AP-1 transcription targets overlaps substantially with that of NF- κ B^[90]. AP-1 comprises a group of heterodimeric or homodimeric basic leucine zipper (bZIP) transcription factors composed of proteins that belong to the c-Fos, c-Jun, ATF, and JDP families that recognize DNA sequence ATGAGTCAT^[90]. Of possible heterodimeric AP-1 variants, c-Fos/c-Jun heterodimers have the highest affinity for AP-1 binding sites^[97]. Jnk-dependent phosphorylation of c-Jun subunit induces formation of the transcriptionally active c-Jun/c-Fos heterodimer^[98].

In macrophages, TLR-4 stimulation with LPS induces TNF- α production, which provides a positive feedback for maintaining transcriptional activity of AP-1 through binding to the receptor TNFR1 and activation of JNK^[99]. In M1 polarized macrophages, AP-1 and NF- κ B share many signaling networks and transcription targets that can suggest a concomitant stimulation and cooperative activity of both factors^[100]. Since both AP-1 and NF- κ B can be activated LPS, the regulatory regions of many LPS-inducible genes such as CXCL2, CXCL9, CXCL10, CCL4, and iNOS contain coupled AP-1/ κ B binding elements.

LPS can also induce the removal of transcription repressor complexes from mixed AP-1/ κ B sites to initiate transcription. In resting macrophages, the activity of promoters of a variety of inflammatory genes is blocked by repressor complexes. For example, NCoR associates with silencing mediator of retinoic or thyroid hormone receptors (SMRT; also known as NcoR2) to form a corepressor complex, which binds either to c-Jun or p50 and inhibits transcription from AP-1/ κ B sites^[101]. LPS-dependent stimulation leads to the recruitment of p65 that mediates activation of IKK- ϵ followed by inhibitory phosphorylation of NcoR and derepression of adjacent AP-1/ κ B sites^[94]. LPS can also induce the removal of corepressor complexes through ubiquitylation/proteasomal-dependent degradation^[102,103]. TLR2, which binds various lipid- and carbohydrate-containing microbial products and induces AP-1 and NF- κ B activation, stimulates Ca²⁺/calmodulin-dependent protein kinase II (CaMKII)-dependent phosphorylation of the TBLR1 component of NCoR complexes which leads to the dissociation of the NcoR/NcoR2 corepressor complex^[94]. Therefore, regulation of the NcoR/NcoR2 checkpoint plays a central role in TLR-2 and TLR-4-induced activation of expression of pro-inflammatory genes.

HYPOXIA-INDUCIBLE FACTORS

Bacterial invasion commonly leads to oxygen deprivation and induction of hypoxic conditions in the local environment. Pro-inflammatory macrophages are resistant to oxygen tissue deficits in part due to the metabolic preference to use glycolysis, which does not require aerobic conditions^[104]. It appears that hypoxia and hypoxia-inducible factors (HIF) trigger inflammatory/anti-inflammatory activation of macrophages^[105].

Th1 cytokines induce the activity of HIF-1 α isoform during M1 macrophage activation whereas Th2 cytokines stimulate HIF-2 α up-regulation during M2 formation^[106]. NF- κ B is involved in the induction of HIF-1 α , which in turn stimulates the expression of iNOS. Even in normoxic conditions, HIF-1 α continues to induce NO production in macrophages along with other molecules, including TNF- α , antimicrobial peptides, and endoproteases such as granzyme B, indicating that hypoxia is not mandatory for HIF-1 α up-regulation^[106]. In mice, HIF-1 α deficiency is associated with weakened antimicrobial responses in myeloid immune cells that cannot prevent systemic expansion of bacterial infection^[107].

In contrast to HIF-1 α , HIF-2 α controls the expression of arginase-1, which restricts the NO production in macrophages by limiting the substrate availability^[108]. With regard to tryptophan metabolism, which is a hallmark of either M1 or M2 macrophage phenotypes, HIF-2 α antagonizes HIF-1 α by inducing arginase-1-dependent production of ornithine, urea, and polyamines, resulting in a M2-specific metabolic signature.

HIF-1 α controls the glycolytic capacity in myeloid cells, and its deficiency leads to depletion of the cellular ATP pool. Indeed, HIF-1 α deficient macrophages exhibit decreased cell motility, phagocytic capacity, aggregation, and bacterial killing due to serious metabolic impairments^[109]. Therefore, HIF-1 α is essential for survival and efficient function of macrophages in the inflammatory microenvironment. Myeloid-specific deletion of HIF-2 α does not seem to affect the intracellular ATP levels suggesting for a dispensable role of this factor in the metabolic control. Tumor-associated macrophages have elevated expression of HIF-2 α , which supports their migration (through induction of M-CSFR and CXCL4), expression of anti-inflammatory cytokines such as IL-10, and increased invasiveness^[110].

STEROID HORMONE RECEPTORS

Glucocorticoid hormones are important regulators of gene expression associated with binding of activated steroid hormone receptors (SHRs; also known as glucocorticoid receptors) to hormone response elements (HREs) in the target promoters. SHRs are homodimeric nuclear receptors produced in the adrenal glands in response to stress signals such as infection, injury or starvation^[111]. In normal non-stressful conditions, circadian rhythms regulate SHR production for supporting systemic homeostasis^[112]. Exposure to glucocorticoids can induce the M2c phenotype characterized by high expression of arginase-1 (in mice), mannose receptor (in humans), IL-10, pattern-recognition receptor pentraxin-3, Mer receptor kinase (MerTK) essential for efferocytosis. M2c cells, which possess anti-inflammatory and immunoregulatory properties, are involved in the inflammation resolution, regulatory T cells induction, phagocytosis of apoptotic cells, and wound healing^[113,114].

Human macrophages activated with glucocorticoids release anti-inflammatory cytokines IL-4, IL-10 while having a decreased production of pro-inflammatory cytokines. The expression of a scavenger receptor CD163, which binds hemoglobin-haptoglobin complexes, is up-regulated in such cells. In glucocorticoid-treated macrophages, expression of IL-1RII, a decoy receptor for IL-1, is also up-regulated. Activity of AP1 and NF- κ B is reduced thereby leading to suppression of expression of pro-inflammatory genes^[115]. Like M1 macrophages, these macrophages maintain high mobility and are able to migrate quickly into inflamed tissue where they may implicate their unique anti-inflammatory and scavenging properties to inhibit inflammation, remove died cells, and induce tissue repair^[116].

Anti-inflammatory properties of SHRs affect a variety of inflammatory signaling mechanisms. SHRs inhibit

TLR-4/TLR-9-dependent NF- κ B activation by binding to p65 that in turn prevents the formation of a transcriptionally active complex NF- κ B/IRF3^[117]. The association of SHR with p65 disrupts the recruitment of the positive transcription elongation factor b, which also interrupts fast induction of NF- κ B-dependent transcription^[118]. Transrepressor activity of SHRs is applicable to the negative regulation of AP-1. SHR binding to c-Fos abolishes the formation of an active AP-1 complex^[119].

SHR interacts with steroid receptor coactivator-2 (SRC-2, also known as a nuclear receptor coactivator 2, NCoA2). NCoA2 possesses intrinsic histone acetylase activity, which makes downstream DNA more accessible to transcription^[120]. NCoA2/SHR complex can also act as a transcription repressor. For example, recruitment of this complex to HRE sites of NF- κ B-inducible genes blocks their transcription^[121]. However, systemic challenge with LPS can depress some NF- κ B target genes in mouse NcoA2-deficient macrophages and induce LPS-dependent inflammatory responses^[122]. Together, these observations suggest for potent anti-inflammatory effects of glucocorticoids and SHRs and their marked role in the anti-inflammatory activation of macrophages.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARS)

The family of peroxisome proliferator-activated receptors (PPARs) comprises a broadly expressed group of nuclear factors. PPAR- γ is preferentially expressed in the adipose tissue, adrenal gland, and spleen. In macrophages, PPAR- γ acts as a key anti-inflammatory factor, which blocks activation of major effector contributors to M1 polarization such as NF- κ B, AP-1, and STAT^[123]. Transcriptional suppression mediated by PPAR- γ is based on stabilization of transcription corepressor complexes at the promoters of the inflammatory genes. PPAR- γ sumoylation targets this factor to NCoR and histone deacetylase-3 (HDAC3)-containing corepressor complexes. This protects the corepressor complex from the ubiquitylation/19S proteasome-dependent degradation and stabilizes repression of inflammatory genes^[124]. It was demonstrated that PPAR- γ -dependent stabilization of the inhibitory complex at the NF- κ B-inducible promoters in apoptotic cells facilitates their further clearance by macrophages during the inflammation resolution^[102].

Basal expression of PPAR- γ is observed in non-activated macrophages, but it could be significantly up-regulated in response to Th2 cytokines IL-4 and IL-13, indicating for a role of PPAR- γ as M2 polarization factor^[125]. IL-4-induced STAT-6 assembles with PPAR- γ that in turn results in enhanced transcription of fatty acid binding protein 4 (FABP4) and other PPAR- γ target genes^[126]. Inflammatory cytokines inhibit PPAR- γ -dependent gene transcription by functional inactivation of this factor, while PPAR γ remains associated with the DNA though unable to initiate gene expression^[127].

The activity and expression of PPAR- γ is regulated metabolically. PPAR- γ induces the expression of a set of genes involved in fatty acid catabolism and oxidation, a major metabolic energy source in M2 macrophages. Free fatty acids are well known as PPAR- γ agonists. Indeed, PPAR- γ -mediated up-regulation of FABP4, a fatty acid carrier, increases influx of endogenous fatty acids to macrophages and further stimulates PPAR- γ activity. In IL-4 stimulated macrophages, FABP4 inhibition leads to the down-regulation of PPAR- γ activity and suppresses formation of foam cells in hyperlipidemic conditions^[128]. Deficiency of PPAR- γ in macrophages prevents generation of the M2 phenotype, as was shown in PPAR- γ -deficient mice, which were prone to obesity and glucose resistance when fed a fat-rich diet^[129]. Since macrophages play a prominent role in lipid transport and metabolism, PPAR- γ -mediated alternative activation of macrophages plays a protective role against obesity-induced adipose inflammation and impaired glucose metabolism in the skeletal muscle^[129-131]. Synthetic PPAR- γ agonists showed their efficiency in the treatment of metabolic diseases such as diabetes, atherosclerosis, and obesity, where inflammatory activation of macrophages leads to detrimental chronic tissue inflammation^[132,133].

PPAR- δ , another member of the PPAR family of transcriptional regulators, cooperates with PPAR- γ in

the induction of macrophage differentiation towards M2^[134]. PPAR- δ activity is induced and mediated by the IL-4/STAT-6 axis^[135]. PPAR- δ agonists promote the development of the anti-inflammatory IL-4-like morphological phenotype in macrophages. Activation of PPAR- δ induces the repression of multiple NF- κ B and STAT-1-dependent inflammatory genes along with down-regulation of immunosuppressive molecules such as IDO, programmed cell death ligand, and inhibitory Fc γ receptor IIB thereby suggesting that PPAR- δ -primed macrophages possess anti-inflammatory, but not immunoregulatory (i.e., immunosuppressive) properties^[136]. Unlike PPAR- γ , activated PPAR- δ is unable to induce classical M2 macrophages from monocytes^[137] indicating a major involvement of PPAR- γ . The role of PPAR- δ is rather more important in the metabolic control. Accordingly, PPAR- δ deficiency was associated with obesity, insulin resistance, and fatty liver disease^[134].

KRÜPPEL-LIKE FACTORS

Krüppel-like factors (KLFs) belong to the family of zinc-finger DNA-binding proteins that have three characteristic zinc fingers on the C-terminus. Among multiple KLF members, KLF2, KLF4, and KLF6 are involved in the transcriptional control of monocyte/macrophage activities^[117]. IL-4-induced STAT-6 and KLF-4 suppress M1 polarization through inhibition of NF- κ B and KLF-4-dependent induction of MCP-1-induced protein, which in turn stimulates CCAAT/enhancer-binding protein- β and PPAR- γ to promote M2 polarization^[138,139]. KLF2/4-induced impairment of NF- κ B function involves alteration of the recruitment of the NF- κ B coactivator complex p300/CBP-associated factor (PCAF)/p300 to the target promoter^[140]. KLF2/4-deficient macrophages are especially prone to M1 polarization upon LPS challenge followed by increased antimicrobial activity. Targeted KLF2 deletion in murine myeloid cells is associated with greater sensitivity of mice to LPS-induced sepsis, acute inflammatory responses, and increased pathogen clearance in an experimental peritonitis model^[141]. Low density lipoprotein-receptor (Ldlr)-deficient mice with specific myeloid deletion of KLF2 showed advanced atherosclerosis indicating the atheroprotective role of KLF2 in myeloid cells, which suppresses inflammatory polarization of macrophages in atherosclerotic lesions^[142]. However, KLF4 deficiency in mammary tumor cells led to diminished tumor growth and expansion due to impaired pro-metastatic function of myeloid-derived suppressor cells suggesting a role of KLF4 in tumorigenesis^[143].

Although both KLF2 and KLF4 have synergistic effects in dampening pro-inflammatory activity in macrophages, the outcomes of their activity on the differentiation towards M2 are different. KLF4-deficient macrophages exhibited altered expression of M2-specific markers in response to IL-4/IL-13 while KLF2-deficient macrophages did not^[138,141]. Thus, KLF4 is primarily involved in IL-4-dependent polarization of macrophages towards M2, while the role of KLF2 appears to be less significant.

Myeloid KLF2 is a negative regulator of HIF-1 α expression since this factor inhibits NF- κ B-dependent HIF-1 α expression. Hypoxia or exposure to bacterial products/endotoxins reduces KLF2 expression in macrophages thereby promoting expression HIF-1 α and HIF-1 α -inducible targets^[141]. These findings show a crucial role of HIF-1 α /KLF2 balance in the regulation of myeloid cell inflammatory responses in hypoxic and normoxic conditions^[144,145].

In contrast to KLF2 and KLF4, KLF6 positively regulates pro-inflammatory phenotype in macrophages. KLF6 is up-regulated by pro-inflammatory stimuli such as LPS and IFN- γ but is suppressed by Th2 cytokines IL-4 and IL-13. During M1 polarization, KLF6 cooperates with NF- κ B in potentiating transcription of inflammatory genes^[146] and inhibits PPAR- γ expression^[147]. KLF6 can also prevent PPAR- γ binding to the promoters of target genes, including CCL20^[148] and thioredoxin-interacting protein (TXNIP)^[149].

In summary, the KLF family of transcription factors exhibits diverse effects on M1/M2 activation of

macrophages, with the prominent role of KLF6 in M1 polarization and KLF4 in the generation of M2 phenotype.

NUCLEAR FACTOR (ERYTHROID-DERIVED 2)-LIKE 2

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor which belongs to the family of bZIP proteins^[150] regulating antioxidant proteins that protect against oxidative stress triggered by injury and inflammation^[151]. Nrf2 is highly expressed in hematopoietic progenitors and cells of the myeloid lineage^[152]. In its inactive state in the absence of stimuli, Nrf2 has a cytoplasmic localization as a complex with two other proteins, an adaptor Kelch like-ECH-associated protein 1 (KEAP1) and ubiquitin ligase cullin 3^[153]. Cullin 3 mediates Nrf2 degradation through the mechanism of ubiquitination facilitated by KEAP1. Ubiquitinated Nrf2 then is transported to the proteasome where it is degraded. Oxidative stress or electrophile stress abolishes critical cysteine residues in the KEAP1 molecule thereby causing liberation of Nrf2 from the repressive complex^[154]. Nrf2 then moves to the nucleus where it assembles with c-Maf and initiates transcription of target genes from the antioxidant response element^[155].

Among Nrf2-dependent targets are numerous antioxidant genes, including heme oxygenase (HO-1), NAD(P)H quinone oxidoreductase 1, sulfoxedoxin 1 (SRXN1), thioredoxin reductase 1 (TXNRD1), glutamate-cysteine ligase (catalytic and modifier subunits), glutathione S-transferases, and UDP-glucuronosyltransferases. Nrf2-mediated up-regulation of expression of these genes leads to the mobilization of the intracellular cytoprotective antioxidant and detoxifying system. In macrophages, Nrf2 is an important redox regulator of inflammatory activation and polarization^[156]. In response to plaque lipids (i.e., oxidized phospholipids), Nrf2 mediates transformation of macrophages to a new phenotype (Mox) expressing large amounts of HO-1 and other Nrf2-dependent antioxidant genes, as well as IL-1 β and IL-10^[157]. Nrf2 appears to be important for the induction of HO-1 overproduction observed in anti-inflammatory M2 and Mhem macrophages^[151]. By contrast, BTB and CNC homolog 1 (Bach1), a DNA-binding factor, acts as a transcriptional repressor of HO-1 expression^[158]. Heme binding to Bach1 induces derepression of the HO-1 gene and promotes recruitment of Nrf2 to the HO-1 promoter^[159]. Therefore, redox signaling and heme are crucially involved in the Nrf2-dependent up-regulation of HO-1. Induction of HO-1 stimulates several pathways including production of the anti-inflammatory compounds bilirubin and carbon monoxide, which contribute to the phenotypic switch of macrophages towards M2^[160]. In macrophages, Nrf2 primes expression of several ATP-binding cassette (ABC) transporters involved in bile and cholesterol efflux^[161]. M2 macrophages and specialized heme/iron-handling macrophage subsets such as HA-mac, M(Hb), and Mhem exhibit increased the expression of ABC transporters^[23].

Therefore, Nrf2 primes the anti-inflammatory polarization of macrophages in response to oxidative injury and plaque lipids. Induction of Nrf2 in response to oxidative stress has a cytoprotective and cardioprotective effect since this factor is involved in the generation of anti-atherogenic macrophage subsets that are involved in hemoglobin/heme/iron utilization and recycling thereby decreasing the intraplaque oxidative stress and damage^[162]. However, in the microenvironment rich in oxidative low-density lipoprotein and pro-inflammatory cytokines, Nrf2 up-regulation may have pro-atherosclerotic consequences because it stimulates expression of several iron-metabolizing genes such as HO-1, ferroportin, ferritin, and hepcidin that increases iron trapping and oxidative stress in macrophages, enhances lipid accumulation and formation of foam cells^[163,164].

CONCLUSION

In this review, we considered a role of principal transcriptional regulators in either M1 or M2 differentiation of macrophages. The transcriptional regulation of macrophage plasticity in response to various stimuli is very complex and involves global changes in the macrophage transcriptome. There are many key

checkpoints in the transcriptional control and signaling network that trigger either pro-inflammatory or anti-inflammatory polarization.

There are known factors that can mediate early “predifferentiation” of monocytes towards either inflammatory or anti-inflammatory phenotype in further differentiation to macrophages. However, it is still disputed whether monocytes can be presented in the “pre-inflammatory” or “pre-anti-inflammatory” state. In mice, it is likely that monocytes are generated as a *Lyc6C*-positive population in a bone marrow that is focused on the “classical” inflammatory differentiation to macrophages. The loss of this marker can designate the subset of blood monocytes that can survive in the bone marrow but develop expression of higher *Lyc6C* surface expression to patrol the circulation in order to observe the endothelial integrity in steady-state in response to inflammation. In humans, it is difficult to examine these modifications due to the mature state of monocytes coming from the bone marrow to the circulation.

DECLARATIONS

Authors' contributions

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All authors declared that there are no conflicts of interest.

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20

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Editorial

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Vascular remodeling 2018: the updates

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Cardiovascular research is fundamentally important to human health, and research progress in this field could not be overemphasized. Recently we were encouraged by the editors of *Vessels Plus* to invite vascular biologists to submit their research and review articles to the special issue on “Vascular remodeling 2018: the updates” that would show up some overview of recent research from biomedical vascular science. In this special issue, we assembled five reviews and one original research paper devoted various areas of vascular biology and denoted recent advances in clinically relevant cellular and signaling mechanisms in vascular remodeling.

The review of Strassheim *et al.*^[1] 2018, describes the role of G proteins-coupled receptors (GPCRs) in pulmonary hypertension (PH) and potential for future targeted therapies. Pulmonary hypertension involves stiffening of pulmonary arteries and increased remodeling of small vessels. Increased pressure in the pulmonary circulation promotes hypertrophy of the heart and, eventually, cardiac failure. GPCRs signaling play a prominent role in maintaining homeostasis and dysregulated signaling contributes to cardiovascular pathology. Current therapies predominantly target vasodilatory pathways and include cGMP-protein kinase G signaling, calcium channels, endothelin receptors, and prostacyclin receptors. The pathways, by which GPCRs control transcription factors involved in vascular smooth muscle cell (VSMC) proliferation and vascular remodeling and those controlling cardiac myocyte hypertrophy or transition to cardiac failure, are far from clear. Understanding the role of GPCR-mediated signaling in PH will lead to discovering better therapies than is currently realized.

Pulmonary hypertension in neonates is (PHN) is associated with high mobility and mortality and is involved in the pathogenesis of various pediatric pulmonary disease states, such as intrauterine growth



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restriction and preeclampsia. Balistreri^[2] reviewed current view on the mechanisms of PHN and its relationship with adult PH and other adult diseases. According to the recent findings, adult PH may be result of developmental programming (DP). Vice versa, it was demonstrated that PHN could be a result of several adverse events during perinatal life. Common risk factors include drugs and alcohol abuse, high altitude living, and pollution. Importantly, that identification of specific risk factor may facilitate the development of effective therapies. Genomic imprinting is the principal driver of DP. It affects newborns primarily through maternal DNA methylation, however, other epigenetic mechanisms such as histone modifications, non-coding RNA-mediated gene silencing, and chromatin remodeling are also likely to be involved. Mechanisms and models of PHN summarized in the current review.

Epigenetic mechanisms have emerged as a one of the major drivers of vascular remodeling. Histone deacetylases (HDACs) modify core histones around DNA by removing acetyl groups from hyper-acetylated histones. More recent findings reveal that HDACs can not only deacetylate histones but many non-histone proteins and are able to regulate numerous cellular functions such as transcription, cytoskeletal polymerization, and signal transduction. Recently, the therapeutic potential of inhibiting HDACs for the treatment of cardiovascular diseases has been appreciated. Kovacs *et al.*^[3] summarized the current view on the role of HDAC-mediated vascular mechanisms associated with acute lung injury (ALI) progression/preservation. ALI arises from a wide range of lung injuries such as toxins or inflammatory mediators, resulting in significant morbidity and frequently in death. A major cause of ALI is dysfunction of the pulmonary vascular endothelial barrier resulting in pulmonary infiltrates, hypoxemia and pulmonary edema. It was recently demonstrated that pharmacologic inhibition of several HDACs leads to enhancement of pulmonary vascular barrier, thereby, preventing the development of ALI. However, the mechanisms of HDAC-mediated vascular barrier preservation are ill defined. The current article provides the functional characterization of HDACs with the emphasis on their role in the regulation of endothelial barrier.

VSMC are the predominant cell type controlling large blood vessel stiffness and blood pressure. They switch between alternate phenotypes of contractile in non-pathological settings to the pathological synthetic-proliferative phenotype, associated with cardiovascular disease. In their review, Ahmed *et al.*^[4] 2018 focus on the role of VSMC under physiological conditions and blood vessel physiology and describe how stiffening of large arteries could be transmitted to the microcirculation of organs such as the heart and lungs. The authors describe in a simplistic manner how different molecules and structures result in the transition between contractile vs. synthetic-proliferative VSMC phenotype, through the mechanisms that involve of cytoskeletal proteins, myosin light chains (MLC-20, MLC-17), and myosin isoforms. They emphasize complex crosstalk between VSMCs and their surrounding matrix in healthy and in pathological conditions thus providing new insights into the mechanisms that regulate the phenotypic switch.

Thrombospondin (TSP) is a family of structurally related proteins with five distinct members (TSP1-5) that bind to surface receptors such as CD36 or the $\alpha v\beta 3$ and $\alpha IIb\beta 3$ integrins to regulate diverse biological processes like inflammation, immunity, control of extracellular matrix properties and composition, as well as glucose and insulin metabolism. The article of Stenina-Adognravi *et al.*^[5] reviews the contribution of TSPs to regulation of cancer growth and describes various functions of TSPs that link these proteins by modulating multiple physiological and pathological events that prevent or support tumor development. TSP1 and TSP2 have major role in vascular tissues, participate in platelet aggregation, and are anti-angiogenic whereas TSP4 is pro-angiogenic and pro-inflammatory in tumor models. TSP4 has shown to have a role in tumor growth and angiogenesis. TSP3 and 5 have major roles in bone development and deletion of the gene impairs skeletal development in mice. The authors summarize studies of TSP functions and roles in different systems of the organism and the complex nature of TSP interactions and functions, including their different cell- and tissue-specific effects.

The circadian clock is the cellular signaling mechanism, which controls a daily cycle of biological activities based on 24 h rhythmic oscillations. It well established that circadian clock components control cardiovascular remodeling and disruption of circadian clock genes results in alterations in rhythmic blood pressure, endothelial dysfunction thus contributing to cardiovascular diseases like atherosclerosis. The original research article of Anea *et al.*^[6] analyses expression of circadian clock proteins through cardiovascular tissues using immuno-histochemical approach. The studies revealed tissue-specific differences in expression and localization of circadian clock proteins in vasculature suggesting the existence of fine-tuned tissue-specific mechanisms of circadian clock regulation.

DECLARATIONS

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The authors are equally contributed to the article.

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Review

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Usefulness of chronic total occlusion devices and techniques in other complex lesion subsets

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Abstract

Percutaneous coronary interventions (PCI) in chronic total occlusion (CTO) have been for long time considered as “last frontier” in interventional cardiology. Among the different subset of complex targets for PCI, CTO lesions represent a challenge for the interventional cardiologist. CTO techniques and devices have evolved in last few years together with the training of specialized interventional cardiologist in such complex field. All these factors have markedly increased procedural success of CTO procedures and have the potential to be applied in other settings. In this paper, we provide an update on the technical aspects and the devices developed for CTO PCIs that can be applied in complex PCI on non-CTO lesions.

Keywords: Chronic total occlusion, complex percutaneous coronary interventions, complications, interventional devices

INTRODUCTION

Chronic total occlusion (CTO) lesions are defined as a coronary lesion with Thrombolysis In Myocardial Infarction grade 0 flow distal to the occluded segment and, although often difficult to determine, clinical evidence of occlusion duration more than 3 months^[1].

CTO represent the most technically challenging procedure subset for the interventional cardiologists. They show a high prevalence between patients referred for coronary angiography in various real-world registries, with an incidence increasing with age^[2,3]. Some recent observational studies have shed a new



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Table 1. American Heart Association/American College of Cardiology lesion classification system

Anatomic risk groups		
Low risk	Moderate risk	High risk
Discrete (length < 10 mm)	Tubular (length 10-20 mm)	Diffuse (length < 20 mm)
Concentric	Eccentric	Excessive tortuosity of proximal segment
Readily accessible	Moderate tortuosity of proximal segment	Extremely angulated segments > 90°
Nonangulated segment (< 45°)	Moderately angulated segment (> 45°, < 90°)	Total occlusions > 3 months old and/or bridging collaterals
Smooth contour	Irregular contour	Inability to protect major side branches
Little or no calcification	Moderate or heavy calcification	Degenerated vein grafts with friable lesions
Less than totally occlusive	Total occlusions < 3 months old	
Not ostial in location	Ostial in location	
No major side branch involvement	Bifurcation lesions requiring double guidewires	
Absence of thrombus	Some thrombus present	

light about CTO procedures demonstrating a clinical benefit in term of reduced angina symptoms^[4], improved left ventricular ejection fraction^[5] and improved long term survival^[6]. However, when looking to recent randomized clinical trials, there are still some concerns about effective clinical impact of CTO revascularization, showing conflicting results^[7-10].

Above all these clinical and prognostic considerations, only in the last few years, the developing of new techniques and new devices and guidewires has raised the procedural success to near 90%^[11-15] in experienced centers. However, observational reports still showed that CTO procedural success rate is lower in less skilled hands, reaching in some cases only 70%^[12]. Following technical advancement and procedural increased success, interventional cardiologists have been recently more involved in CTO procedures. Hence, it is now advisable for the interventional cardiologist to follow specific training programs and to consider on site proctoring before starting to perform CTO procedures. Moreover, operators involved in such courses and on-site CTO programs could improve their learning curve even in complex percutaneous coronary interventions (PCI), bringing their experience about CTO devices and techniques to everyday practice.

Hence, in this review, we focus the attention on specific insights on CTO devices and techniques, which could enhance interventional cardiologist capability to overcome many challenges and complications encountered during daily PCI.

PLANNING THE PROCEDURE: WEIGH THE PROS AND CONS

Accurate angiographic review with complete quantification of possible hazard during the procedure remains a cornerstone of successful CTO PCI. In this context, dual injection angiography should be performed in all cases except in the complete absence of contralateral collaterals. A complete evaluation of CTO lesion characteristic before the procedure is the key for the success.

A first extensive evaluation of anatomic predictors of PCI success was reported in AHA/ACC Guidelines for Percutaneous Interventions [Table 1]^[16]. However, regarding CTO procedures, many scores have been proposed, but the most commonly used for its simplicity in identifying main characteristics that may impact procedural success is the “J-CTO” score^[17]. Patients with higher J-CTO score have significantly lower success rate. The four angiographic parameters of this score are: (1) proximal cap location and morphology, with a clearly defined and “tapered” proximal cap favoring antegrade approach; (2) lesion length, with a value > 20 mm clearly more challenging to cross; (3) calcification; (4) bending > 45° within CTO lesions, which lower procedural success.

More recently many other scores, such as RECHARGE CTO score^[14] and PROGRESS CTO score^[15], have demonstrated a similar predictive ability of CTO procedural success when compared with J-CTO score.

The above described score tools are very useful to guide clinical and procedural decision during CTO procedures. In addition, they could be very helpful when planning complex PCI as they underlined all angiographic and clinical predictors of every PCI technical success.

CAN COMPLEX PCI AND CTO PROCEDURES FIT TRANSRADIAL APPROACH?

In usual PCI setting 6F Guiding Catheter (GC) is enough to obtain a complete procedural success in the vast majority of cases. However, CTO lesions could better be afforded using large (7F or 8F) GC in order to obtain larger inner space together with higher back-up support and stability. In last decade, this strategy was almost exclusively performed by transfemoral approach. However, the selection of radial arteries as vascular access for complex PCI has substantially grown in the last ten years as several studies have demonstrated a marked reduction of access site-related bleedings^[18]. Recently, several technical improvements in PCI's materials have boosted utilization of radial approach even in more complex interventions, such as CTO procedures, also adding the option of hybrid vascular approach (6F radial access for contralateral injection and 7F or 8F for antegrade/retrograde approach)^[19]. First, a new family of GC, called "Sheathless" (first available type: Eucath, Asahi, Japan), has been introduced. The "Sheathless" GC has a highly hydrophilic coating and can be inserted without a sheath thus showing an increased inner lumen when compared to standard GC (6.5F vs. 6F or 7.5F vs. 7F). More recently, a brand-new sheath, called "Slender" (Terumo, Tokyo, Japan), has been produced and showed an increased inner lumen despite an outer diameter still in the range of radial compatibility. Therefore, in the setting of complex PCI, a 6-in-7 F "Slender" sheath could be easily used to insert a 7F GC into radial artery thus combining technical feasibility with lower vascular access related complications.

The development of such devices have progressively make radial approach more common during CTO procedures. Indeed, in such procedures, the need for larger GC is essential as only two microcatheters or one microcatheter and a monorail balloon fit together into 6F GC, while all frequently used devices can be only inserted alone.

In the setting of CTO intervention and more extensively in all PCIs, material compatibility is a critical issue and operators should focus their attention on materials' compatibility, considering also that same material (for example same size of semi-compliant balloon) of different manufactures may have different diameter. Therefore, a careful procedure planning which includes a tailored selection of radial equipments may help safely carry on PCI through radial approach.

GUIDING CATHETER AND MOTHER-IN CHILD DEVICES: THE NEED FOR MORE SUPPORT

When planning a PCI, one of the first thing to decide is the GC you would use to obtain enough backup and support to perform the procedure. In the setting of CTOs, a large (7 or 8 F) GC offers more support and greater inner diameter which allows insertion of such complex materials, even in combination (such as dual over-the-wire microcatheters) and now can be inserted by radial approach. In last few years, several techniques and materials have been developed to increase procedural support to overcome difficulties encountered in crossing CTO or complex lesions and delivering PCI materials.

Nowadays, the need for more support in CTO procedures could be afforded by two different strategies: usage of devices with improved backup force ("passive support") or techniques to directly enhance support ("active support").

The better way to enhance passive support is to choose a larger GC with more supportive backup curves. Therefore, Amplatz left GC for the right coronary artery and XB (eXtraBackup) or EBU GC for the left coronary artery are commonly used to improve backup. In order to avoid such complications, side holes are

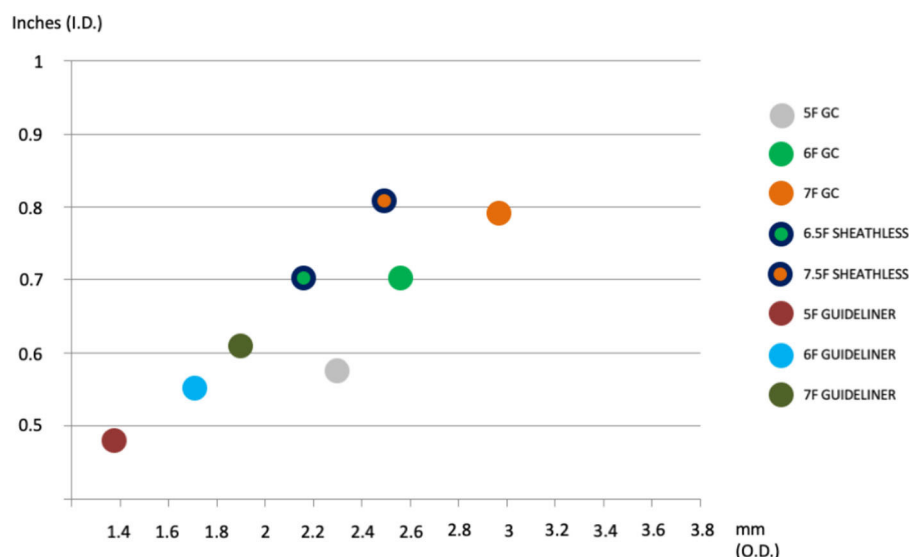


Figure 1. Comparative chart of different guiding catheter/GuideLiner systems which may be considered to perform percutaneous coronary interventions through radial approach. On X-axis outer diameter in millimeters; on Y-axis inner diameter in inches

often used to minimize dampening, but should not be used in unprotected left main as they can provide a false sense of security and cause severe ischemia. More recently, several mother-in-child devices have been introduced to maximize passive support during CTO and even complex PCI. The GuideLiner catheters (Vascular Solutions, Inc., MN, USA) were the first to be introduced in 2009^[20]. All these devices are coaxial “mother and child” guiding catheter extension delivered through a standard guiding catheter on a monorail system with flexible distal extension and radiopaque markers near the distal tip. These characteristics, together with a tight design, allow deep intubation into the coronary arteries without slippage thus favoring the delivering of materials behind areas of narrowing or tortuosity, which could reduce GC backup [Figure 1].

On the other hand, active support could be improved with some techniques developed specifically for CTO procedures. A first unspecific option does exist and consist to perform a deep intubation of the GC. However, this old technique carries out a consistent risk of proximal vessel dissection and has been overcome by using mother-in-child devices. More recently, a new technique, called “mother-daughter-granddaughter” has been developed using a 6F Guideliner into an 8F Guideliner to allow navigation of very tortuous segments, such as saphenous graft, unfolding the equipment rather than pushing it. However, these technique should be handled more carefully as the risk of coronary dissection is very high.

The widespread use of 7F or 8F GC led to the development of another active support option: the “anchoring balloon” technique^[21]. Sometimes antegrade guidewire crossing of a CTO lesions is not followed by successful advance of microcatheters or balloon through the lesion. The first step of such technique is to wire a risk free side branch target before the lesion. Subsequently, inflating a balloon into the side branch provided a stable “anchor” which increase support to advance materials through CTO. This technique could be also useful in daily PCI procedures, in any case of difficult advancement of materials (such as balloon or stents) through tight or calcified lesion. More recently, some variations have been introduced to the original “anchoring balloon” technique. Out of these, the most used technique in daily PCI scenario is the distal mother-in-child devices anchoring^[22]. In any case of difficult stent delivery, the mother-in-child device is advanced just before or even through the target lesion over a distally placed inflated balloon. Such balloon was then deflated and withdrawn safely and the selected stent could be delivered at the site of the lesion [Figure 2].

In any case of highly complex PCI situations, the use of mother-in-child devices to increase passive support or the application of active support techniques could improve success rate, even in case of PCI that may have previously been considered technically unapproachable.

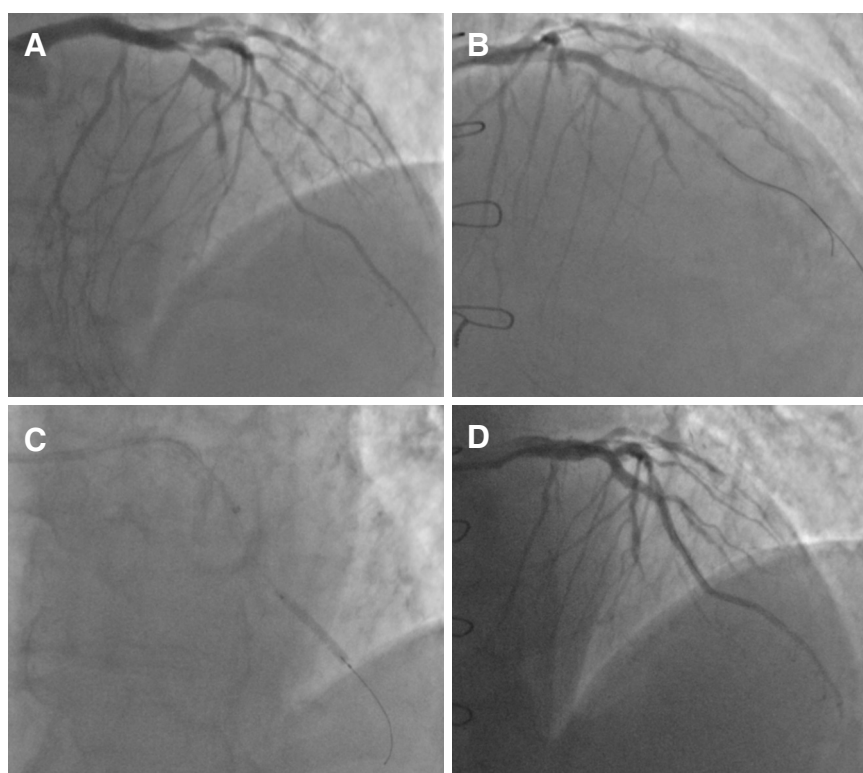


Figure 2. “Anchoring balloon” technique example. A: Basal angiographic assessment; B: angiographic evaluation after proximal stent implantation; C: GuideLiner delivery with distal anchoring balloon; D: final angiography after three more overlapping stents implantation

GUIDEWIRES AND MICROCATHETERS: A BAG OF TRICKS

The toolbox of CTO operators has rapidly increased over the last decade as new tools and equipments have been developed. A complete knowledge of such equipment is now essential for every interventional cardiologist in order to improve performance in everyday PCI.

Specialized CTO guidewires are now commonly available in every cathlab as they represent the key tool to afford antegrade crossing of CTO lesions. Indeed, antegrade wire escalation is the most commonly used technique to cross the lesion throughout the true vessel lumen and involves progressive utilization of guidewires with various stiffness and/or penetrating force. Moreover, with the development of global interest over CTO procedures, such guidewires have been extensively publicized even in international conventions and their characteristics are now familiar to every interventional cardiologist [Table 2]. In everyday practice, soft, tapered and polymer-jacketed wires, such as the Fielder family (Asahi Intecc, Japan), could be very useful to cross very narrowed lesions, especially in calcified vessels. In this setting, the polymeric coating together with the tapered tip of such guidewires may be helpful in navigating a narrow true lumen of a tortuous calcified vessel minimizing the risk of causing a dissection. However, routine use of polymer jacketed guidewires with higher tip weight should be avoided in everyday practice. Beyond CTO guidewires, a new family of workhorse wires called Sion (Sion, Sion Blue and Sion Black; Asahi Intecc, Japan), characterized by a higher direction and torque response, have been developed and have showed such ability in retrograde collaterals crossing. Such wires are essentially driven by a new technology called “Composite Core”: the parallel placement of a classic wire and a twist wire linked at the distal tip determine a more precise transmission of torque rotation. Such characteristics make Sion guidewires very useful in daily PCI in every case of difficult distal wire positioning in tortuous and/or calcified vessels and in bifurcation PCI when wiring or re-wiring of side branch is difficult due to a narrow angle.

Table 2. Main characteristics of chronic total occlusion guidewires commonly used in daily percutaneous coronary interventions

Family	Guidewire	Shaft coating	Tip coating	Tip diameter	Tip weight (g)	Main purpose
Fielder (Asahi)	Fielder XT-A	Polymer jacketed	Hydrophilic	0.010 (tapered)	1	Sliding of microchannels
	Fielder XT-R	Polymer jacketed	Hydrophilic	0.010 (tapered)	0.6	Sliding of microchannels
Sion (Asahi)	Sion	Hydrophilic	Hydrophilic	0.014	0.7	Navigation of tortuous vessels
	Sion Black	Polymer jacketed	Hydrophilic	0.014	0.8	Surfing of small vessels (collaterals)
	Sion Blue	Hydrophilic	Hydrophobic	0.014	0.5	Higher torque control for vessel wiring
Sentai (Boston Scientific)	Fighter	Polymer jacketed	Hydrophilic	0.009 (tapered)	1.5	Sliding of microchannels
	Hornet	Hydrophilic	Hydrophilic	0.008 (tapered)	1	Navigation of microchannels
	Samurai	Hydrophilic	Moderated Hydrophilic	0.014	0.5	Higher torque control for vessel wiring

A correct manipulation of CTO guidewires should always be performed using an over-the-wire system such as microcatheters. Main characteristics of such devices are summarized in Table 3. Among these, Finecross (Terumo, Tokyo, Japan) and Corsair Pro or Caravel (Asahi Intecc, Japan) are the most commonly used. The key role of microcatheter in CTO procedures could be summarized in three essential steps: (1) to safely place CTO guidewire just in front of the lesion; (2) to increase support and precision in CTO guidewire manipulation during antegrade crossing; (3) to allow guidewire exchange once the lesion has been crossed. All these steps could be used even in complex PCI procedures when a CTO dedicated guidewires is used to cross a heavy calcified and/or narrowed lesion as explained before or when a workhorse guidewire should be manipulated more precisely with facilitate torque in the tip response. Finally, microcatheters could be very useful to reduce guidewire kinking and prolapse while trying to cross a lesion immediately after a large side branch.

More recently, dual lumen microcatheters, such as Fineduo (Terumo, Tokyo, Japan), Crusade (Asahi Intecc, Japan), Twinpass (Vascular Solutions, USA) and NHancer RX (IMDS, The Netherlands), with both a rapid exchange and over-the-wire lumen, have been developed. During CTO procedures, dual lumen microcatheters are useful in some scenarios: (1) to allow a more precise engagement of the cap located at the level of a side branch (in antegrade approach) or located too close to the connection with interventional collateral (in retrograde approach); (2) to preserve side branch when a bifurcation is located into CTO body; (3) to perform “parallel wire” technique. In everyday practice, the employment of dual lumen microcatheters is increasing, as they could be very useful in bifurcation PCI to wire a side branch with difficult take-off angle or to re-wire side branches after crossover stenting^[23]. Moreover, in any case of main vessel dissection without a protection guidewire into side branch during bifurcation PCI, a dual lumen microcatheter could help to wire the side branch limiting the risk of dissection expansion after second “free” guidewire advancement.

BALLON UNCROSSABLE AND BALLOON UNLIDATABLE LESIONS: PUSHING THE LIMIT

After successful guidewire crossing, in 5%-10% the microcatheter is not able to cross CTO body^[24]. This will usually occur in the highly calcified lesions, which are also challenging to cross with guidewires; however, even in simpler cases, this problem could arise unexpectedly. In this setting, some techniques may be adopted to increase support, such as “buddy wire”, the employment of a mother-in-child system or the anchoring balloon technique, with uncertain results. In the past, in case of persisting uncrossability, the first widely used option was “grenadoplasty” (during which a small balloon is advanced as far as possible and then inflated at high pressures until it ruptures), with conflicting results in plaque modification.

Table 3. Main characteristics of microcatheters

Name	Main specification	Length (cm)	Distal inner lumen	Entry tip profile	Crossing profile	Delivering technique
Finecross (Terumo)	Single lumen, hydrophilic, floppy tip	130 or 150	0.018	1.8 Fr	1.8 Fr	Push without rotation
Corsair Pro (Asahi)	Single lumen, moderated hydrophilic, high support	135 or 150	0.015	1.3 Fr	2.5 Fr	Counter-clockwise Rotation (max 10 consecutive)
Caravel (Asahi)	Single lumen, highly hydrophilic with low crossing profile	135 or 150	0.016	1.4 Fr	1.9 Fr	Push without rotation
Venture (Vascular Solution)	Single lumen with tip deflection system of 90°	145	0.014	1.8 Fr	2.2 Fr	Push without rotation
Crusade (Asahi)	Double lumen (OTW 6.5 mm before tip)	140	0.017	2.2 Fr	2.9 Fr	Push without rotation
Twin Pass (Vascular Solutions)	Double lumen (OTW 11 mm before tip)	135	0.014	1.9 Fr	2.7 Fr	Push without rotation
FineDuo (Terumo)	Double lumen (OTW 6.5 mm before the tip)	140	0.014	2.2 Fr	2.9 Fr	Push without rotation

Nowadays, new low-profile rapid-exchange balloons, such as Tazuna (Terumo, Tokyo, Japan) and Ikazuchi (Kaneka Corporation, Japan) are available. The main feature of these balloons is the extremely low entry tip profile (between 0.015 and 0.017), lower than microcatheters, combined with a higher pushability given by the rapid-exchange system. Therefore, the successful crossing of the lesion with a balloon rather than microcatheter strongly increased in last few years. In cases of low-profile balloon failure, lesion modification techniques still represent a remarkable option, as plaque debulking subsequently facilitate balloon crossing. More specifically, if microcatheters successfully cross the lesion, the distal guidewire could be exchanged with a rotawire and rotational atherectomy ablation could be performed. Where available, 0.9 mm excimer laser atherectomy over a conventional guidewire is also an option. However, it must be highlighted that lesion modification techniques should be considered “last resort” measures where standard techniques have proved unsuccessful, as they could arise complications, such as coronary perforation and/or rupture and even devices entrapment with possible procedural failure or catastrophic consequences. More recently a new generation of microcatheters, the Turnpike family (Vascular Solutions, USA), show a higher capability in lesion crossing as they contain threads enabling a “screw-like” approach. However, these devices are quite aggressive and their employment is still relegated to CTO procedures. As expectable, most of these equipments are now widely used in everyday PCI in every case of lesion uncrossable (due to calcification, tortuosity or extremely narrowing) by a common semi-compliant balloon and have increased procedural success even in daily setting.

In CTO procedures, as in everyday PCI, an adequate predilation is essential before stent deployment. Among CTO lesions, undilatable lesions still represent a challenge for the interventional cardiologist. In the past, the first dedicated device designed to obtain successful dilation of calcified plaque was the Flexptome™ Cutting Balloon™ Dilatation Device (Boston Scientific, USA). In such device, three or four microblades are mounted over a non-compliant balloon with several diameter and length. During the dilation, microblades create three or four plaque incisions facilitating subsequent expansion with conventional balloons. In last two decades, however, rotational atherectomy (Rotablator, Boston Scientific, USA) have represented the most remarkable option to obtain lesion modification and to facilitate balloon dilation. The diamond burr causes “differential cutting” of inelastic tissues preserving integrity of normal elastic segments while the high rotational speed (usually > 60,000 rpm) eliminate the contact between the burr and the arterial wall thus allowing crossing of tortuous segments without damage. Seldom, excimer laser coronary atherectomy could be used to perform plaque debulking by delivering of rapid ultraviolet B pulses to coronary lesion with subsequent tissue breakdown by photoacoustic mechanism. More recently, the idea to use local and high-energy lithotripsy waves for the treatment of coronary calcification lead to the development of a new dedicated device. Shockwave IVL system (Shockwave Medical Inc., USA) consist of a semicompliant, rapid-exchange balloon, connected to a pressure-waves generator by a cable. After lesion crossing, balloon

is inflated at 4 atm and lithotripsy cycle in activated and pulses once per second for ten seconds (for a maximum of 8 repeatable cycles), thus cracking intraplaque calcium. This device has the potential to become a cornerstone in PCI, but only limited clinical data about outcome and safety are available^[25] and further randomized trials are needed. Another valuable option is the employment of recent developed high pressure, non-compliant balloons, such as OPN NC (SIS Medical, Switzerland) which could be inflated till 35-40 ATM, usually for about 30-60 seconds. Moreover, Angiosculpt (AngioScore, Inc.), a semi-compliant balloon covered by three nitinol coils, have been recently developed and represents a sort of evolution of cutting balloon with lower profile and higher number of cuts by millimeter of plaque.

In common everyday practice, complex fibro-calcific coronary lesions still represent a challenge for the interventional cardiologist and commonly require adjunctive techniques and devices to facilitate successful PCI. However, only correct and experienced employment of such devices could lower the risk of complications, especially more catastrophic ones. Therefore, all the above described devices should be available in cath lab and specific training programs should be attended by single operators.

RADIATION EXPOSURE IN THE CATH LAB: FACING THE PUBLIC ENEMY

Over the past decade, CTO interventions progressively spread all over the world leading to an increase awareness of radiation exposure. CTO procedures are quite often long and complex procedures with associated longer fluoroscopy times, as reported in several registries^[11]. However, the absence, among interventional cardiologists, of consciousness of radiation injuries lead to development of a position paper about medical radiation in cardiovascular imaging^[26]. Radiation-associated complications can be categorized as deterministic (which have a threshold above which injury occurs, e.g., skin injury) and stochastic (that have no threshold for injury to occur, e.g. cancer, infertility). Nowadays, the accepted threshold for skin injuries is 5 Gray, so operators should follow the so-called ALARA rule (radiation as low as reasonably acceptable) in order to minimize radiation injuries to the patient. Therefore, moving from CTO procedures, some tricks to reduce radiation exposure are now commonly followed in every day practice: (1) reduction of the fluoroscopy frame rate from 15/s to 7.5/s (probably the most effective way); (2) use of fluoro-store function; and (3) optimization of x-ray tube collimation.

PROCEDURAL COMPLICATIONS: LESSONS LEARNED FROM CTO PROCEDURES

In the setting of CTO interventions, the incidence of complication is usually higher than everyday PCI^[27]. Perforation, acute vessel thrombosis and device entrapment are the most common cardiac complications and are more common during retrograde procedures. Moreover, extracardiac complications, such as arterial embolization, radiation injury, contrast induced nephropathy and vascular access complication should be taken into account when performing both CTO procedures and everyday PCI.

In the everyday PCI setting, acute vessel thrombosis is the most common cardiac complication and could be caused by five main mechanisms: dissection, new thrombus formation, no reflow, inadvertent air injection and vasospasm^[28]. Coronary dissection usually happens in long, complex and calcific lesions and rarely cause vessel closure. Moreover, in the vast majority of cases, a guidewire could be advanced throughout the vessel true lumen and this complication is easily managed by stent deployment. Seldom, acute coronary dissection followed by abrupt vessel closure could be caused by sub intimal tracking of hydrophilic guidewire with some difficulties to re-gain vessel true lumen and perform PCI. In such cases, the use of stiffer and/or easily directing CTO guidewires in experienced hands could be very useful even in combination with dissection and re-entry techniques.

Nowadays, arterial perforation is still the most common interventional cardiologist's nightmare and is not only restricted to CTO procedures. The incidence of coronary artery perforation is less than 1%, but is a very

Table 4. Ellis classification of coronary perforation^[30]

Ellis class	Definition
I	Extraluminal crater without extravasation
II	Pericardial or myocardial blushing
III	Width of perforation ≥ 1 mm with contrast streaming and cavity spilling

catastrophic complication which could lead to sudden cardiac tamponade or acute myocardial infarction^[29]. Aggressive predilation or postdilation, usually in more calcified lesions, could determine large vessel perforations. Indeed, one of the most predictive factors for large vessel perforation is a high balloon-to-artery ratio > 1.2 combined with a high inflation pressure^[29]. Moreover, rotational atherectomy is another common cause of such complication.

Ellis score is usually used to grade the severity of such perforation and Ellis grade III (defined as contrast extravasation from > 1 mm perforation) is associated with the highest mortality^[30] [Table 4]. However, coronary perforation severity and subsequent management is strongly affected by clinical scenario and perforation site.

In any case of proximal vessel perforation without jet extravasation (Ellis 1), treatment is limited to careful observation for 15-30 min and no further actions are needed if does not increase. In contrast to previous common practice, now is strongly avoided to reverse heparin with protamine until all equipments are removed from coronary artery. When even minimum pericardial or myocardial blushing occurs, the optimal strategy is to inflate a balloon for about 10 min at low pressure over the site of perforation to stop the flow. To avoid prolonged ischemia during this phase, some operators suggested the “microcatheter distal perfusion technique”, which consist of placing over another guidewire a microcatheter distal to perforation in order to inject patient’s blood. When balloon inflation does not achieve vessel sealing, a covered stent should be placed in the site of perforation from the same catheter or by using the so-called “ping-pong technique”. Indeed, in case of consistent jet extravasation, removal of balloon and subsequent deliver of covered stent could determine massive blood loss. Moreover, only recently developed covered stents shows compatibility with 6F GC (PK Papyrus, Biotronik, Berlin, Germany) but they still not allow coexistence with balloon shaft, thus determining the need for GC replacement. In such cases, this CTO derived technique conveys the use of a second GC to engage the coronary ostium while the first is withdraw few millimeters without deflate balloon at perforation site. Therefore, during rapid balloon deflation and reinflation, a second guidewire is placed distally. Such balloon is finally deflated and retrieved only while covered stent is delivered at the site of perforation.

In the setting of CTO interventions, routine use of heavier, polymer jacketed and tapered guidewires results in a higher incidence of distal perforation related to wire manipulation. However, this complication could even happen during daily PCI when a CTO (or simply a workhorse hydrophilic) guidewire is chosen to cross a complex lesion. When distal perforation happen, proximal prolonged balloon inflation should be considered as first line treatment. Nevertheless, a higher dose of heparin is usually administered during CTO or complex PCI thus lowering the success of such strategy. In past decades, complete sealing of distal perforation was usually obtained with local injection of subcutaneous fat through an inflated over-the-wire balloon in order to prevent spreading of such material in other coronary arteries or systemic circulation. Nowadays, micro detachable coils for neuroradiology interventions have been developed and could be safely used to seal distal coronary perforation. Indeed, such coils are 0.010 compatible and could be easily delivered through all microcatheters commonly used in cathlab, which show an inner diameter between 0.015 (Nhancer Pro X, IMDS, The Netherlands) and 0.018 (Finecross, Terumo, Japan). The best option is a recently described technique, called “balloon-microcatheter technique” which allow fast and easy sealing of distal coronary perforation in everyday setting^[31]. After proximal balloon inflation, they suggest to advance

a microcatheter over a second wire beyond the inflated balloon (during very short balloon deflation) in order to facilitate distal micro-coils delivering. However, it should be considered that, during standard PCI performed by 6F GC, only Finecross (Terumo, Japan) and Nhancer Pro X (IMDS, The Netherlands) microcatheters could be advanced parallel to the inflated balloon without increase GC inner diameter. One or more micro-coils are then delivered through the microcatheter and repeated checking of distal sealing may be done during short balloon inflations.

In everyday practice, coronary perforation occurs quite infrequently when compared to CTO procedures, but still carry out a higher incidence of adverse outcome. A complete knowledge and cath lab availability of dedicated devices, such as micro-coils and covered stent is essential. In case of such complication even more important is a step-by-step approach together with employment of CTO derived rescue methods, such as “ping-pong” or “balloon-microcatheter” techniques, which could improve patient’s survival.

CONCLUSION

CTO intervention is a rapidly evolving area and is commonly considered the last frontier of interventional cardiology. Specific equipment and techniques showed a rapid evolution in last decade, thus increasing procedural success even in less experienced centers. Nowadays, global knowledge of CTO tools is widespread and they have become a strong and precise weapon in interventional cardiologist’s hand to face obstacles encountered in daily PCI scenario.

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Authors’ contributions

All authors have contributed equally to the article.

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Ethical approval and consent to participate

Not applicable.

Consent for publication

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Original Article

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Computational evaluation of mitral valve repair with MitraClip

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Abstract

Aim: This paper aims to evaluate the effectiveness of MitraClip implantation as a solution to severe mitral regurgitation (MR) in the case of posterior leaflet prolapse due to hypertrophic obstructive cardiomyopathy and chordae rupture.

Methods: NX CAD software was used to create a surface geometric model for the mitral valve (MV). A hyperelastic material model, calibrated against experimental results, was used to describe stress-strain responses of the MV leaflets, and a spring element approach was used to describe chordae response. Abaqus CAE was employed to create a finite element model for diseased MV suffering from MR. The effectiveness of MitraClip implantation on valve function was investigated by simulating the deformation of diseased valve, with and without MitraClip repair, during peak systole and diastole. Leaflet deformation and stress distributions were used to assess the effectiveness of the procedure.

Results: Overall, significant improvement was achieved for the diseased valve after MitraClip implantation. Prior to the introduction of the clip, the diseased valve was subjected to posterior leaflet prolapse which would induce a jet of MR. Once the MitraClip was included in the simulation, the valve leaflets were able to close and seal off, almost entirely at peak systolic condition without a significant impact on the stress distribution of the valve leaflets.

Conclusion: The results in this study provide further evidence to support MitraClip repair as a viable treatment for high-risk patients suffering from severe MR, and also highlight the need for further research into such an advanced, minimally invasive surgery technique.



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Keywords: Finite element, mitral valve, MitraClip, mitral regurgitation, hypertrophic obstructive cardiomyopathy, hyperelastic model

INTRODUCTION

The mitral valve (MV) is a complex apparatus, mainly consisting of mitral annulus, anterior and posterior leaflets, chordae tendineae and two sets of papillary muscles. The MV is situated between the left atrium and the left ventricle, which, under regular healthy conditions, ensures that blood can only flow in one direction. Therefore, there are two key stages to understand the function of the MV. Firstly, the valve allows blood to flow from the left atrium to the left ventricle during diastolic ventricular filling, and secondly, the valve prevents backflow of blood into the left atrium during systolic ventricular ejection^[1]. If the valve is diseased, it cannot ensure a unidirectional flow of blood into the left ventricle, causing the back flow of blood into the left atrium during systole, a condition known as mitral regurgitation (MR). There are multiple notable causes of MR, but this study will focus on posterior leaflet prolapse and ruptured chordae tendineae in the context of hypertrophic obstructive cardiomyopathy (HOCM)^[2].

A population-based study into the burden of valvular heart diseases highlighted that MR is the most common heart disease in the western population, and the prevalence of the disease rose strikingly with advancing age^[3]. For this reason, there is a lot of interest in developing safer and more repeatable procedures for the treatment of MR. More specifically, there is a push to develop procedures which are less invasive than conventional open-chest surgery, as a large number of patients (as many as 49%) with MR in need of repair or replacement are considered at high risk for surgical intervention^[4]. Reasons for this high risk association can be due to the patients' age and other comorbidities (the presence of additional diseases co-occurring with the primary disease) and the result is that the patients simply do not qualify for conventional open-chest surgery^[5].

The MitraClip system is a minimally invasive procedure to treat MR in the case where a patient isn't eligible for open-chest surgery. Unlike conventional surgery, the MitraClip procedure does not require opening of the chest. Instead, clinicians access the MV with a catheter that is guided through a vein in the patient's leg to reach the heart^[6]. There are also a lot of cases which suggest that the MitraClip system is in fact an effective approach for the treatment of severe MR in practice. The initial Egyptian experience study into percutaneous mitral repair with MitraClip system demonstrated that, out of five patients, procedural success was achieved in all patients (100%). There was no procedural mortality after 30 days. In addition to the reduction in MR severity, the clinical status improved in 4 patients (80%) at discharge^[7]. Furthermore, the initial French experience provided additional evidence to support the positive effects of MitraClip implantation. The study was based on the treatment of 62 patients (72.7 ± 11.4 years; 71.7% men; New York Heart Association (NYHA) class III or IV; MR \geq grade 3) and assessed their conditions pre and post treatment. The study concluded that the in-hospital mortality rate was 3.2%, survival rate at 6 month follow up was 83.1%, with 90.9% of patients in NYHA class I or II and residual MR \leq grade 2 in 80% of cases^[8]. So, despite being an initial learning phase, the results should be seen as promising for the patients who are ineligible for open-chest surgery. That being said, there is a clear indication for further improvement of the MitraClip system, particularly the use of simulation work and clinical trials to further understand the MV and its interaction with the MitraClip.

Therefore, the aim of this paper is to investigate the effects of MitraClip implantation on regurgitant MV function, in terms of the valve's ability to close entirely during systole as well as stress distribution across the valve leaflets at peak systole and diastole, in a finite element (FE) environment.

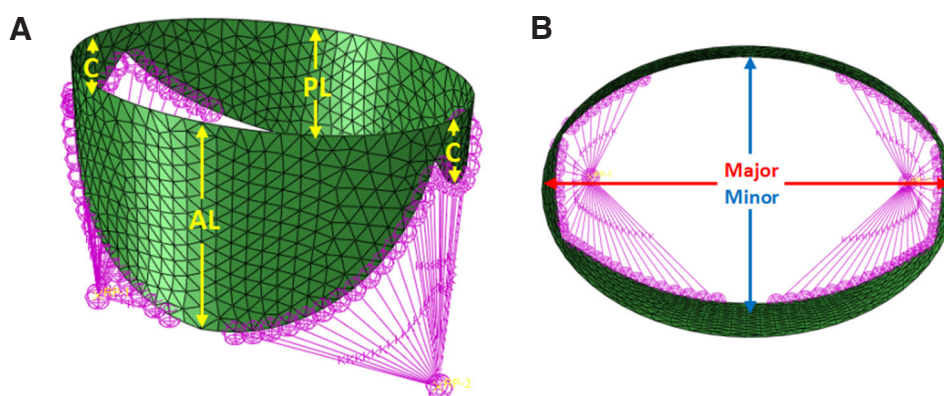


Figure 1. Initial configuration for diseased valve without MitraClip: (A) isometric view; (B) aerial view

METHODS

Description of mitral valve model

Simulations were carried out by Abaqus explicit solver^[9]. The step time was 1 s for the systolic step in all simulations. The stable time increment was of the order 10^{-6} s throughout the analysis. The MV geometry was created using surface modelling tools available in NX^[10] and dimensions were based on an anatomic study into intact and excised valves^[11]. Notably, the anterior leaflet, posterior leaflet and commissures have a height of 18 mm, 11 mm and 6 mm, respectively. The annulus was approximated to an elliptical profile with a major axis of 34 mm and a minor axis of 24 mm [Figure 1]. The main steps to create the geometric model were extruding an ellipse to form a solid body, then creating planar sketches for the anterior and posterior leaflet profiles, and finally projecting these sketches around the solid body. The result was a planar surface contained within a 3D modelling space which could then be imported into Abaqus to complete the pre-processing stages.

The geometry was discretised into 1,023 S3 shell elements (3-node triangular general-purpose shell, finite membrane strains) and a thickness of 1 mm was assigned to each element (the thickness was assumed uniform across the tissue). A series of spring elements were attached to the free edge of the leaflets to represent the chordae tendineae. A stiffness of 1.6 N/mm was assigned to each spring^[12]. Two reference points were used to account for the papillary muscles.

The simulations were carried out for two separate models. The first configuration was the diseased valve without a clip, aiming to understand how the MV is failing to operate [Figure 1].

The second configuration employed a reconstructed geometry, where the central regions of the anterior and posterior leaflets were clamped together to represent the MitraClip implanted [Figure 2].

Interaction, loading and boundary conditions

The motion and pressures in which the MV apparatus experiences during systole and diastole are completely governed by its interaction with blood flow as it passes from the left atrium to the left ventricle. Since blood will not be explicitly modelled within the FE simulation, appropriate boundary conditions and loading will need to be applied to the valve to capture this interaction.

In both simulations, a linearly increasing ventricular pressure from 0 to 120 mmHg (0.016 MPa) was applied to the outer surfaces of the valve to represent systolic peak during the first step. In the second step, a linearly increasing pressure from 0 to 5 mmHg (0.0007 MPa) was applied to the inner surfaces of the valve to represent diastole. The annulus and papillary muscles were fully constrained, and the effect of papillary

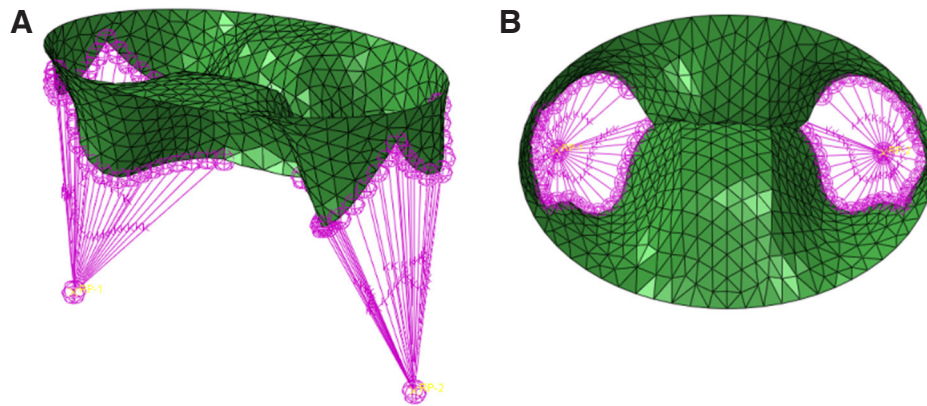


Figure 2. Initial configuration for diseased valve with MitraClip: (A) isometric view; (B) aerial view

muscle displacement and annular motion on the stress pattern was shown negligible at systolic peak^[13]. For the second simulation, the central-region nodes were also fully constrained to represent the MitraClip. In this study, MitraClip was not modelled explicitly in the FE simulations. Instead, full constraints have been used to define the MitraClip's interaction with the leaflets. The amount of interaction between the anterior and posterior leaflets was decided based on the dimension of the MitraClip, taken from Abbott's product specification^[14]. Specifically, a total of 114 elements in the central region of the leaflets (78 elements for anterior and 36 elements for posterior) were fully constrained to define the interaction between the anterior and posterior leaflets as a result of the clip.

A surface to surface contact condition was defined between all the inner surfaces of the MV. For the normal behaviour, hard contact was used to model the overclosure response, and separation was allowed after contact. For the tangential behaviour, a penalty friction formulation was used, and the value of friction coefficient assigned was taken as 0.05. Directionality of friction was assumed to be isotropic. This interaction has been previously justified, as it characterises the contact between soft and wet surfaces, such as hydrogels, whose surface behaviour may be considered a good approximation for the leaflets in the absence of further experimental data. The use of the penalty contact algorithm (assumes surfaces start to interact just before they actually touch each other) is also justified as *in-vivo* leaflets do actually start to interact before coapting, since just before actual contact a blood film is trapped between them and then moved away by the leaflets^[15].

Material model

A 5th order hyperelastic reduced polynomial model (available in Abaqus CAE) was adopted to describe the mechanical behaviour of the MV tissue. This model assumes that the deformation of the material can be described by a strain energy density formulation, from which the stress-strain relationship can be derived. The strain energy density equation is defined as^[16]:

$$U = \sum_{i=1}^N c_{i0}(\bar{I}_1 - 3)^i + \sum_{i=1}^N \frac{1}{D_i}(J^{el} - 1)^{2i} \quad (1)$$

where U is the strain energy per unit of reference volume, N is a material parameter, C_{i0} and D_i are temperature-dependent material parameters. Here, \bar{I}_1 is the first deviatoric strain invariant defined as:

$$\bar{I}_1 = \bar{\lambda}_1^2 + \bar{\lambda}_2^2 + \bar{\lambda}_3^2 \quad (2)$$

where the deviatoric stretches $\bar{\lambda}_i = J^{-\frac{1}{3}}\lambda_i$, J is the total volume ratio, J^{el} is the elastic volume ratio and λ_i are the principal stretches.

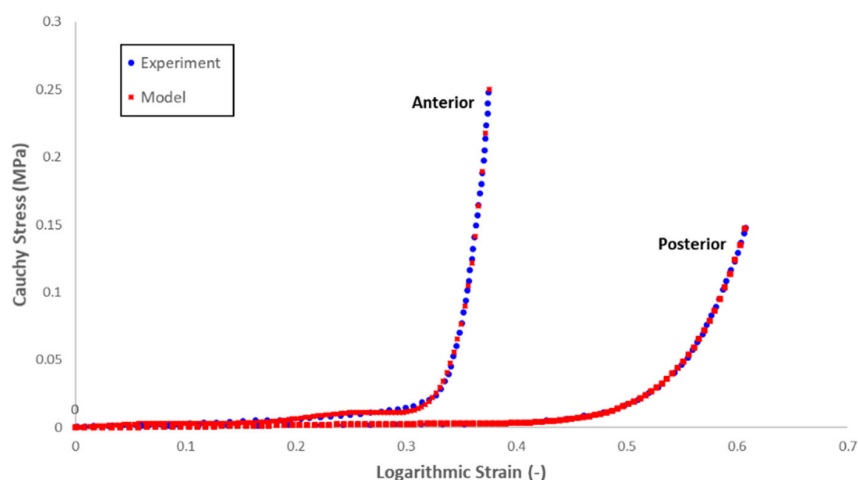


Figure 3. Radial stress-strain response of human hypertrophic obstructive cardiomyopathy anterior and posterior leaflet samples, experimental data vs. model simulation

Table 1. Reduced polynomial material parameters used to define the mechanical behaviour of the mitral valve tissue

Leaflet	C_{10}	C_{20}	C_{30}	C_{40}	C_{50}	D_1	D_2	D_3	D_4	D_5
Anterior	0.00964	-0.106	0.788	-2.364	2.514	0.001	0	0	0	0
Posterior	0.00110	0.00152	-0.00389	0.00222	0.00079	0.001	0	0	0	0

For the anterior leaflet, the model parameters were obtained by fitting the constitutive equation to experimental radial stress-strain data of HOCM anterior valve tissue provided in the literature^[17]. It should be noted that the anterior and posterior leaflets exhibit different mechanical behaviour, with the posterior leaflet having higher extensibility (i.e., lower stiffness). Due to a lack of stress-strain data for the HOCM posterior leaflet, a shift, based on the difference measured for healthy anterior and posterior leaflets^[17], has been applied to the stress-strain data for HOCM anterior leaflet in order to capture the increased extensibility of a posterior leaflet. The shifted stress-strain data were then used to calibrate the model parameters for the HOCM posterior leaflet. The resulting curve fit and model parameters can be seen in Figure 3 and Table 1, respectively, and were obtained by conducting single-element simulation using the described material model. The compressibility (D) parameters were fixed based on the assumption of MV tissue being “nearly incompressible”^[18], whilst the C values were refined iteratively to obtain the closest fit. A density of 10.4 g/cm^3 was assigned to all MV tissue. This value is ten times higher than the actual value to account for the inertial effects of blood flow^[13].

As aforementioned, the mechanical behaviour of the chordae tendineae was described using a simplified spring stiffness approach. The value of 1.6 N/mm was estimated according to chordae properties given in the literature^[12]. The use of built-in SPRING elements from the Abaqus library is ideal for simulating the response of the chordae tendineae, as this element is able to transmit axial load through a line of action and this line of action is able to rotate when subjected to large-displacement analysis^[19], which is characteristic of the simulations within this study.

RESULTS

Continuum mechanics can be used to describe the deformation of structures through the use of a nine-component stress tensor. However, individually, these values can be difficult to interpret and use in a practical sense. Therefore, more informative stress values can be obtained through the use of mathematical operations, derived from the original nine-component tensor. In this study, the results were provided with

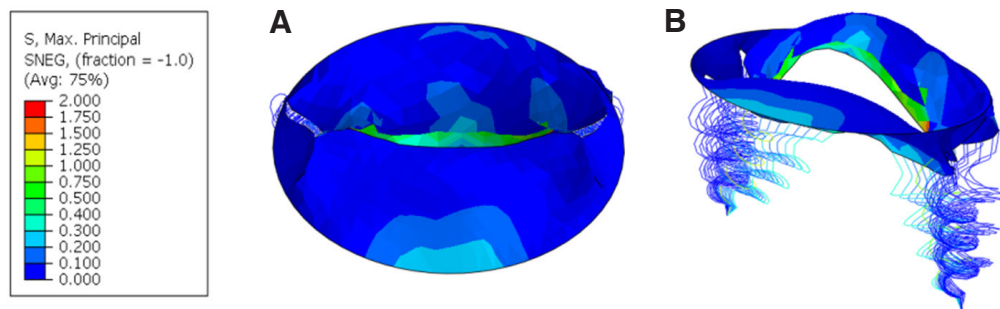


Figure 4. Maximum principal stress (MPa) contour plot for mitral valve at peak systole without MitraClip: (A) aerial view; (B) isometric view

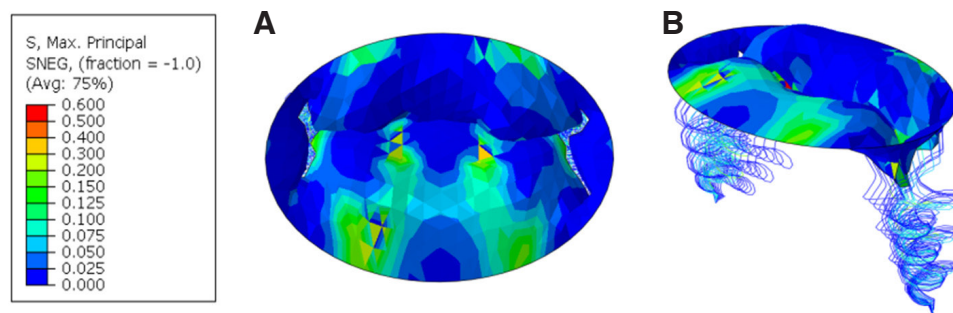


Figure 5. Maximum principal stress (MPa) contour plot for mitral valve at peak systole with MitraClip: (A) aerial view; (B) isometric view

respect to the maximum principal stresses in the leaflets. The principal stresses indicate the minimum and maximum normal stresses an element will undergo for the given external loading conditions.

Systole

Figures 4 and 5 show the simulation results for the diseased valve operating at peak systole, without and with the MitraClip, respectively. The deformation scale factor is set to 1 for true representation.

It can be seen from Figure 4 that the posterior leaflet has prolapsed due to a combination of the increased extensibility of HOCM leaflets and the ruptured chordae presented in the model. The area created by this prolapse would allow for a regurgitant jet to pass blood back into the left atrium and subsequently cause MR. In terms of the stress distribution, it generally falls in the range of 50-300 kPa across most of the valve's surface. However, there is a large stress concentration of approximately 2.0 MPa in the region where the prolapsed leaflet is attached to the chordae. This can be expected due to the unnaturally large amount of deformation experienced by the posterior leaflet during these prolapsed conditions. That being said, the key finding highlighted by Figure 4 is the displacement of the posterior leaflet, and how its position at peak systole is preventing complete closure of the MV leaflets.

From Figure 5, it can be seen that the introduction of the MitraClip has affected the MV function at peak systole. The central region of the leaflets, fixed together as a result of clip implantation, has solved the previous issue of prolapse. From the aerial view, it can also be seen that the valve has almost completely sealed off any gaps which were present before. The order of magnitude of stress has not been affected significantly by the introduction of the MitraClip across the majority of the surfaces.

In terms of specific values, the stress distribution generally falls in the range of 50-300 kPa across the majority of the valve's surface, similar to the simulation without MitraClip [Figure 4]. However, the introduction of the clip has significantly reduced the peak stress experienced by the valve to a value of

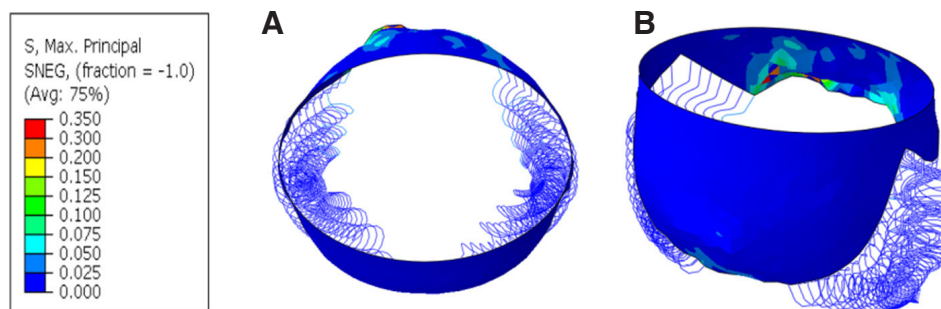


Figure 6. Maximum principal stress (MPa) contour plot for mitral valve at peak diastole without MitraClip: (A) aerial view; (B) isometric view

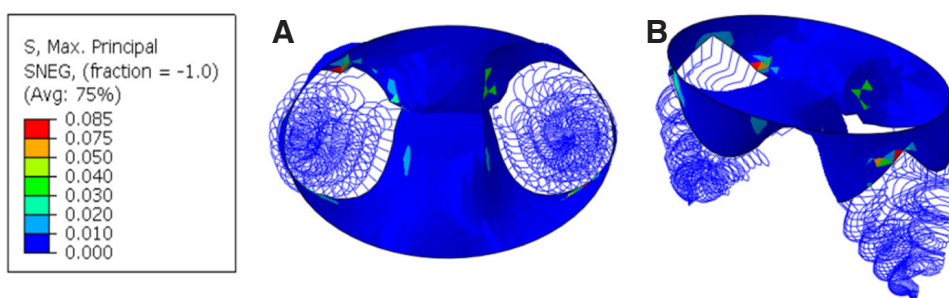


Figure 7. Maximum principal stress (MPa) contour plot for mitral valve at peak diastole with MitraClip: (A) aerial view; (B) isometric view

approximately 600 kPa (compared to 2.0 MPa for the simulation without MitraClip; [Figure 4](#)). This is because the fixed central region of the leaflets eliminated the stress caused by posterior leaflet prolapse. [Figure 5](#) highlights that the MitraClip has not only improved the MV function in terms of its ability to coapt successfully, but also alleviated the local increase in stress due to prolapse.

Diastole

[Figures 6 and 7](#) show the valve operating during diastole, without and with the MitraClip, respectively. The MV is subjected to a reduced level of stress during diastole due to the lower atrial pressure applied and the lack of surface interaction between the leaflets.

In [Figure 6](#), the region where the chordae are ruptured leads to an increase in stress relative to the rest of the valve. This follows a similar pattern to the stress concentration presented in [Figure 4](#); however, the order of magnitude is much lower. The stress distribution across the leaflets generally falls in the range of 0 to 75 kPa, and peak stress occurs in the regions on the posterior leaflet where the chordae was attached, with a magnitude of approximately 350 kPa. The increased extensibility of the HOCM leaflets and ruptured chordae has also caused the posterior leaflet to displace further in the ventricular direction than what would be seen under healthy conditions.

[Figure 7](#) highlights the “double orifice area” induced by the clip during diastole. This is a key feature that arises as a result of the anterior and posterior leaflets being clamped together through the use of a clip. As the central region of the valve leaflets are fixed together, a small gap is created on either side of the clip during diastolic ventricular filling. The double orifice area is a crucial characteristic after edge-to-edge repair of the MV, as it acts as passageway for blood to flow through during diastole but allows for the leaflets to completely close up and seal off backflow of blood during systolic ventricular ejection^[20].

Even with the presence of a double orifice area, the majority of the valve is still subjected to a relatively low level of stress (less than 30 kPa), with a few small areas of stress concentration due to the attachment of the

chordae. Again, the peak stress has been reduced by the introduction of the MitraClip to a value of 85 kPa during diastole.

DISCUSSION

Previous clinical trials have reported the successful use of the MitraClip system as a solution for severe MR in the context of HOCM. A study of six patients suffering from the disease concluded that percutaneous mitral repair using the MitraClip is feasible and may be performed safely in HOCM, and this technique can be effective in reducing MR and improving symptoms^[21]. This is supported by clinical trial which reported that patients experienced a reduction in MR and a reduction in the left ventricular outflow tract (LVOT) gradient from a mean of 75.8 ± 39.7 to 11.0 ± 5.6 mmHg^[22]. Nearly all patients demonstrated improvements in symptoms by either new NYHA class designations or improved exercise tolerance^[22].

The results of our FE simulations provided further evidence to support that MitraClip implantation is a viable approach for solving MR in the case of posterior leaflet prolapse due to HOCM leaflet extensibility and chordae rupture. The introduction of the clip has prevented the leaflet from prolapsing and aided in almost complete closure of the valve during peak systole. The general order of magnitude of stress across the leaflets has not been affected significantly by the clip during systole and diastole. In fact, in the previously prolapsed region where the chordae are present, the MitraClip has alleviated stress significantly.

There have been a number of previous studies into FE modelling of the MV apparatus with a focus on topics such as nonlinear tissue response, annulus dilation and relative papillary muscle motion. However, the literature is limited regarding simulations of the MitraClip system in an FE environment, and also addressing the diastolic stage. The novelty of the current study lies with the concise side-by-side comparison of the same diseased valve, operating under the same loading conditions, with and without the MitraClip, respectively. The performance of the valve has been assessed under both peak systolic and peak diastolic conditions, and a quantifiable improvement in MV function has been established after introduction of the clip. Therefore, the results should aid in a better understanding of the implications that the MitraClip system has on both the stress distribution and deformation of the MV leaflets. This information may aid in further development of the MitraClip and should encourage further research into advanced, minimally invasive treatments for severe MR in high risk patients.

Further work is also needed to refine the FE method employed in the current study to more accurately capture the impact of HOCM and produce more comprehensive results in the future. Notably, the effects of annulus dilation and relative papillary motion will have to be accounted for to produce a less conservative model. Furthermore, a deeper analysis into simulating systolic anterior motion would be beneficial due to the risk of LVOT obstruction and MR, which is associated with a 20% risk of sudden death^[23]. That being said, the current study has provided a solid ground to extend to these related areas. In addition, a fluid-structure interaction (FSI) model would be more helpful for analysing MitraClip behaviour during diastolic stage. Some previous computational studies have employed FSI approach in modelling and understanding the function of mitral valve^[24,25], but not in the context of MitraClip repair. To run an appropriate FSI analysis for mitral valve repaired with MitraClip, it will require tremendous additional efforts and times, and seems beyond the scope of current study. Nevertheless, the research group are currently undertaking such analyses and the results will be reported in future.

DECLARATIONS

Authors' contributions

Contributed to the research and the preparation of the manuscript: Prescott B, Abunassar CJ, Baxevanakis KP, Zhao L

Carried out the work, processed the results and drafted the manuscript: Prescott B

Supervised the technical work and also contributed to discussions, writing and editing of the manuscript: Abunassar CJ, Baxevanakis KP, Zhao L

Availability of data and materials

Further data and materials are available upon request to Professor Ligu Zhao at Loughborough University (email: L.Zhao@Lboro.ac.uk).

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Conflicts of interest

The authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Original Article

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Carotid atherosclerosis-related mutations of mitochondrial DNA do not explain the phenotype of metabolic syndrome

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Abstract

Aim: This study was undertaken to explore the relationship between metabolic syndrome (MetS) and atherosclerosis-related mitochondrial DNA (mtDNA) mutations, since MetS shares conventional and genetic risk factors with atherosclerosis.

Methods: The study involved 220 participants; the carotid ultrasonography followed by intima-media thickness (cIMT) measurement was used for quantitative diagnostics of carotid atherosclerosis. The diagnosis of MetS was set according to International Diabetes Federation criteria (IDF-2009). The level of mtDNA heteroplasmy in leukocytes was determined by qPCR. The severity of MetS was estimated on combination of serum HDL cholesterol, triglycerides and fasting glucose, systolic and diastolic blood pressure, and waist circumference measurements.

Results: MetS was present in 44 study participants. Ten mtDNA mutations were tested, and m.3336T>C and m.652delG heteroplasmy levels correlated with the clusterization of MetS symptoms, in particular the cardiovascular and metabolic risk factors, of triglyceride and fasting glucose levels. The other mtDNA mutations each only correlated with one symptom (i.e., m.652delG and m.12315G>A-with triglycerides; m.3256C>T, m.1555A>G, and m.15059G>A-with systolic blood



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pressure; m.14846G>A-with fasting glucose). There was no correlation between integral severity of MetS and cIMT.

Conclusion: In this study, the MetS phenotype was not explained directly by atherosclerosis-related mtDNA variants. It is possible to hypothesize that mtDNA-related mechanisms in atherosclerosis and MetS may be different, in spite of the similarity of several phenotypic characteristics.

Keywords: Metabolic syndrome, carotid atherosclerosis, mitochondrial DNA mutations, risk factors

INTRODUCTION

Excessive body mass, obesity, metabolic syndrome (MetS) and Type 2 diabetes are metabolic disturbances affecting the population, often occurring in parallel with atherosclerosis development, contributing to morbidity. All of these pathologies are characterized by excessive deposition of body fat and health impairment. Cardiovascular and metabolic diseases are in part of genetic, in part of behavioral origin. Genetic influences are either hereditary or due to somatic (acquired) mutations. Pathogenic mutations can occur either in the nuclear DNA or in the mitochondrial DNA (mtDNA). A number of genome-wide association studies (GWAS) have been carried out in order to discover genes related to metabolic diseases^[1-4]. However, commercially available GWAS arrays used in such studies rarely cover mitochondrial variants in the population very well. Therefore, the role of mitochondrial genes in metabolic diseases has been less well studied, even though the mitochondria play the definitive role in energy production and metabolism, and in the development of oxidative stress. Despite the ever increasing prevalence and high heritability of atherosclerosis and metabolic diseases, as well as intensive and long research efforts, the causal genes remain poorly known. Mitochondria are the center of energy metabolism in the human body; therefore, variations of their genes and function are expected to influence metabolism and reactive oxygen species (ROS) production.

Epidemiological and clinical studies have revealed a clustering of conventional cardiovascular risk factors including obesity, MetS, Type 2 diabetes mellitus, atherosclerosis, and coronary artery disease. Each conventional risk factor alone increases the risk of clinical manifestations of atherosclerosis with the combination of several risk factors exacerbating clinical sequelae. This is also true in the setting of MetS, with multiple risk factors resulting in heightened risk of atherosclerotic disease together with cardiometabolic abnormalities. MetS is also generally defined in clinical studies and clinical practice as a cluster of risk factors associated with Type 2 diabetes mellitus and cardiovascular disease^[5-10]. It is further generally recognized that conventional risk factors possess a significant genetic component, but the evidence of the role of genetic factors in risk factor clustering in individuals remains uncertain^[11].

It has recently been shown that several mutations of mtDNA are associated with atherosclerosis. Namely, heteroplasmic mutations m.652delG, m.1555A>G, m.3336T>C, m.3256C>T, m.5178C>A, m.12315G>A, m.13513G>A, m.14459G>A, m.14846G>A, and m.15059G>A were found in atherosclerotic plaques of human aortic intima, and there were significant differences in the heteroplasmy level between unaffected and atherosclerotic tissues. These mutations occurred in mitochondrial genes MT-RNR1 (rRNA 12S); MT-TL1 and MT-TL2 (tRNA-Leu); MT-ND1, MT-ND2, MT-ND5, and MT-ND6 (subunits 1, 2, 5 and 6, of NADH dehydrogenase, respectively), and MT-CYB (cytochrome b)^[12-15]. Further, most of these mutations were found to be associated with the severity of carotid atherosclerosis and, with lesser extent, the presence of coronary heart disease^[16-20].

Since MetS shares common risk factors with atherosclerosis and, moreover, is considered itself as the independent risk factor for atherosclerosis, this study was performed to test the hypothesis that MetS and atherosclerosis-related heteroplasmic mtDNA mutations are associated.

METHODS

Patients

This study was kept in accordance with the *Helsinki Declaration* of 1975 as revised in 1983, 2008 and 2013, and was approved by the local ethics committee. The study participants were recruited from the visitors' flow at municipal outpatient clinics, who have passed a routine screening for cardiovascular risk factors. In total, 220 study participants were recruited (97 men, 123 women) with a mean age of 65.1 years (SD 9.4). The written informed consent was obtained from all participants prior to inclusion in the study.

MetS diagnostics

For identification of patients with clustered risk factors and MetS, IDF 2009 criteria were used^[10]. In brief, waist circumference > 94 cm in men and > 88 cm in women, triglycerides ≥ 150 mg/dL or drug treatment for elevated triglycerides, HDL cholesterol ≤ 40 mg/dL in men and ≤ 50 mg/dL in women, or drug treatment for reduced HDL cholesterol, systolic blood pressure ≥ 150 mmHg and/or diastolic blood pressure ≥ 85 mmHg, or antihypertensive drug treatment, and fasting glucose ≥ 5.6 mmol/L or drug treatment of elevated glucose were taken in consideration. The presence of at least 3 of above criteria was required for the diagnosis of MetS. The group of MetS-free study participants consisted of subjects who had 0-1 of the above criteria. The subjects with 2 MetS criteria were not eligible for inclusion in the study to avoid possible uncertainties. To formally describe the severity of MetS (the extent of clusterization of conventional risk factors), an unofficial integral MetS index was calculated as a simple multiplication of the absolute values of waist circumference, triglycerides, systolic and diastolic blood pressure, and fasting glucose, divided by HDL cholesterol.

Ultrasonographic examination

For diagnostics of carotid atherosclerosis, high-resolution B-mode carotid arterial ultrasonography imaging was used (SSI-6000 ultrasound system, SonoScape, China, equipped by 7.5-MHz L741 linear array probe). The protocol of ultrasound examination developed earlier by Salonen *et al.*^[21], 1995 was used. The cIMT measurements were carried out with M'ATH software package (IMT, France). The extent of carotid atherosclerosis and the size of atherosclerotic plaques were evaluated as described elsewhere^[17,22].

MtDNA genotyping

DNA was isolated from circulating leukocytes (whole venous blood) using a commercial kit for DNA isolation and purification (QIAGEN GmbH, Germany) according to manufacturer's instructions. DNA concentration in samples was determined by NanoPhotometer Pearl UV/Vis SDRAM P-34 (IMPLEN, Germany); the samples were kept in TE buffer at a concentration of 0.03 $\mu\text{g}/\mu\text{L}$. Heteroplasmy levels of mtDNA mutations m.652delG, m.1555A>G, m.3336T>C, m.3256C>T, m.5178C>A, m.12315G>A, m.13513G>A, m.14459G>A, m.14846G>A, and m.15059G>A were analyzed on Real Time PCR System 7500 Fast (Applied Biosystems, USA) by qPCR (5'-terminal tag excision, TaqMan Assay). The nucleotide sequences for primers and probes are shown in Table 1.

For qPCR, 4 μL DNA was added to 25 μL of standard reaction mixture [1x TaqMan Buffer, 3 mmol/L MgCl_2 , 250 $\mu\text{mol/L}$ of each dNTP, 300 nmol/L primers, 300 nmol/L hybridization probes, 0.5 units Taq-polymerase (Helicon, Moscow, Russia)]. Denaturation was held for 2 min at 94°C; amplification stage with fluorescence detection included denaturation for 10 s at 94°C, and annealing for 15 s at a temperature specified for each pair of primers and probes (range 61°C-67°C).

Statistical analysis

The statistical analysis was done using the IBM SPSS 22.0 software package (IBM Corp., Chicago, IL, USA). The methods of descriptive statistics, correlation analysis by Spearman and Pearson, and one-way analysis of variance (ANOVA) were used. Mean values were compared by *T*-test or *U*-test by Mann-Whitney for continuous variables, and χ^2 Pearson's test for categorical variables. To assess the homogeneity of variance,

Table 1. Nucleotide sequences for primers and probes for qPCR

Mutation	Primers	Probes
m.652delG	F actgaaatgttttagacgggct R ggggatgcttgcattgtgtaa	5'-ROX- aatagggttggctctagcctttctattagctc -BHQ-2-3' 5'-FAM- aatagggttggctctagcctttctattagctc -BHQ-1-3'
m.1555A > G	F aggacatttaactaaaaccctacg R agctacactctggttctgcca	5'-ROX- agaggaaacaagtcgtaacatgtaagtgtac -BHQ-2-3' 5'-FAM- agaggagacaagtcgtaacatgtaagtgtac -BHQ-1-3'
m.3256C > T	F ataccacacccaccaag R aagaagaggaattgaacctctgact	5'-ROX- gcagagcccgtaaatcgataaaactta -BHQ-2-3' 5'-FAM- agagcccgtaaatcgataaaacttaaa -BHQ-1-3'
m.3336T > C	F acagtcagaggttcaattctctt R ttctgttcgtaagcattagga	5'-ROX- tactctctatcgtagccatttctaatcgc -BHQ- -3' 5'-FAM- ttctcattgtaccatttctaatcgaat -BHQ-2-3'
m.5178C > A	F cttaactccagcaccacgac R aggctctcaggagagga	5'-ROX- atctcgacactgaaacaagataacatga -BHQ-2-3' 5'-FAM- cgcacctgaaacaagctaacatgactaa -BHQ-1-3'
m.12315G > A	F cagctatccattggtcttaggc R ggaagtcagggttaggtggt	5'-ROX- ccaaaaatttttagtgcaactccaaataaaag -BHQ-2-3' 5'-FAM- ccaaaaatttttagtgcaactccaaataaa -BHQ-2-3'
m.13513G > A	F gcagcctagcattagcagga R atagggtcaggcgtttgt	5'-ROX- caggttttactccaaaaccacatcatc -BHQ-2-3' 5'-FAM- caggttttactccaaaaccacatcatc -BHQ-2-3'
m.14459G > A	F ccactaaaacactcaccaagacc R tttaggggaatgatgttg	5'-ROX- ctgagatactctcaatagccatcactgt -BHQ-2-3' 5'-FAM- ggatactctcaatagccatcgtgtag -BHQ-2-3'
m.14846G > A	F aaccactcattcatgcacctc R cctgtggtgatttgaggat	5'-ROX- gcatgatgaaactcagctcactcctt -BHQ-2-3' 5'-FAM- catgatgaaactcagctcactcctt -BHQ-2-3'
m.15059G > A	F caatggcgctcaattctt R caggaggataatgccgatgt	5'-ROX- gggcgaggcctattatcagatcatttct -BHQ-2-3' 5'-FAM- gcgaggcctattatcagatcatttct -BHQ-2-3'

F-Test was used. The data were presented in terms of mean and SD, where appropriate. The significance of differences was defined at the 0.05 level of confidence.

RESULTS

In total, 220 participants were recruited in the study. Forty-four study participants (20% of the sample) met the criteria of MetS. The data on anthropometric, clinical and biochemical data are presented in [Table 2](#).

As expected, the two groups differed significantly in parameters taken as the criteria for MetS (waist circumference, the presence of arterial hypertension and Type 2 diabetes mellitus, systolic and diastolic blood pressure, blood glucose, serum triglycerides and HDL cholesterol). Additionally, there was a difference in body mass index, the prevalence of left ventricular hypertrophy, the prevalence of CHD, and family anamnesis of Type 2 diabetes mellitus. At the same time, there were no statistically significant differences in age, smoking, total and LDL cholesterol, and family anamnesis of myocardial infarction and hypertension.

Instrumental data on the extent of carotid atherosclerosis assessed by high-performance ultrasound examination are given in [Table 3](#). Mean cIMT and mean maximum cIMT were significantly higher in MetS patients, thus demonstrating higher predisposition to atherosclerosis in patients with cardiometabolic abnormalities. However, the difference in the extent of carotid atherosclerosis assessed by the size of atherosclerotic plaques did not reach statistical significance. Moreover, the correlation coefficient between cIMT and integral MetS index did not reach statistical significance ($r = 0.119$, $P = 0.10$).

The data on genotyping of mtDNA are given in [Table 4](#). Among 10 mtDNA mutations studied, only heteroplasmy levels for m.3336T>C and m.14846G>A mutations were significantly different between MetS patients and MetS-free study participants. The correlation analysis revealed significant correlations between the severity of MetS assessed by integral MetS index and m.652delG heteroplasmy ($r = 0.213$, $P = 0.003$), and m.3336T>C heteroplasmy ($r = 0.323$, $P < 0.001$), but not m.14846G>A heteroplasmy.

Several correlations were revealed between mtDNA heteroplasmy and stand-alone components of MetS. Systolic blood pressure correlated with heteroplasmy m.1555A>G ($r = -0.144$, $P = 0.046$), m.3256C>T ($r = 0.197$, $P = 0.006$), and m.15059G>A ($r = 0.218$, $P = 0.002$). Serum triglycerides correlated with heteroplasmy m.652delG ($r = 0.190$, $P = 0.008$), m.3336T>C ($r = 0.291$, $P < 0.001$), and m.12315G>A ($r = 0.153$, $P = 0.034$).

Table 2. Anthropometric, clinical and biochemical characteristics of study participants

Variable	MetS-free study participants, <i>n</i> = 176	MetS patients, <i>n</i> = 44	<i>P</i> for the difference
Age, years	65.0 (9.9)	65.7 (7.5)	NS
Gender, m:f	76:100	21:23	NS
BMI, kg/m ²	25.8 (3.9)	30.9 (4.8)	< 0.001
Waist circumference, cm	85.9 (4.1)	100.9 (5.6)	< 0.001
Systolic BP, mmHg	146 (13)	137 (18)	< 0.003
Diastolic BP, mmHg	81 (11)	88 (10)	< 0.001
Current smokers, %	8	11	NS
Hypertension, %	60	87	0.002
LVH, %	31	50	0.024
T2DM, %	4	47	< 0.001
CHD, %	19	45	0.001
Family history of AMI, %	27	34	NS
Family history of HT, %	40	37	NS
Family history of T2DM, %	13	34	0.002
Total cholesterol, mg/dL	240 (48)	234 (47)	NS
Triglycerides, mg/dL	113 (47)	182 (72)	< 0.001
LDL cholesterol, mg/dL	148 (43)	144 (42)	NS
HDL cholesterol, mg/dL	69 (14)	53 (14)	< 0.001
Fasting glucose, mmol/L	5.3 (0.9)	6.4 (1.1)	0.004
Integral MetS index	5267 (3181)	18069 (12495)	< 0.001

Family history, the presence of the disease in first degree relatives diagnosed at age before 60. BMI: body mass index; BP: blood pressure; LVH: left ventricular hypertrophy; T2DM: Type 2 diabetes mellitus; CHD: coronary heart disease; LDL: low density lipoprotein; HDL: high density lipoprotein; AMI: acute myocardial infarction; HT: hypertension; NS: not significant; MetS: metabolic syndrome

Table 3. Characteristics of carotid atherosclerosis

Variable	MetS-free study participants, <i>n</i> = 176	MetS patients, <i>n</i> = 44	<i>P</i> for the difference
Mean cIMT, mm	0.853 (0.151)	0.935 (0.209)	0.006
Mean maximum cIMT, mm	0.986 (0.187)	1.086 (0.285)	0.009
Atherosclerotic plaques, score	0.78 (0.85)	1.08 (0.91)	0.071 (NS)

Note: to calculate the score for atherosclerotic plaques in carotid arteries, the 4-point scale was used (0, no plaques; 1-2, lesions occluding up to 10% or 10%-30% lumen diameter, respectively; 3, plaques occluding > 30% lumen diameter)[17]. NS: not significant; MetS: metabolic syndrome

HDL cholesterol correlated with heteroplasmy m.1555A>G ($r = 0.151$, $P = 0.037$) and m.14459G>A ($r = -0.165$, $P = 0.022$). Fasting blood sugar correlated with m.3336T>C heteroplasmy ($r = 0.180$, $P = 0.013$), and m.14846G>A heteroplasmy ($r = 0.142$, $P = 0.050$). None of the mutations correlated with waist circumference or diastolic blood pressure.

Linear regression analysis was performed to explain the variability of integral MetS index by the presence of heteroplasmic mtDNA mutations. The linear regression model explained 14.2% variability of integral MetS index ($R = 0.377$, $P = 0.003$). The most potent explanatory variable was T3336C heteroplasmy ($P = 0.003$); other mutations did not reach explanatory level by statistical significance.

As mentioned above, the difference between MetS-free study participants and MetS patients reached statistical significance for BMI, waist circumference, systolic and diastolic BP, hypertension and left ventricular hypertrophy, Type 2 diabetes prevalence and family history, triglycerides, HDL cholesterol, fasting glucose, integral MetS index, mean and mean-maximum cIMT, plaque score, and heteroplasmy for m.3336C>T and m.14846G>A mutations. Therefore, the null-hypothesis on the absence of difference was rejected with more than 95% probability, and the groups size was sufficient to demonstrate the observed differences. We have also checked the statistical power of the study for those mtDNA mutations, for which

Table 4. Heteroplasmy level of mtDNA (% of mutant allele) in leukocytes from MetS patients and MetS-free study participants

Heteroplasmic mtDNA mutation	MetS-free study participants, <i>n</i> = 176	MetS patients, <i>n</i> = 44	<i>P</i> for the difference
m.652delG	2.8 (7.7)	5.3 (9.0)	NS
m.1555A>G	17.1 (11.6)	14.3 (7.4)	NS
m.3256C>T	22.6 (14.3)	26.0 (16.1)	NS
m.3336T>C	7.9 (5.1)	13.1 (15.5)	0.036
m.5178C>A	15.4 (9.9)	16.7 (13.3)	NS
m.12315G>A	22.0 (12.5)	25.3 (12.3)	NS
m.13513G>A	24.6 (19.1)	20.3 (17.1)	NS
m.14459G>A	17.2 (12.9)	17.2 (12.1)	NS
m.14846G>A	14.6 (11.5)	20.8 (13.7)	0.047
m.15059G>A	6.2 (5.7)	6.7 (5.8)	NS

NS: not significant; MetS: metabolic syndrome; mtDNA: mitochondrial DNA

the differences in heteroplasmy levels did not reach statistical significance. Statistical power varied from 28% to 76%; the analysis has shown that the sample size was insufficient to exclude type 2 error for mutations m.652delG, m.1555A>G, m.12315G>A, and m.13513G>A. For all remaining mutations, the increase of sample size turned unreasonable, since it could not lead to statistically significant between-group differences.

DISCUSSION

In this study, we have found that only a few mtDNA mutations previously described as atherosclerosis-related ones^[13-18], are also associated with MetS. Namely, these are m.3336T>C mtDNA mutation and, to some extent the m.652delG mutation. Since MetS is a constellation of several risk factors, the observed associations should be explained by correlations with single cardiometabolic risk factors, that is the higher the correlation of risk factors with mtDNA heteroplasmy, the higher the probability of association with MetS. This seems to be especially true for m.3336T>C mtDNA mutation, which occurs in MT-ND1 gene (encodes subunit 1 of NADH dehydrogenase). It is known that the m.3336T>C mutation is a silent point mutation, resulting in an ATT to ATC substitution without changing the amino acid sequence of the transcribed protein. The association of m.3336T>C variant with metabolic syndrome may be speculatively explained by the linkage with some still unknown mutant haplotype associated with mitochondrial dysfunction. In any case, it is not at present possible to suggest any mechanistic role of m.3336T>C mutation in the formation of a pathologic phenotype; one can only consider this mtDNA variant as a potential biomarker or even as a bystander. It remains questionable, if this mutation may have something to do with disruption to mitochondrial glutathione redox status due to oxidative stress; the analysis of sequence variation in mitochondrial complex I genes did not describe m.3336T>C variant as possibly, probably or almost certainly pathogenic one^[23]. It should be mentioned that because of highly polymorphic nature of mtDNA, the establishing of polymorphic either pathogenic nature of any detected sequence change is still a major difficulty^[23].

As for the other mutations, there are some theoretical pathways whereby they may promote metabolic consequences, altering the expression of cardiometabolic risk factors via increased oxidative stress, increased ROS production, and mitochondrial dysfunction, which may be generally described as the inhibition of mitochondrial consumption of oxygen, the changes in the mitochondrial membrane potential, and the reduction of adenosine triphosphate levels due to an disbalanced intake and expenditure of energy^[24].

Mutation m.652delG (guanine deletion at position 652) in MT-RNR1 gene affects the function of 12S ribosomal RNA, even leading to complete mitochondrial dysfunction due to decrease in expression of respiratory chain enzymes, reduction of the amount of these enzymes and increase of oxidative stress^[25]. Mutation m.1555A>G in the same gene leads to a single nucleotide substitution, and is known to be associated with mitochondrial deafness and aminoglycoside-induced sensorineural hearing loss^[26,27]. Interesting, this mutation is also thought to stabilize the ribosome and provide some beneficial effect^[20].

In support of this, within our study, m.1555A>G heteroplasmy level negatively correlated with systolic blood pressure and positively correlated with HDL cholesterol, thus trending to be associated with lower cardiometabolic risk.

Mutation m.3256C>T occurring in coding sequence of the MT-TL1 gene (encodes tRNA leucine) leads to impaired protein synthesis due to reduced number of cellular organelles^[28,29]. This effect can be enhanced by mutation m.12315G>A, which is located in the coding sequence of another gene encoding tRNA leucine, namely, the MT-TL2 gene. So, the impairments in tRNA leucine production may act as a previously unknown mechanism for the development of metabolic abnormalities, with the m.12315G>A mutation known to be associated with mitochondrial encephalomyopathy^[30].

Three mutations (m.14459G>A, m.14846G>A, and m.15059G>A) occur in coding regions of two genes responsible for the synthesis of subunit 6 of NADH dehydrogenase and cytochrome B (MT-ND6 and MT-CYB genes, respectively). The impairments of these respiratory chain enzymes can attenuate NADH oxidation and ubiquinone (CoQ) reduction and promote oxidative stress. Mutation m.14459G>A leads to alanine to valine substitution in a conserved region of ND6 protein, and is associated with several mitochondrial disorders (Leber's hereditary visual neuropathy, hereditary ocular neuropathy, dysfunction of basal ganglia, atrophy of visual nerve, musculoskeletal syndrome and encephalopathy)^[31,32]. Mutations m.14846G>A and m.15059G>A induce the damage of cytochrome B. The former (glycine to serine substitution at position 34) affects intermediate transfer of electrons in mitochondrial respiratory chains. The latter (glycine to stop codon substitution at position 190) stops translation and leads to the loss of 244 amino acids at C-terminal of protein. Both mutations reduce enzymatic function of cytochrome B, and are associated with mitochondrial disorders in various myopathies^[33,34].

The list of mtDNA mutations associated with metabolic and atherosclerotic diseases obviously needs to be supplemented with new variants deserving further investigation. As an example, the T/C substitution at position 16189 in the hypervariable D-loop of the control region is suspected to be associated with various multifactorial diseases; the frequency of this mtDNA variant in patients with coronary artery disease and type 2 diabetes mellitus was higher as compared to healthy individuals of Middle European descent in Austria^[35].

Defective cell metabolism is considered as the main mechanism of MetS development, due to the disbalance of nutrient intake and utilization for energy^[36]. It is supposed that decreased fatty acid oxidation, in turn, increases the accumulation of fatty acyl-CoAs and other fat-derived molecules in various organs and cells, this causing the inhibition of insulin signaling, resulting in hyperinsulinemia, which targets various organs and tissues in metabolic diseases. It is known that mtDNA mutations correlate with increased ROS production in cells^[37,38]. Thus, oxidative stress induced by genetic factors, aging and mitochondrial biogenesis can affect mitochondrial function, leading to insulin resistance and related pathological conditions, such as MetS, Type 2 diabetes mellitus, cardiovascular and atherosclerotic disease^[39-42]. However, it is still not clear whether mitochondrial dysfunction is one of the primary causes of cardiometabolic disturbances, or merely a secondary effect^[43].

Beyond doubt, this study has certain limitations. First, the atherosclerotic state of study participants was evaluated only by carotid ultrasonography and cIMT measurement, the latter being widely used as a surrogate marker for detecting subclinical atherosclerosis for risk prediction and disease progress^[44]. Ultrasound-derived atherosclerosis metrics are independent predictors of cardiovascular events and improve risk prediction when added atop of conventional cardiovascular risk factors^[45]. However, it can be argued that cIMT and carotid plaque measurement are insufficient for the diagnostics of systemic atherosclerosis, and therefore may be supplemented by other diagnostic techniques, like computer tomography or magnetic resonance imaging. However, it could be true if only these methods possessed much better resolution to

measure the plaque size and/or the intima-media thickness in a quantitative manner. In our study, the principal point was the use of cIMT as the continuous variable, which allows further correlation and regression analyses. Second, statistical analysis has shown that the sample size was insufficient to exclude type 2 error for mutations m.652delG, m.1555A>G, m.12315G>A, and m.13513G>A. It means that the null-hypothesis on the presence of the differences for these variables in the case when we failed to observe them in our study due to insufficient sample size, cannot be rejected.

In conclusion, the phenotype of MetS in this study was not explained directly by atherosclerosis-related mtDNA variants, or the known proatherogenic mtDNA mutations. By far, it may be hypothesized that mtDNA-related mechanisms in atherosclerosis and MetS are different, in spite of the similarity of several phenotypic characteristics. However, there is a convincing evidence that mtDNA damage may play a mechanistic role in arising and development of cardiovascular and metabolic disorders. Complexity of the MetS phenotype should be taken into account, as well as the uncertainty about the common pathogenic mechanisms explaining the clustering of metabolic abnormalities, and modulating effects of lifestyle factors^[46]. The conventional role of modified low density lipoprotein in the development of atherosclerosis should be also assumed^[47,48]. It may be speculated that the sets of mtDNA mechanistic biomarkers may be different in MetS and atherosclerosis. As an example, in the East Finland Founder Population Hypertension Genetics Study (EFFGE) the whole mtDNA was sequenced in 1,204 adult subjects, and the variants with the strongest association with obesity were retested in 1,656 subjects from the Young Finns Study. At least 7 novel mtDNA variants were found to be associated with body mass index, and 6 - with obesity (body mass index above 30 kg/m²) (J.T. Salonen, personal communication). Interestingly, none of these mtDNA variants were ever shown to be associated with atherosclerosis or its clinical manifestations (or, more correct, tested for such association). So, the search for genetic determinants of the MetS remains the challenge.

DECLARATIONS

Authors' contributions

Concept of the study, general coordination and supervision of the research project, data analysis, statistical analysis, and manuscript writing: Sobenin IA

Concept elaboration and discussion, and manuscript editing: Salonen JT

MtDNA genotyping: Khasanova ZB, Sinyov VV, Melnichenko AA

Patients' recruitment, clinical examination and clinical data acquisition: Kirichenko TV, Prokudina AI, Orekhova VA, Grechko AV

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was kept in accordance with the *Helsinki Declaration* of 1975 as revised in 1983, 2008 and 2013. It was approved by the local ethics committee of the Institute for Atherosclerosis Research, Skolkovo Innovation Center, Moscow, Russia. All participants gave their written informed consent prior to their inclusion in the study.

Consent for publication

Not applicable.

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Original Article

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Profiling of risk of subclinical atherosclerosis: possible interplay of genetic and environmental factors as the update of conventional approach

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Abstract

Aim: To explore whether geographical location, genetic and environmental factors are associated with carotid atherosclerosis in high-risk individuals.

Methods: In Moscow 470 apparently healthy, asymptomatic volunteer subjects with a high cardiovascular disease risk were recruited to participate in a cross-sectional study. Carotid intima-media thickness (cIMT), a validated biomarker for present and future cardiovascular disease risk, was assessed by means of high resolution ultrasound scans in subjects.

Results: The total burden of conventional cardiovascular risk factors explained 21% of the cIMT variability; the mutational burden of mitochondrial genome defined by heteroplasmic mutations m.652delG, m.3256C>T, m.13513G>A, m.14459G>A, and m.15059G>A independently explained 23% variability; the combination of conventional and genetic risk factors increased explanatory level to 36%. Further exploratory statistical analyses showed air pollution as an independent risk factor for cIMT.

Conclusion: In our study we confirmed and expanded the existence of a European geographic gradient of atherosclerosis risk and its association with cardiovascular disease risk. Geographical, environmental (particularly, air pollution) -



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and genetic risk factors (particularly, mutant variants of mitochondrial genome) may interplay in the formation of susceptibility to atherosclerosis.

Keywords: Carotid atherosclerosis, high-risk patients, mitochondrial DNA mutations, air pollution, convenient cardiovascular risk factors

INTRODUCTION

Early detection and treatment of patients with a high risk of atherosclerosis is an urgent medical problem. Within this aspect, the identification of markers of subclinical atherosclerosis is an essential factor^[1]. The thickness of the intima-media layer of the carotid arteries [carotid intima-media thickness (cIMT)], determined by high-resolution ultrasonography, is considered to be a validated and conventionally accepted non-invasive marker of subclinical atherosclerosis, that is used in clinical and epidemiological studies to assess the effect of traditional and new cardiovascular risk factors on atherosclerosis^[2]. Since there is a correlation between cIMT and the degree of development of coronary atherosclerosis, and this factor has a prognostic significance in relation to the clinical manifestations of atherosclerosis, it is proposed as a surrogate marker of systemic atherosclerosis, including coronary one^[3,4]. The classic cardiovascular risk factors are weakly associated with the cIMT of the carotid arteries, thus suggesting the presence of other factors that determine the risk of developing atherosclerosis. The results of a recent European multicenter study [The Carotid Intima Media Thickness (IMT) and IMT-Progression as Predictors of Vascular Events in a High Risk European Population Study, or IMPROVE Study] revealed the existence of a geographical gradient of the cIMT, coupled with a known gradient of cardiovascular mortality^[5]. The detected south-north geographic gradient of cIMT did not depend on interpopulation differences in the cumulative effects of conventional cardiovascular risk factors. It has been suggested that other mechanisms play a role in the origin of this gradient, including hereditary, socio-economic and environmental factors. In order to verify this assumption, we conducted our own population-based cross-sectional study in Moscow, the methodology of which could allow us to regard the results as a significant addition to the data from the European IMPROVE study. In particular, we aimed to explore whether geographical location, genetic and environmental factors are associated with carotid atherosclerosis in high-risk individuals, thus focusing on the possibility of specific interplay of genetic and environmental factors.

METHODS

Patients

The study was performed at municipal outpatient clinics in Moscow, Russia. The study was organized in accordance with international and domestic standards of quality clinical practice, namely, Helsinki Declaration of 1975 as revised in 1983, 2008 and 2013, and was approved by the local ethics committee. The study involved 470 subjects (200 men and 270 women) without clinical manifestations of atherosclerosis randomly recruited from the visitors' flow who have passed a routine screening for cardiovascular risk factors, as a screening subpart of Atherosclerosis Monitoring and Atherogenicity Reduction Study (ClinicalTrials.gov Identifier: NCT01734707) and Monocyte Activation in Preclinical Atherosclerosis Study (ClinicalTrials.gov Identifier: NCT02126280). The criteria for inclusion and exclusion were designed from those of IMPROVE Study, which allowed to form an observation group that is completely comparable with samples from European populations.

We have recruited men and women aged 55-79 years old who had at least three conventional risk factors for cardiovascular diseases. The latter included: hypercholesterolemia (low-density lipoprotein cholesterol level >160 mg/dL, or prescribed to cholesterol-lowering medications), hypertriglyceridemia (triglycerides > 200 mg/dL, or prescribed to triglyceride-lowering drugs), low HDL-cholesterol (below 40 mg/dL), arterial

hypertension (diastolic blood pressure > 90 mmHg and/or systolic blood pressure > 140 mmHg, or prescribed to antihypertensive drugs), the presence of diabetes mellitus or impaired glucose tolerance (blood sugar > 110 mg/dL, or prescribed to regular insulin injections and/or sugar-lowering drugs), smoking (consumption of > 10 cigarettes per day for thirty preceding months), and the presence of family history of cardiovascular diseases. For women, an additional inclusion criterion was the presence of menopause (spontaneous or surgical) of more than 5-years duration.

Exclusion criteria were: abnormal anatomical configuration of the neck and neck muscles, severe tortuosity or unusual layout of the carotid arteries and its branches, the history of clinical manifestations of atherosclerosis (myocardial infarction, angina pectoris, acute and transient cerebral ischemic attacks, aortic aneurisms, revascularization of carotid, coronary or peripheral arteries), chronic heart failure of 3-4 functional class, and the presence of severe concomitant diseases.

Clinical examination of study participants included a biochemical analysis of serum lipid profile, identification of the main cardiovascular risk factors, calculating a prognostic 10-year risk of developing coronary heart disease, myocardial infarction and sudden coronary death^[6], and quantitative ultrasound diagnostics of carotid atherosclerosis.

Lipid analysis

Venous blood was taken after overnight fasting. To obtain serum, the blood was incubated for 1 h at 37 °C and centrifuged for 15 min at 1,500 g. Cholesterol and triglyceride levels were measured by commercial enzymatic kits (Cholesterol-32-Vital, and Triglycerides-22-Vital, respectively; Vital Diagnostics SPb, St. Petersburg, Russia). Serum HDL cholesterol concentrations were measured after precipitation with magnesium chloride phosphotungstic acid reagent (HDL-Cholesterol-04-Vital, Vital Diagnostics SPb, St. Petersburg, Russia). Serum LDL cholesterol was calculated by Friedewald formula as the difference between total cholesterol and the sum of HDL cholesterol and 1/5 triglycerides, and the ratio LDL-C/HDL-C was calculated.

Ultrasonographic examination

For diagnostics of carotid atherosclerosis, high-resolution B-mode carotid arterial ultrasonography imaging was used (SSI-6000 ultrasound system, SonoScape, China, equipped by 7.5-MHz L741 linear array probe). The protocol of ultrasound examination developed earlier by Salonen *et al.*^[7], was used. The cIMT measurements were carried out by a certified operator with M'Ath software package (IMT, France). The extent of carotid atherosclerosis were evaluated as described elsewhere^[8,9].

MtDNA genotyping

DNA was isolated from whole venous blood with commercial kit for DNA isolation and purification (QIAGEN GmbH, Germany). DNA concentration in samples was determined by NanoPhotometer Pearl UV/Vis SDRAM P-34 (IMPLEN, Germany); the samples were kept in TE buffer at a concentration of 0.03 µg/µL. Genotyping of mtDNA was performed for heteroplasmic mutations m.652 delG, m.1555A>G, m.3336T>C, m.3256C>T, m.5178C>A, m.12315G>A, m.13513G>A, m.14459G>A, m.14846G>A, and m.15059G>A. For the amplification of mitochondrial DNA fragments, PCR method followed by pyrosequencing, with earlier described primers and conditions were used^[9]. In brief, to quantitatively evaluate mutant allele, a method of pyrosequencing was adapted for the condition when normal and mutant alleles may be present in a biological specimen; the defective allele was quantified by analyzing the peak heights in the pyrogram of one-chained PCR-fragments of a mitochondrial genome^[9-11].

Statistical analysis

Data processing was performed using the SPSS software package, version 22.0 (IBM Corp., Chicago, IL, USA). Subprograms of descriptive statistics, variational analysis, parametric and nonparametric statistics,

Table 1. Descriptive data on study participants

Variable	Men, <i>n</i> = 200	Women, <i>n</i> = 270	<i>P</i> value	Total, <i>n</i> = 470
Age, years	61.4 (59.9-62.5)	66.1 (65.5-67.1)	< 0.001	64.1 (63.6-65.1)
Body mass index, kg/m ²	27.0 (26.5-27.5)	27.8 (27.3-28.3)	0.033	27.5 (27.1-27.9)
Systolic blood pressure, mmHg	148 (145-150)	144 (142-146)	0.026	145 (144-147)
Diastolic blood pressure, mmHg	89 (87-90)	85 (84-86)	< 0.001	86 (85-87)
Current smokers, %	15	12	0.4	13
Never smokers, %	56	78	< 0.001	70
Smoking cessation, %	29	10	< 0.001	17
Smoking experience, years	10.0 (7.8-12.3)	4.8 (3.4-6.1)	< 0.001	6.8 (5.6-8.0)
Diabetes mellitus, %	8	5	0.20	6
Arterial hypertension, %	76	77	0.80	77
Menopause, years	-	16.1 (15.2-17.0)	-	-
Family history of:				
Myocardial infarction	25	33	0.059	30
Arterial hypertension	35	57	< 0.001	49
Diabetes mellitus	14	12	0.60	13
Total cholesterol, mg/dL	238 (230-245)	261 (255-267)	< 0.001	252 (248-257)
Triglycerides, mg/dL	152 (140-165)	134 (127-140)	0.004	140 (134-147)
HDL cholesterol, mg/dL	60 (58-62)	70 (69-72)	< 0.001	66 (65-68)
LDL cholesterol, mg/dL	147 (141-154)	164 (159-169)	< 0.001	158 (154-162)
LDL/HDL ratio (Atherogenic index)	2.6 (2.4-2.7)	2.5 (2.4-2.6)	0.20	2.5 (2.4-2.6)

covariance analysis and linear regression were used. Data are presented as the mean and 95% confidence interval^[12]. The significance of differences was defined at the 0.05 level of confidence.

RESULTS

Clinical and anthropometric characteristics, and lipid profile of study participants are presented in [Table 1](#).

As follows from the presented data, the cohort of study participants was, in general, at early retirement age, with overweight but not obesity, and with mild systolic arterial hypertension. The inclusion criteria used in this study predetermined a high proportion of participants with diagnosed hypertension (77%). Accordingly, there was a high proportion of participants with a family history of myocardial infarction (30%), hypertension (49%) and diabetes (13%). The proportion of subjects with diabetes (6%) was comparable to that in population (reported occurrence, 4.5%-6%). The share of smokers (13%) was rather low, possibly due to the features of the surveyed contingent (older people, mostly with higher education, and to a certain extent focused on maintaining a healthy lifestyle).

Men and women differed by most of clinical and anthropometric parameters. Men were younger ($P < 0.001$), had a lower body mass index ($P = 0.033$) presumably due to lower amount of abdominal fat, were characterized by higher blood pressure along with less likely family history of hypertension. The differences in family history of myocardial infarction and diabetes did not reach statistical significance.

Men had a higher proportion of smokers, the mean duration of smoking, and a lower proportion of never-smokers. At the same time, they also had a higher proportion of past-smokers, that also indicates the higher compliance of the surveyed population to the healthy lifestyle.

According to lipid measurements, moderate deviations in the lipid profile of the blood serum were observed. The presented mean values indicate the presence of moderate hypercholesterolemia with normal triglycerides. Elevated levels of total cholesterol were caused primarily by LDL cholesterol. At the same time, generally normal levels of HDL cholesterol were observed; as a result, LDL-C/HDL-C ratio remained within the normal range (the upper limit of normal values was determined as 3.0), thus indicating that balanced lipid metabolism was maintained. High levels of HDL cholesterol (taking the recommended lower

Table 2. Ultrasound characteristics of carotid atherosclerosis

Variable	Men, <i>n</i> = 200	Women, <i>n</i> = 270	<i>P</i> value	Total, <i>n</i> = 470
Mean cIMT, mm	0.819 (0.798-0.840)	0.827 (0.813-0.842)	0.5	0.824 (0.812-0.836)
Carotid artery internal diameter, mm	7.66 (7.55-7.78)	7.24 (7.17-7.31)	< 0.001	7.40 (7.34-7.47)

Table 3. Inter-population comparison of ultrasound characteristics of carotid atherosclerosis

Population-derived sample	Mean cIMT, mm	Latitude	Longitude
*Perugia, Italy, <i>n</i> = 542	0.70 (0.69-0.71)	43°	12°
*Milan, Italy, <i>n</i> = 553	0.72 (0.71-0.73)	45°	9°
*Paris, France, <i>n</i> = 501	0.68 (0.67-0.69)	48°	2°
*Groningen, The Netherlands, <i>n</i> = 532	0.72 (0.71-0.73)	53°	7°
*Stockholm, Sweden, <i>n</i> = 533	0.79 (0.78-0.80)	59°	18°
*Kuopio, Finland, <i>n</i> = 1,050	0.76 (0.75-0.77)	62°	28°
Moscow, Russia, <i>n</i> = 472	0.82 (0.81-0.84)	56°	38°

*The data are derived from published results of IMPROVE Study^[5]

threshold of 50 mg/dL) may also indirectly indicate the adherence to a healthy lifestyle, in particular, regular consumption of fresh vegetables and fruits.

Women differed significantly from men by all lipid parameters. They had higher cholesterol levels in both lipoprotein fractions (and as a result, in total cholesterol), and lower triglyceride levels. However, their LDL-C/HDL-C ratio remained normal, as in men.

The direct quantitative characteristics of atherosclerosis, resulted from ultrasound scanning of the carotid arteries followed by cIMT measurements, are provided in Table 2. In men, the diameter of the carotid arteries was significantly larger than in women ($P < 0.001$). However, there were no significant differences between men and women in the mean cIMT of the carotid arteries. The absence of such differences allowed us to carry out a regression analysis of the dependence of cIMT on conventional risk factors without taking into account sex differences. This analysis showed that the mean cIMT correlates with age ($P < 0.001$), systolic blood pressure ($P < 0.001$), HDL cholesterol ($P = 0.039$) and LDL cholesterol ($P = 0.003$). The regression model was significant at $P < 0.001$; the adjusted R^2 value was 0.209.

The data on the direct quantitative characteristics of cIMT were compared with the results of the IMPROVE Study^[5]. The results of the comparison are presented in Table 3. Despite the fact that the inclusion criteria used in the study allowed us to form an observation group comparable to European samples for the cumulative effects of traditional cardiovascular risk factors, the mean cIMT in the high-risk patients from Moscow population was significantly higher than in any one from European populations at $P < 0.001$.

Correlation analysis confirmed the existence of a geographic gradient of cIMT revealed in the IMPROVE Study. The correlation coefficient between the mean cIMT values and the geographical position of the research center, calculated as the geometric mean of geographical latitude and longitude, was 0.905 ($P = 0.005$).

The analysis of the relationship between the mean cIMT arteries and the standardized mortality from coronary heart disease, obtained from the WHO database (<http://www.euro.who.int>) was performed. The correlation coefficient between these variables was 0.853 ($P = 0.015$). The logarithmic model of this regression was reliable at $P = 0.003$; the adjusted R^2 value was 0.860.

We have also performed the linear regression analysis, where cIMT was taken as dependent variable, and convenient cardiovascular risk factors (age, sex, body mass index, systolic and diastolic blood pressure,

smoking status, LDL cholesterol, HDL cholesterol, serum triglycerides, menopausal status for women, family history of myocardial infarction) as independent variables. It was found that the total burden of these risk factors explains 21% of the cIMT variability. Since conventional risk factors do not fully explain the variability of cIMT in the samples from European populations and the Moscow high-risk group, it should be assumed that other factors play a role in the formation of atherosclerosis predisposition.

One of these factors may be the adverse effects of the external environment. As a possible characteristic of the environmental situation, we used the mean annual integrated air pollution index. It was calculated as the sum of the ratios of the concentrations of the five major pollutants (nitrogen oxide, nitrogen dioxide, carbon monoxide, ozone and formaldehyde) to the maximum permissible concentration in the atmosphere. It was found that standardized mortality from coronary heart disease tends to correlate with the atmospheric pollution index: the correlation coefficient was 0.742 with $P = 0.056$. At the same time, the air pollution index correlated with the mean cIMT: the correlation coefficient was 0.812 with $P = 0.026$.

The other acting non-beneficial factor may be the genetic background. In our study, we have assessed the mutation burden of mitochondrial DNA by those heteroplasmic variants that have been previously shown to be associated with atherosclerotic lesions in human aorta and also with carotid atherosclerosis^[9-11]. As it was shown in linear regression model, 5 of 10 mutations, for which the level of heteroplasmy was measured, independently provided the explanatory level of 23% for cIMT variation by adjusted R^2 at $P < 0.001$ (Fisher's exact test 11.21; $P < 0.001$); these were m.652delG, m.3256C>T, m.13513G>A, m.14459G>A, and m.15059G>A. The combined model, which included conventional risk factors and the above mutations provided significantly better explanatory level (36%, Fisher's exact test 8.28; $P < 0.001$).

DISCUSSION

In this study, we used one of the generally accepted methods for assessing susceptibility to atherosclerosis, namely, non-invasive ultrasound scanning of the carotid arteries in high-resolution mode. This approach allowed to obtain direct quantitative estimate of the degree of development of subclinical atherosclerosis in individuals with a high risk of CHD. The criteria used for inclusion in the study allowed us to form a sample well comparable to the European samples in the IMPROVE Study on the cumulative effects of traditional cardiovascular risk factors. A direct comparison of the measurement of cIMT with European data showed that in spite of the same cumulative risk, the Moscow population-derived sample was more prone to atherosclerosis.

We were able to expand significantly the hypothesis on the existence of a geographic gradient of the variability of cIMT, and this gradient was associated not only with geographical latitude, as it was demonstrated in the IMPROVE Study, but also with geographical longitude. The association of the cIMT gradient with the gradient of cardiovascular mortality was confirmed, thus allowing to describe cIMT as the risk factor for atherosclerotic disease and its clinical manifestations.

It was confirmed that the variability of cIMT is not sufficiently explained by the cumulative effect of conventional risk factors: the explanatory nature of the used models for assessing the association of risk factors with cIMT was 21%. Therefore, it was rather logical to assume that previously unexplored (or insufficiently studied) mechanisms of susceptibility to atherosclerosis may be of importance. The latter may include hereditary, socio-economic and environmental factors. The cross-sectional study in the sample from Moscow population, the methodology of which allowed it to be regarded as an addition to the data from the IMPROVE Study, showed that the ecological situation can be one of the factors associated with both the development of subclinical atherosclerosis and its clinical manifestations and complications.

The hypothesis on the possible role of the damage of mitochondrial DNA in atherosclerosis development has raised relatively recently but gains more and more experimental and clinical background. It is known that

genetic factors evolved from variation of nuclear genome can be attributed approximately to 5% variability of atherosclerotic diseases^[13]. On the opposite, the latest findings on the role of mitochondrial DNA variation in individual predisposition to atherosclerosis provide a growing body of evidence and the expectation of the breakthrough in understanding of molecular mechanisms and pathways of atherogenesis^[14,15]. Heteroplasmic mtDNA mutations represent a promising molecular biomarker of genetic susceptibility to atherosclerosis and related pathologies^[16-19]. In our study, we have demonstrated that several atherosclerosis-associated mutations of mitochondrial DNA taken together as a mutation burden of mitochondrial genome possess their own explanatory power and give a significant increase atop of assessment of conventional risk factors.

Undoubtedly, this study has certain limitations. First, the study was performed on a limited and fairly specific contingent (Muscovites, the elderly persons, mostly with higher education, focused on maintaining a healthy lifestyle, but with high level of convenient cardiovascular risk factors). Data from IMPROVE Study show that the cIMT varies significantly even between populations that are geographically close to each other. It is clear that in Russia in the areas differing not only geographically, but also in socio-economic, environmental and ethnic aspects, the variability of cIMT, as well as cardiovascular mortality, can differ significantly. The only possible resolution of this restriction may be the performance of similarly designed cross-sectional studies in various regions of Russia.

The second limitation is that the study did not assess socio-economic and ethnic variables. These unaccounted factors may also play a role in the formation of susceptibility to atherosclerosis and, undoubtedly, should be studied in further population-based studies.

The third limitation comes from the inability to quantitatively explore the interaction of mitochondrial DNA variants with conventional cardiovascular risk factors and environmental ones; therefore, the hypothesis on their interplay in the development of atherosclerosis remains the plausible idea, which needs further investigations. Besides, the fundamental problem still exists, since the molecular mechanisms whereby mitochondrial genome mutations may promote atherogenesis remain obscure. The most intriguing solution of this problem may be related to generation of cellular lines with edited mtDNA, which should reproduce pathologic phenotype and serve as the model for studying the effects of certain mtDNA mutations on cell functionality and mitochondrial dysfunction.

Finally, of all possible environmental factors theoretically capable of influencing the formation of phenotypic susceptibility to atherosclerosis (namely, diffuse intimal thickening, diagnosed ultrasonographically as an increased cIMT), only the integral indicator of atmospheric pollution was evaluated in our study. This indicator is neither standardized nor generally accepted. There is still no single worldwide database on air pollution. Each country uses its own standards, maximum permissible levels for the content of pollutants in the atmosphere, and methods for their determination. One of the adverse environmental factors is the dust load, which is not regulated and is not evaluated in the vast majority of countries around the world. In our study, the association of the cIMT with the content of dust particles in the atmosphere was not evaluated, mainly due to the lack of valid data. Therefore, the data on correlation between cIMT and air pollution index should be verified in a specially designed multicenter study; otherwise, these findings lead to rather speculative conclusions. However, a recent study in Germany showed that the content of solid dust particles of exhaust gases in atmospheric air is related to the degree of subclinical atherosclerosis, the prevalence of coronary heart disease and the incidence of myocardial infarction^[20].

Despite the above limitations, the results of our study suggest that environmental and genetic factors are involved in the formation of susceptibility to atherosclerosis and should be considered as plausible risk factors for cardiovascular diseases. However, the question on the plausibility of findings in routine clinical practice and preventive medicine remains disputable^[21]. It is likely that early awareness on individual genetic predisposition to atherosclerosis may be the motivating factor for higher personal compliance to healthy

lifestyle in more beneficial ecologic environment. On the other hand, it will also give a rise to earlier and more focused medicinal preventive measures.

DECLARATIONS

Authors' contributions

Concept of the study, general coordination and supervision of the research project, data analysis, statistical analysis, and draft manuscript writing: Sobenin IA

Patients' recruitment, clinical examination and clinical data acquisition: Myasoedova VA, Kirichenko TV, Orekhova VA, Grechko AV

MtDNA genotyping: Khasanova ZB, Sinyov VV, Melnichenko AA

Concept elaboration, general coordination and discussion: Orekhov AN

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethics approval and consent to participate

This study was kept in accordance with the Helsinki Declaration of 1975 as revised in 1983, 2008 and 2013. It was approved by the local ethics committee of the Institute for Atherosclerosis Research, Skolkovo Innovation Center, Moscow, Russia. All participants gave their written informed consent prior to their inclusion in the study.

Consent for publication

Not applicable.

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Original Article

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Lipid profile of children with glycogen storage disease

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Abstract

Aim: To determine the lipid profile patterns in children with different types of glycogen storage disease (GSD).

Methods: The study included 62 children with GSD (43 boys, 19 girls), mean age 8.29 years. All patients underwent anthropometry, assessment of physical development, lipid profile analysis.

Results: The children were divided into three groups depending on the type of GSD. Nineteen children (31%) had type I GSD (Group 1), 16 (26%) - type III (Group 2) and 27 (43%) - types VI and IX (Group 3). Dyslipidemia of varying severity was more specific to patients with type I and III GSD. Higher levels of triglycerides were associated with type I GSD, while higher levels of LDL cholesterol were common to type III GSD ($P < 0.05$). No changes in the lipid profile were observed in 18 (29%): one with type I, 4 with type III, and 13 with types VI and IX.

Conclusion: Lipid metabolism disorders were detected in 71% of children with GSD, especially with types I and III. The elevated levels of total cholesterol and LDL cholesterol are associated with the early progression of atherosclerosis and an increased cardiovascular risk in the general population. But there is a lack of evidence of a link between lipid metabolism



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disorders detected in childhood and an increased risk of cardiovascular diseases in patients with GSDs. More studies needed to investigate this issue.

Keywords: Lipid profile, glycogen storage disease, children, triglycerides, LDL cholesterol

INTRODUCTION

Glycogen storage disease (GSD, glycogenosis, dextrinosis) is a common term for a group of hereditary metabolic disorders associated with impaired glycogen metabolism. The prevalence of GSD varies from one case per 20,000 to 300,000 births depending on the type of disease^[1].

The major part of GSDs has an autosomal recessive inheritance (e.g., type IX is X-linked recessive disorder) and implemented through specific enzyme deficiencies. Furthermore, some cases of simultaneous several enzymes failure have been described. Defects in synthesis enzymes, as well as glycogen utilization enzymes, can be observed depending on the GSD type. Genetic disturbances of glycogen metabolism lead to the accumulation of normal and/or pathologically changed glycogen in internal organs and tissues. The liver, heart, skeletal muscles and blood cells are affected in GSD I (von Gierke disease), III (Cori or Forbes disease), VI (Hers disease), VII (Tarui disease), VIII and IX types^[2]. The pronounced metabolic changes cause growth and developmental delay at an early age^[2].

The cardiac pathology in GSDs especially in type II (Pompe disease) is well studied^[3]. Among other types, the most common cardiovascular (CV) disorders are found in children with type III GSD. Myocardial involvement in GSD is performed by left ventricular hypertrophy, less frequently cardiomegaly, or isolated right ventricular hypertrophy and, in rare cases, life-threatening arrhythmia and hypertrophic cardiomyopathy as non-compact ventricular myocardium^[4]. Pulmonary arterial hypertension is another CV complication of GSD^[5,6].

The crucial mechanism of CV complications in GSD is dyslipidemia. GSD-associated lipid disorders indicate close interactions between the metabolism of carbohydrates and lipids in these patients^[7]. In case of such severe metabolic disorders as GSDs, especially types I and III, affect lipid metabolism in early childhood.

According to the Rake study, hypercholesterolemia and hypertriglyceridemia are more common in type I GSD, particularly subtype Ia^[8]. Furthermore, pancreatitis and cholelithiasis are prevalent complications of hyperlipidemia in GSD. Moreover, these patients characterised by the higher relationship of high triglyceride levels (> 5.6 mmol/L) with hepatic adenoma development^[9].

Hypertriglyceridemia in GSD type I occurs as a result of a number of reasons. First, a violation of gluconeogenesis leads to the accumulation of fatty acid precursors, from which triglycerides are subsequently synthesized. Secondly, a decreased lipoprotein lipase activity causes disturbances in physiological lipolysis and a decrease in the clearance of triglycerides. In addition, the conditions described above can be exacerbated by a chronically low level of insulin, which normally inhibits the synthesis of triglyceride-rich particles in the liver^[10,11]. Finally, a fatty liver is a common condition in patients with GSD I as a consequence of excess fatty acid transport from the adipose tissue to the liver and increased lipogenesis de novo^[12]. Increased cholesterol synthesis in combination with disturbances of lipoprotein and triglyceride elimination leads to severe hypercholesterolemia and hypertriglyceridemia in patients with GSD I^[13,14].

In GSD III, hypertriglyceridemia is detected in 67% of patients, while only one-third demonstrates hypercholesterolemia. In general, lipid disorders are not as common comparing to GSD I^[15].

The impact of oxidized lipoproteins on atherogenesis is well known^[16]. The role of oxidative stress and total antioxidant protection in patients with GSD I was examined in a number of studies. In one study, total reactive antioxidant potential was compared between children with GSD, diabetes, and familial hypercholesterolemia. This biomarker was much higher among GSD patients^[17].

Some studies demonstrated the development of premature atherosclerosis and an increased risk of CV complications associated with GSD^[18,19], whereas others did not reveal the association of GSD with premature atherosclerosis^[1,20]. Moreover, it was found that LDL-cholesterol particles are paradoxically more resistant to oxidative stress in patients with GSD I compared to the control group^[21].

A series of studies show the connection between high cholesterol levels in children and adolescents in the general population and the risk of atherosclerosis and CV complications in adult life^[22]. However, despite the fact of the existence of dyslipidemia in GSD has been known for several decades, the need for medical treatment of lipid disorders in GSD is still disputable.

Fibrates, statins, niacin, bile acid sequestrants, ezetimibe, lomitapide, phytosterols, fish oil, etc. are currently used to correct lipid disorders in adults. The issue of prescribing these medicines to children remains controversial. Apparently, lipid-lowering therapy should be prescribed if there are strict indications, when diet therapy does not eliminate high levels of blood lipids, threatening the development of acute pancreatitis or atherothrombotic complications^[23].

Thus, the aim of our study was to determine lipid profile patterns in children with different types of glycogen storage disease.

METHODS

The study included 62 children with GSD (43 boys, 19 girls), mean age 8.29 years. All patients underwent anthropometry including weight, height adjusted as Z-score to the age with the calculation of body mass index Z-score and percentiles. The assessment of physical development for overweight and obesity was carried out using the WHO Anthro and AnthroPlus programs. Fasting total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides plasma levels were analyzed by Konelab Prime 60i auto-analyzer (Thermo Scientific, Wilmington, DE, USA) with the internal age-adjusted normal range. Dyslipidemia was defined as any abnormal total cholesterol, HDL cholesterol, LDL cholesterol or/and triglycerides plasma levels. Statistical analysis was performed using Statistica for Windows 6.0 (StatSoft Inc., USA). The statistical significance level was taken as sufficient for $P < 0.05$.

The study was conducted in agreement with the Declaration of Helsinki, GCP. The special approval from the Independent Local Board of Ethics Committee was received.

RESULTS

The children were divided into three groups depending on the type of GSD. Nineteen children (31%) had type I GSD (Group 1), 16 (26%) - type III (Group 2) and 27 (43%) - types VI and IX (Group 3). The groups were comparable in age. In the group of children with type VI and IX GSD, boys prevailed, since the vast majority of patients had type IX GSD, which is characterized by the X-linked pattern.

Overweight and obesity were found in 10.5% of children in Group 1, 6.3% in Group 2 and 11.1% in Group 3. Weight deficit was observed in 21%, 12.5%, and 14.8%, respectively.

Lipid profile disorders were detected in 44 (71%) of 62 children. Dyslipidemia of varying severity was more specific to patients with type I and III GSD. Higher levels of triglycerides were associated with type I GSD,

Table 1. Lipid profile of children with GSD (*n* = 62)

GSD type	Total (<i>n</i> = 62)	Type I (<i>n</i> = 19)	Type III (<i>n</i> = 16)	Type VI and IX (<i>n</i> = 27)
Cholesterol, mmol/L	4.7 ± 0.2	4.9 ± 0.5	4.9 ± 0.3	4.4 ± 0.2
HDL cholesterol, mmol/L	0.6 ± 0.03	0.8 ± 0.06	0.8 ± 0.07	1.1 ± 0.1
LDL cholesterol, mmol/L	3.1 ± 0.1	2.8 ± 0.2	3.6 ± 0.3	0.9 ± 0.06
Triglycerides, mmol/L	2.2 ± 0.3	3.9 ± 0.8	2.05 ± 0.3	1.1 ± 0.1

while higher levels of LDL cholesterol were common to type III GSD ($P < 0.05$) [Table 1]. No changes in the lipid profile were observed in 18 (29%): one with type I, 4 with type III, and 13 with types VI-IX.

Hypertriglyceridemia and a decrease in the level of HDL cholesterol were most frequently detected [Figures 1A and B]. Hypertriglyceridemia was specific to type I and III GSD. At the same time, low HDL cholesterol levels were more common in patients with type I GSD than in patients with type III or type VI-IX GSD.

Hypercholesterolemia was diagnosed in more than one-third of patients with type I and type III GSD, and more than one-third of patients with type III demonstrated high levels of LDL cholesterol [Figure 1C].

Thus, hypercholesterolemia, hypertriglyceridemia and low HDL cholesterol are common to type I GSD, while high levels of LDL cholesterol, hypertriglyceridemia and low HDL cholesterol are typical for type III GSD. For type VI and IX GSD, lipid metabolism disorders were observed less frequently, and the most common changes were a decrease in HDL cholesterol.

DISCUSSION

Lipid metabolism disorders, including hypercholesterolemia, hypertriglyceridemia, and decreased levels of HDL cholesterol were detected in 71% of children with GSD. They were mainly observed in patients with types I and III of the disease, in which hypertriglyceridemia often occurred. More than one-third of patients with type I and type III GSD were diagnosed with hypertriglyceridemia, and an equal number of patients with type III GSD had elevated levels of LDL cholesterol. Patients with GSD VI-IX types performed lower rates of lipid disorders.

The elevated levels of total cholesterol and LDL cholesterol are closely associated with the early progression of atherosclerosis and an increased cardiovascular risk in the general population. At the same time, there is still no clear position on hypertriglyceridemia as a risk factor for cardiovascular diseases^[24].

According to some studies hypertriglyceridemia can be associated with atherogenesis, firstly, as a marker of some metabolic disorders, secondly, because of the vascular wall macrophages infiltration due to oxidised triglyceride-rich lipoproteins receptor activation^[11]. There is evidence that elevated triglycerides lead to hypercoagulation through stimulation of a plasminogen tissue activator inhibitor, an increase in the content of VII coagulation factor, and the activation of the transition of prothrombin to thrombin^[24]. The Hokanson and Austin meta-analysis of prospective studies established that triglyceride levels of up to 5 mmol/L (450 mg/dL) predicted the risk of coronary heart disease (CHD), especially in women. Thus, in the Framingham study, the risk of CHD positively correlated with triglycerides levels^[25].

According to the Copenhagen Male Study covering 2,906 men aged 52-74 years without CHD, the first myocardial infarction occurred in 229 of them in 8 years of follow-up and the risk of developing CHD increased as the initial triglyceride levels increased^[26]. A meta-analysis of clinical trials with a total of 46,413 men and 10,864 women examined by Hokanson and Austin showed that triglycerides are an independent risk factor for CHD even after adjusting for HDL cholesterol values^[26].

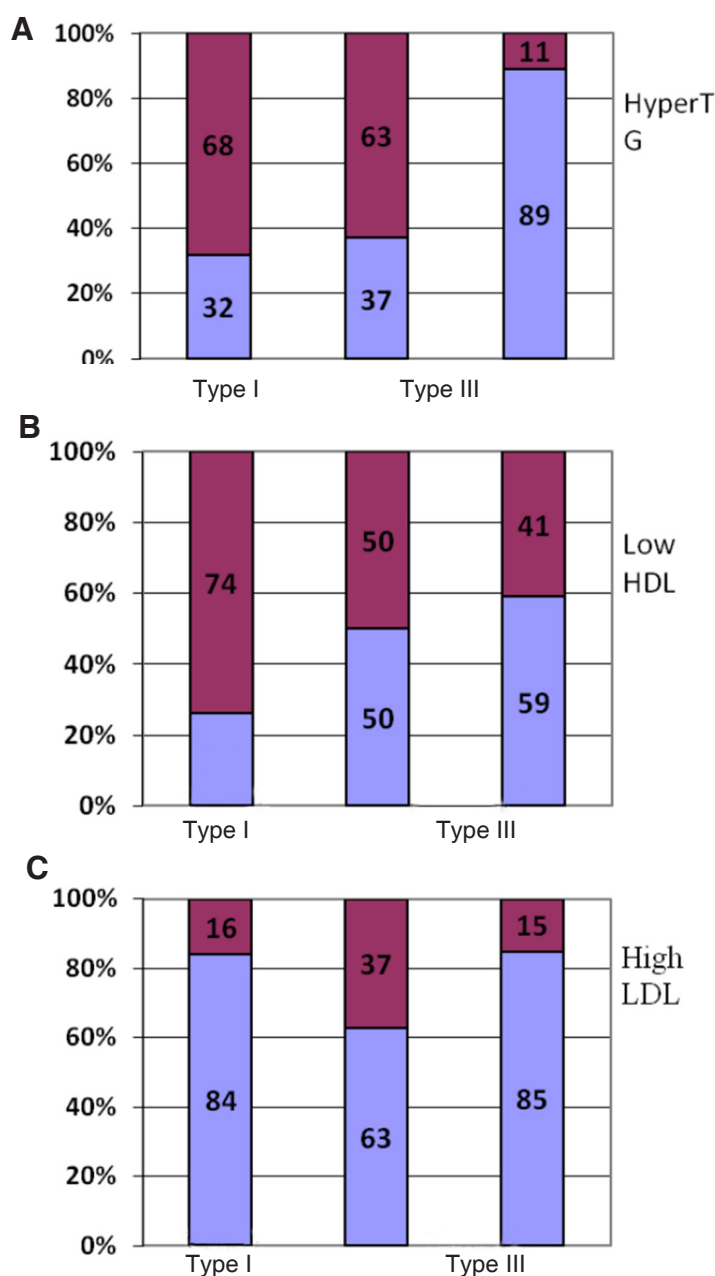


Figure 1. Dyslipidemia patterns in children with different types of GSD ($n = 62$). 1st column - Type I; 2nd column - Type III; 3rd column - Types VI and IX. Dark purple - abnormal levels; light blue - normal levels. A: Triglycerides; B: HDL cholesterol; C: LDL cholesterol

There is currently no convincing evidence of a link between lipid metabolism disorders detected in childhood and an increased risk of cardiovascular diseases in patients with GSDs. More studies needed to investigate this issue.

DECLARATIONS

Authors' contributions

Concept and design: Stroková TV, Pavlovskaya EV, Starodubova AV

Data acquisition: Pavlovskaya EV, Zubovich AI, Bagaeva ME, Surkov AG

Data analysis: Varava YR, Polenova NV, Kosyura SD

Manuscript preparation: Varava YR, Polenova NV, Livantsova EN

Critical revision and finalizing of the manuscript: Pavlovskaya EV, Varava YR, Starodubova AV

Availability of data and materials

Authors confirm that the data were strictly obtained from medical records according to the privacy policy and ethics code of our institute.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study received an approval from the Local Ethic Committee (0529-2016-0007), as it was conducted as a part of the State Program of Basic Research. All procedures were done with accordance to GCP standards and Informed consents from parents or official representatives were received prior to any research procedures.

Consent for publication

Not applicable.

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Letter to Editor

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Subcellular anti-atherosclerotic therapy

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Dear Editor,

Photo-theranostics is a therapeutic and diagnostic approach that uses photosensibilization^[1]. This approach makes part of the medicine of the future-subcellular therapy, including at the level of mitochondria^[2]. Photo-theranostics targets cells and tissues that are affected by pathological changes inducing apoptotic cell death. However, the approach allows targeting cellular organelles, if the photo-sensitizing agent is accumulated selectively. Such selectivity can result from the organelle's functional state, as in the case of mitochondria affected by mitochondrial genome abnormalities. Currently, subcellular therapy is being developed mainly for cancer cells, but we believe that such approaches are applicable to cardiology, in particular, for atherosclerotic diseases. For instance, some pro-atherogenic mitochondrial DNA mutations associated with atherosclerosis can lead to mitochondrial dysfunction^[3].

Some photo-sensitizing agents accumulate selectively in mitochondria making the photodynamic targeting efficient enough. Thus, protoporphyrine IX (PpIX), which is widely used in clinical practice, is produced from a prodrug 5-aminolevulinic acid (5-ALA) in course of heme synthesis cascade in the mitochondria. Exogenous 5-ALA causes excessive formation of PpIX, which cannot be efficiently processed by ferrochelatase and accumulates in the cells. PpIX accumulation directly depends on the cell's metabolic activity.



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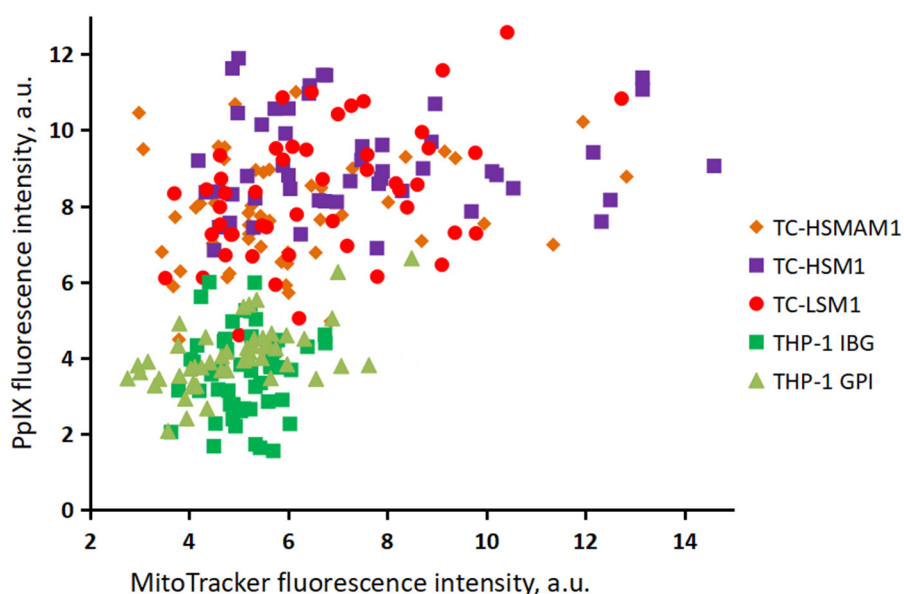


Figure 1. Distribution of protoporphyrin IX (PpIX) and MitoTracker fluorescence intensity in cybrid lines and THP-1 macrophage lines after 4 h of incubation with 5-aminolevulinic acid

We studied 5-ALA-induced PpIX accumulation on cybrid lines derived from rho0 THP-1 macrophages and patients' thrombocytes with mutation-bearing proatherosclerotic mitochondria. We used 3 cybrid lines (TC-HSMAM1, TC-HSM1, TC-LSM1) and 2 native THP-1 monocyte cultures obtained from different sources (THP-1 IBG, THP-1 GPI). The mitochondrial functional state was assessed as mitochondrial membrane potential using fluorescence intensity of mitochondrial dye MitoTrackerTM Orange CMTMRos (Thermo Fisher Scientific). A high level of fluorescence is characteristic of active mitochondria; on the contrary, dysfunctional mitochondria possess a low level of fluorescence. Estimation of fluorescence was performed by laser scanning microscopy with spectral unmixing. MitoTracker and PpIX dyes were excited with a 561 nm laser, and fluorescence was detected at 570-750 nm wavelength. The signal was spectrally unmixed to obtain separate MitoTracker and PpIX signals. Individual cells areas were traced on microscopic images taken under identical conditions, and mean values of PpIX and MitoTracker fluorescence intensity were obtained for each cell.

We found that PpIX accumulation varied significantly from cell to cell within cell populations. Cybrid lines had increased PpIX accumulation in comparison with native THP-1 macrophage lines. A positive correlation between PpIX and mitochondrial potential was observed [Figure 1].

It can be assumed that selective elimination of dysfunctional (mutated) mitochondria can be achieved by adjusting laser intensity sufficient to induce photodynamic destruction of the organelles, while active (normal) mitochondria and the cells as a whole should be preserved at the used intensities.

Photodynamic therapy is a non-invasive treatment method in oncology, dermatology and infectious diseases. The disadvantage of this approach is the low depth of penetration into the tissue of excitation light and insufficient accumulation of photosensitizers in subcellular organelles. On the other hand, subcellular organelles, and in particular mitochondria, are promising therapeutic targets for effective therapy. At the same time, targeting mitochondria approaches do not necessarily have to be associated with the use of laser. It can be assumed that substances accumulating in mitochondria as a result of metabolic disturbances themselves without a laser will contribute to the death of dysfunctional mitochondria if there is an intra-mitochondrial overload of such substances. In addition to 5-ALA, there are many other mitochondria

targeting substances. This is a good arsenal for a broad search for potential drugs for subcellular therapy including anti-atherosclerotic therapy^[4,5].

DECLARATIONS

Authors' contributions

Conducting experiments: Ryabova AV, Romanishkin ID, Skobeltsin AS, Moskalev AS, Makarov VI, Nikiforov NG

Manuscript preparation: Loschenov VB, Orekhov AN

Manuscript editing: Sobenin IA

Availability of data and materials

Not applicable.

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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.

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Review

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Clinical and perioperative applications of three-dimensional echocardiography

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Abstract

The use of three-dimensional echocardiography, both in the clinical cardiology and perioperative settings, has increased thanks to its ability to add important information to the standard bi-dimensional exam and to evaluate structures without geometric assumptions. Both real time three dimensional (3D) transesophageal echo and offline quantitative measurements from 3D acquisitions have become integral for qualitative and quantitative analysis of structures and for surgical and procedural guidance. This review aims to provide an overview on the applications of 3D echo, with particular reference to the perioperative settings.

Keywords: Three dimensional, transesophageal echocardiography, transthoracic echocardiography, perioperative

INTRODUCTION

At the end of the 20th century, The New England Journal of Medicine released a landmark editorial listing the 11 most important medical achievements of the past 1000 years; body imaging was included as one of them^[1]. Among all the imaging modalities developed over the decades, echocardiography is now one of the most widely used diagnostic test.

The history of echocardiography, sprinkled with many interesting events and anecdotes^[2] cataloguing the journey towards currently used echo modalities, is quite intriguing. Standard mono- and bi-dimensional



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echocardiographic examination for transthoracic and transesophageal echocardiography allow the physician to obtain crucial information on cardiac structures, function, and hemodynamics in a remarkably quick and non-invasive or minimally invasive manner. In recent years, the never-ending quest for better quality and more detailed images, fueled by the invaluable contribution of the newest advances in hardware and software technologies, has led physicians to “usher in the third dimension”^[3]. Three-dimensional echocardiography (3D echo) can potentially overcome many of the limitations intrinsic to mono- and bi-dimensional echocardiography. The great opportunities offered by this relatively novel technique have aroused the interest of many: In order to give a sense of it, a total of 9631 papers could be retrieved on Pubmed by searching “three dimensional echocardiography” (April, 2019).

This review aims to provide an overview on the applications of 3D echo, with particular reference to the perioperative settings.

THREE-DIMENSIONAL ECHOCARDIOGRAPHIC EVALUATION OF CARDIAC STRUCTURES

As a general principle, 3D echo is based on the acquisition of pyramidal datasets used to sample the cardiac structures of interest, acquired over a single beat [real-time (RT)] or several beats (multi-beat). Multi-beat acquisitions use ECG gating to “stitch” volume slices together by means of a dedicated software to generate a 3D image^[3].

The accuracy and feasibility of 3D echo in studying valvular and congenital lesions has been largely demonstrated in the operating room, where 3D transesophageal echo (3D TEE) has been able to accurately predict valve morphology and provide complementary anatomic information to two-dimensional TEE (2D TEE)^[4]. Unsurprisingly, 3D TEE has now been widely integrated into the workflow for guiding complex interventional procedures^[5].

Adequate 2D image quality is an essential prerequisite for the evaluation of any cardiac structure by means of 3D echo. Moreover, inclusion of the entire cardiac structure of interest within the pyramidal dataset is of utmost importance. Of note, this can be quite challenging in patients with dilated ventricles (both left or right ventricle).

Once images are acquired, a key advantage of 3D over 2D echo is represented by the fact that measurements do not rely on any geometric assumptions. Dataset analysis is therefore not affected by foreshortened or off-axis views, as opposed to traditional 2D assessment.

However, the higher spatial resolution of 3D echo comes at the expense of lower temporal resolution, as compared to other echo modalities; while this might constitute a disadvantage (especially when assessing regional wall motion), careful technical adjustments of the images might still provide reasonable accuracy^[6]. Of note, poor temporal resolution could be improved by a multi-beat acquisition that stitches volumes together. Notably, this modality requires a motionless patient (without interference of electrocautery, arrhythmias, or ventilation).

With the advent of newer, more powerful TEE and TTE probes, this issue has been significantly improved with adequate frame rates from a single beat acquisition.

Nevertheless, as elegantly summarized by Surkova and colleagues^[7], the specific advantages of 3D echo over other imaging modalities - including its portability, absence of ionizing radiation, and the full compatibility with implanted cardiac devices like pacemakers and defibrillators- outweigh the aforementioned limitations in most cases.

EVALUATION OF THE LEFT VENTRICLE

Subjective interpretation of left ventricular (LV) volumes and function has long been recognized as one of the greatest limitations of mono- and bi-dimensional echocardiography^[8]. Accurate interpretation of LV volumes and function becomes even more difficult in the not uncommon case of patients with regional LV wall motion abnormalities, including aneurysmal LV for which conventional 2D echo has been demonstrated as not accurate in determining absolute LV volumes and ejection fraction (EF) because of LV asymmetry^[9].

Validation of echo against cardiac magnetic resonance imaging (MRI) (regarded as the gold-standard), nuclear imaging, and computed tomography (CT) has consistently shown 3D echo to be more accurate and reproducible compared to conventional 2D evaluation^[10]. In a meta-analysis including 23 studies (1,638 echocardiograms), Dorosz *et al.*^[11] showed that 3D echo, as compared to cardiac MRI, systematically underestimates volumes and has wide limits of agreement; its performance, however, remains better than traditional 2D methods. Of note, greater magnitude of bias was reported in patients with poor quality data sets and/or large ventricles, likely due to the known difficulty to include larger structures within the 3D pyramidal dataset. Similar results have been obtained in other series^[12], confirming the systematic underestimation of MRI-derived volumes by 3D echo.

These concepts have been incorporated into the latest edition of the American Society of Echocardiography (ASE) guidelines on chamber quantification, which now recommend different cutoffs to define normal LV 3D parameters^[6].

A certain degree of underestimation by 3D echo has also been demonstrated with regard to LV mass assessment as compared to cardiac MRI, although improvements in terms of accuracy have been achieved more recently. In a meta-analysis including 25 studies comparing 3D echo vs. cardiac MRI, Shimada *et al.*^[13] found that papers published in or before 2004 reported high heterogeneity ($I^2 = 69\%$) and significant underestimation of LV mass by 3D echo [-5.7 g, 95% confidence interval (95%CI) -11.3 to -0.2, $P = 0.04$], papers published from 2005 to 2007 were still heterogeneous ($I^2 = 60\%$) but showed less systematic bias (-0.5 g, 95%CI -2.5 to 1.5, $P = 0.63$). In contrast, studies published in or after 2008 were highly homogenous ($I^2 = 3\%$) and reported excellent accuracy (-0.1 g, 95%CI -2.2 to 1.9, $P = 0.90$).

EVALUATION OF THE RIGHT VENTRICLE

The complex and highly asymmetric anatomy of the right ventricle (RV) has traditionally represented a challenge for quantitative evaluation by echocardiography. Its trabeculations, along with the retrosternal position and its unique shape (defying any easy geometric approximation) explains the difficult task of RV assessment. Nonetheless, RV size and function have been shown to be important predictors of cardiovascular morbidity and mortality in patients with various conditions^[14].

Conventional 2D echo lacks the possibility to encompass the whole RV structure (inflow, outflow, and apical trabecular area); consistently, RV evaluation must be pursued by means of acquisitions via multiple acoustic windows, as recommended by international guidelines^[6].

Nowadays, different imaging modalities can offer better understanding of the RV anatomy (e.g., cardiac MRI, CT scan) but their use is limited by local availability, higher costs, complexity and greater time-consumption compared to echocardiography^[14].

The previously described ability of 3D echo to acquire the whole structure of interest has opened to new possibilities to overcome the challenges encountered with conventional echocardiography when it comes to exploring “the forgotten ventricle” [Figure 1]^[15].

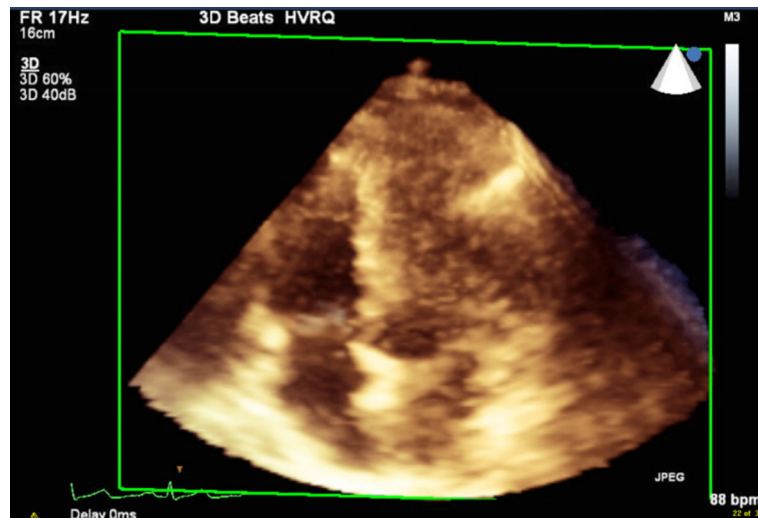


Figure 1. Transthoracic 3D image of the right ventricle in apical four chamber view

Vendors have developed dedicated software packages allowing the operator to perform accurate RV volumes and function evaluation with no need for geometrical assumptions.

As in the case of the LV, underestimation of RV volumes should be expected when comparing 3D echo to cardiac MRI. In a study enrolling 100 consecutive adult patients with normal or pathologic RVs, Leibundgut and associates generated a dynamic polyhedron model of the RV using a dedicated software. EDV, ESV, and stroke volumes were slightly lower on 3D echo imaging than on MRI (124.0 ± 34.4 vs. 134.2 ± 39.2 mL, $P < 0.01$; 65.2 ± 23.5 vs. 69.7 ± 25.5 mL, $P = 0.2$; and 58.8 ± 18.4 vs. 64.5 ± 24.1 mL, $P < 0.1$, respectively), while no significant difference was observed for EF ($47.8 \pm 8.5\%$ vs. $48.2 \pm 10.8\%$, $P = 5.7$)^[16] confirming results of other groups^[17,18].

Various imaging modalities (2D echo, 3D echo, radionuclide ventriculography, cardiac computed tomography, gated single-photon emission CT, and invasive cardiac cine ventriculography) were tested against cardiac MRI with respect to both LVEF and RVEF accuracy by Pickett *et al.*^[19]. For RVEF, CT and 3D echo were shown to have the best data to support their use with a bias $< 5\%$, tight limits of agreement and good correlation coefficients ($r > 0.75$)^[19].

EVALUATION OF CARDIAC VALVES

Conventional 2D echocardiography requires multiple views of the structure of interest along with the ability to mentally reconstruct the 3D image of the item under investigation, which can be particularly challenging in the case of the complex anatomy of diseased cardiac valves.

3D echo allows easier and, most importantly, better understanding of valvular anatomy and morphological disorders. These possibilities have made 3D echo extremely useful in the case of mitral valve (MV) disease, when, for instance, precise location of flail or prolapsed leaflet becomes of great importance both for diagnosis and repair.

Mitral valve

The human MV is a complex 3D device made of independent elements that constitute a dynamic structure where interaction among leaflets, mitral annulus, subvalvular apparatus (chordae tendineae and papillary muscles), and the left ventricle must be perfectly coordinated^[20].

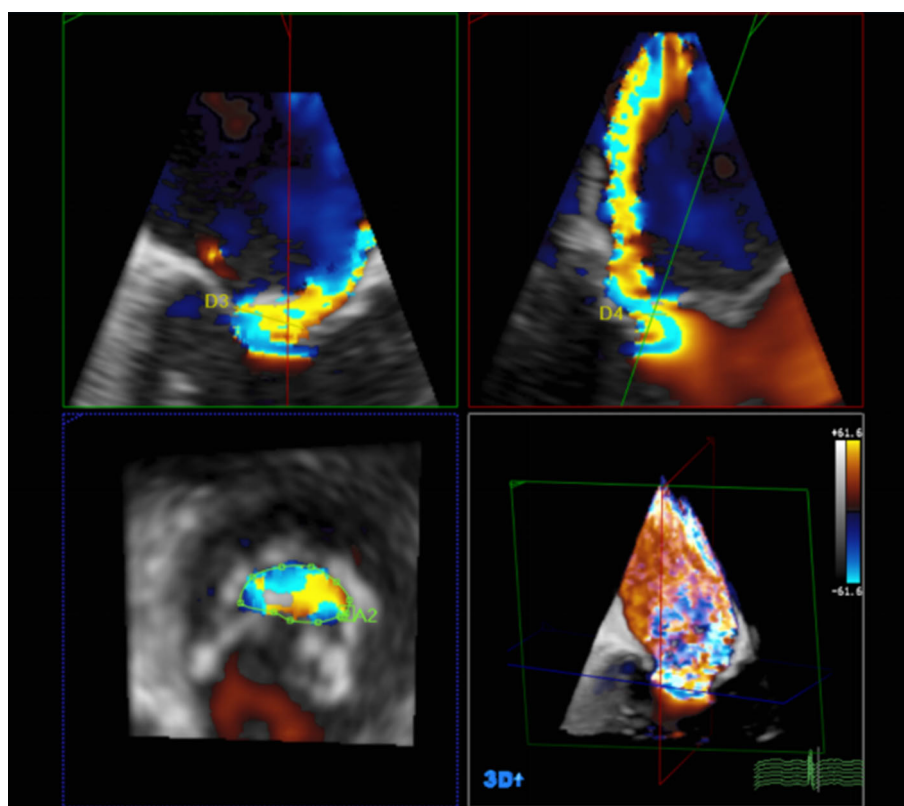


Figure 2. Transesophageal 3D-color assessment of vena contracta in a regurgitant mitral valve, showing its asymmetric shape

The superiority of 3D over 2D echo in the assessment of patients with mitral regurgitation (MR) has consistently been reported^[21]. Pepi *et al.*^[22] evaluated the feasibility and accuracy of 3D transthoracic echocardiography (3D TTE) and 3D TEE in evaluating MV pathology in 112 patients undergoing MV repair surgery. 3D techniques were feasible in a relatively short time (3D TTE: 7 ± 4 min; 3D TEE: 8 ± 3 min), with good (3D TTE 55%; 3D TEE 35%) and optimal (3D TTE 21%; 3D TEE 45%) imaging quality in the majority of cases. 3D TEE allowed more accurate identification (95.6% accuracy) of all MV lesions in comparison with other techniques; of note, 3D TTE and 2D TEE had similar accuracies (90% and 87%, respectively), whereas the accuracy of 2D TTE (77%) was significantly lower.

The use of 3D color enables new level of possibilities of assessment of mechanisms and severity of regurgitant lesions. Accurate evaluation of jet morphology, jet origin and jet volume is easily achieved, since direct visualization of jet characteristics is possible with no need for mental 3D reconstruction. Moreover, 3D color has prompted a true “Copernican revolution” in the quantification of the severity of MR, as it has been convincingly demonstrated that the vena contracta (VC) is often highly asymmetric [Figure 2], therefore making 2D assessment less reliable^[23].

A higher level of accuracy in terms of MV anatomical details, identification of diseased segments, prolapsing or calcified scallops, measurement of leaflet surface, tethering distance, tenting volume (just to cite some of the many parameters one could collect), can be obtained by means of 3D TEE, as compared to TTE. Newly developed semi-automated/ modeling packages for quantitative analysis of MV geometry and function based on 3D echo images acquired during 3D TEE are also available [Figure 3].

These tools are particularly helpful in understanding the pathophysiology and severity of MR, as well as in planning surgical or interventional treatments. In a study evaluating the utility of parametric 3D modeling

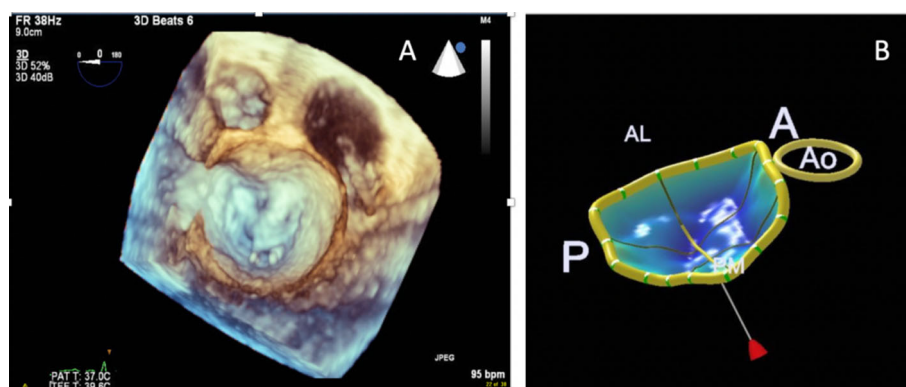


Figure 3. Multi-beat transesophageal 3D view of the mitral valve in en face “surgeons view”, showing a prolapse of the A2 scallop and flail chordae (A); mitral valve navigator model of the mitral valve allowing for complex measurements of the mitral valve apparatus (B)

of the MV, investigators found that modeling with color-encoded display of the MV leaflets, assisted the most inexperienced imagers in MV pathology diagnosis^[24].

3D echo might also provide incremental diagnostic value in patients with MV stenosis. The ability of 3D echo to assess residual MV orifice area has been validated by Zamorano *et al.*^[25]. Authors tested which echo-Doppler method showed the best agreement with MV area invasively evaluated by the Gorlin’s formula in 80 patients with rheumatic MV stenosis. Compared with all other echo-Doppler methods, real time 3D echo had the best agreement with the invasively determined MV area (average difference between both methods and limits of agreement: 0.08 cm^2 [-0.48-0.6]); notably, interobserver and intraobserver variability were both good (intraclass correlation coefficient [ICC] = 0.90 and 0.96, respectively).

Aortic valve

Lower rates of successful 3D echo imaging of the aortic valve as compared to the MV have been reported in the past years. In an initial experience with real-time 3D TEE on 211 patients by Sugeng *et al.*^[21], excellent visualization of the aortic valve was achieved in only 18% of cases, as opposed to the MV for which excellent visualization was reported in 85% to 91% of patients for all scallops of both MV leaflets). Nevertheless, improved technology (both on the hardware and software sides) has nowadays made 3D echo not only capable of providing good quality images but also to overcome another pitfall intrinsic to the traditional evaluation of the aortic valve by means of 2D echo. Standard use of the continuity equation for the evaluation of aortic valve area (AVA) in patients with aortic stenosis assumes a circular shape of the LV outflow tract (LVOT)^[26]. However, similarly to the VC, the LVOT can often be asymmetric [Figure 4]; this, in turn, potentially leads to significant under-estimation of the AVA when continuity equation is applied in the context of 2D imaging.

An interesting study by Gaspar and associates showed that the LVOT was eccentric in 96% of patients studied and that LVOT areas calculated from 2D echo systematically underestimated LVOT area compared to cardiac CT by $17 \pm 16\%$ ^[27]. However, due to inadequate TTE image quality of the LVOT, there was only moderate correlation between 3D LVOT area and cardiac CT ($r = 0.63$).

Multiple studies have shown good correlation between 3D annular and perimeter measurements of the aortic annulus compared to CT. In a meta-analysis by Elkaryoni and colleagues including 14 studies and 1,228 patients, there was excellent agreement between 3D TEE annular area and perimeter with CT-derived measurements ($r > 0.8$)^[28].

There has been interest in using 3D TEE to determine AVA in aortic stenosis - both as 3D planimetry and using 3D planimetry of the LVOT and applying the continuity equation. While 2D echo assessment of AVA

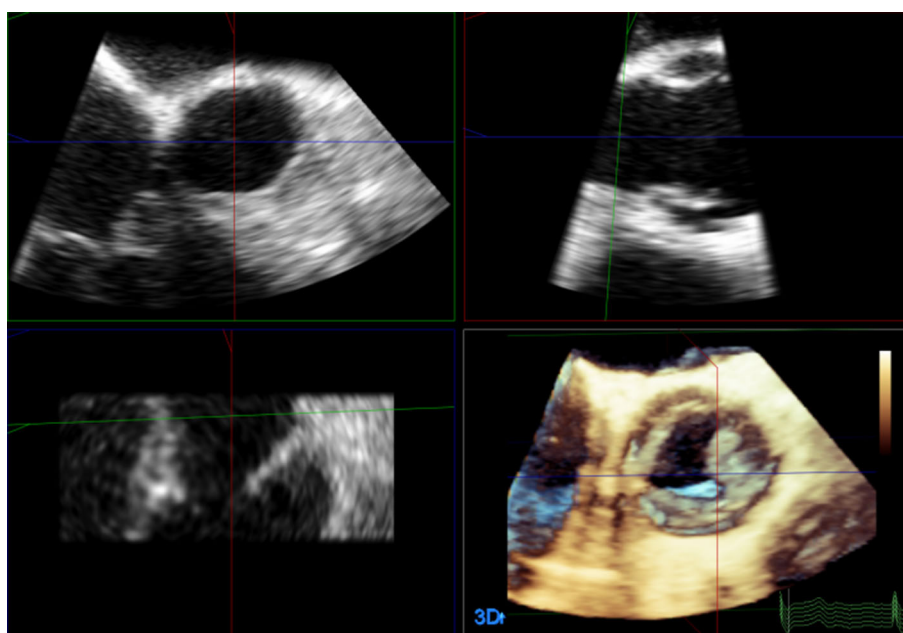


Figure 4. Transesophageal 3D echo: multi-planar reconstruction of the left ventricular outflow tract, showing its asymmetric shape

by planimetry and Doppler derived techniques (via the continuity equation) has been widely validated and studied, 3D AVA values have uncertain clinical significance and these values have yet to be validated.

Evaluation of aortic valve by 3D color echo may also improve the quantification of aortic regurgitation (AR) severity. Perez de Isla *et al.*^[29] used 3D color Doppler of AR to measure 3D VC to quantify severe AR and compared it to the gold standard of MRI. They studied 32 patients and traced the cross-sectional effective orifice area by using multiplane reconstruction of the en-face view (equivalent to VC area). They found excellent linear correlation between 3D echo and MRI: At a 3D vena contracta area cut off of 0.50 cm^2 , the receiver operating characteristic curve demonstrated excellent area under the curve to detect severe AR (3D VC cross sectional area method = 0.97; 3D VC cross sectional area/LVOT cross sectional area method = 0.98), and the authors concluded that 3D color echo is an accurate and highly reproducible diagnostic tool for estimating AR severity. Additionally 3D color echo had better agreement with CMR than 2D color Doppler. The utility of 3D echo in the setting of AR was also explored by Pirat and colleagues, who showed that proximal isovelocity surface area by 3D color-Doppler is feasible and quantification of 3D aortic regurgitant volume was more accurate than the usual 2D methods ($r = 0.83$ and $r = 0.69$ vs. volumes measured by flow meter for 3D and 2D echo, respectively)^[30].

THREE-DIMENSIONAL TRANSESOPHAGEAL ECHOCARDIOGRAPHY IN THE PERIOPERATIVE SETTING

As a modality, “perioperative TEE” is defined as the use of TEE for clinical care of patients before, during and immediately after procedures. 3D echo has enhanced both quantitative and qualitative information of ventricular and valvular function as well as our ability to use TEE as a procedural adjunct during structural heart cases and open cardiac surgery.

PERIOPERATIVE 3D TEE ASSESSMENT OF LEFT AND RIGHT VENTRICULAR FUNCTION

Assessment of ventricular function is one of the cornerstones of perioperative TEE. Though 2D method of disks is currently the gold standard^[6], 3D has been shown to be more accurate than 2D, and 3D

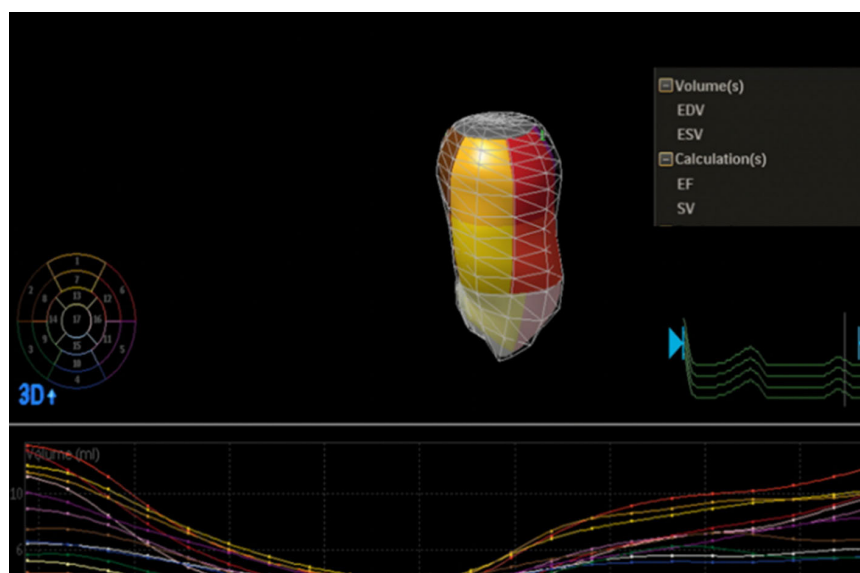


Figure 5. 3D semi-automatic quantification of the left ventricle demonstrating global function and regional wall motion

semi-automated assessment of LV function has recently been incorporated in the ASE guidelines for comprehensive perioperative TEE exam^[31] [Figure 5]. Similar to TTE, recent studies with 3D TEE have shown improved accuracy compared to 2D with the gold standard of cardiac MRI^[32].

3D assessment of the RV intraoperatively is a promising new application of perioperative TEE. There is ample literature suggesting that current 2D indices of RV assessment are limited in assessing overall RV function due to the complex geometry of the right ventricle. There have been TTE studies demonstrating improved accuracy of RV quantification with 3D echo^[19]. In the past, this application was limited by the fact that measurements needed to be made offline through dedicated software and were more time consuming, which is cumbersome in the acute perioperative setting. In earlier generation 3D TEE probes, the large sector size necessary to capture the entire RV created images with poor temporal resolution. However, with newer, more powerful 3D TEE probes, temporal resolution is often preserved [Figure 6] in addition to providing superior spatial resolution. Though 3D RV evaluation is feasible, there remains few intraoperative RV 3D imaging studies to date, and those that exist performed analyses off-line. Of note, a recent article by Grønlykke and colleagues comparing 2D and 3D indices of RV function found that 3D evaluation of RV stroke volume correlated with RV pulmonary artery catheter derived cardiac output^[33]. Nevertheless, as 3D evaluation of the RV becomes more efficient, it is expected to be incorporated to the intraoperative workflow.

PERIOPERATIVE 3D TEE FOR PROCEDURE GUIDANCE AND VALVULAR ASSESSMENT

3D TEE has improved communication between the cardiac anesthesiologist and cardiac surgeon or interventional cardiologist by allowing the structures of interest to be shown in real-time, reconstructed in the en face “surgeon’s view” - how the surgeon sees the structure anatomically in the chest. Studies have shown that 3D imaging of the MV is superior to 2D in assessing the pathology of degenerative MR and predicting the complexity of MV repair^[34-36], as well as the success of the repair^[37]. As valve sparing aortic surgeries become more preferred and commonly performed, it remains to be seen whether 3D will be helpful in predicting success of aortic valve repairs^[38].

Advancements in 3D technology has allowed real time 3D TEE to have a central role in percutaneous structural heart interventions. While guidance during these procedures was originally recommended

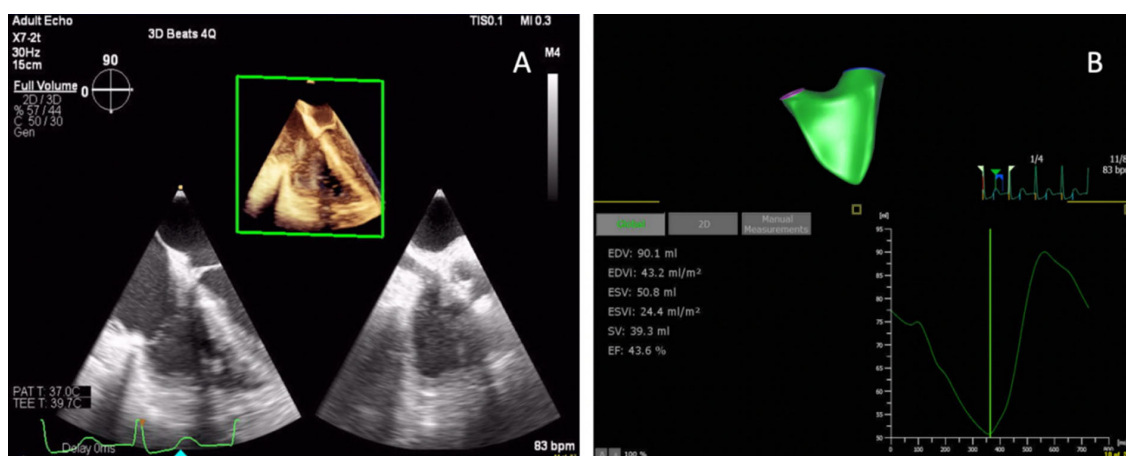


Figure 6. Transesophageal 3D multi-beat acquisition of the right ventricle (A); off-line analysis by dedicated software of right ventricular function (B)

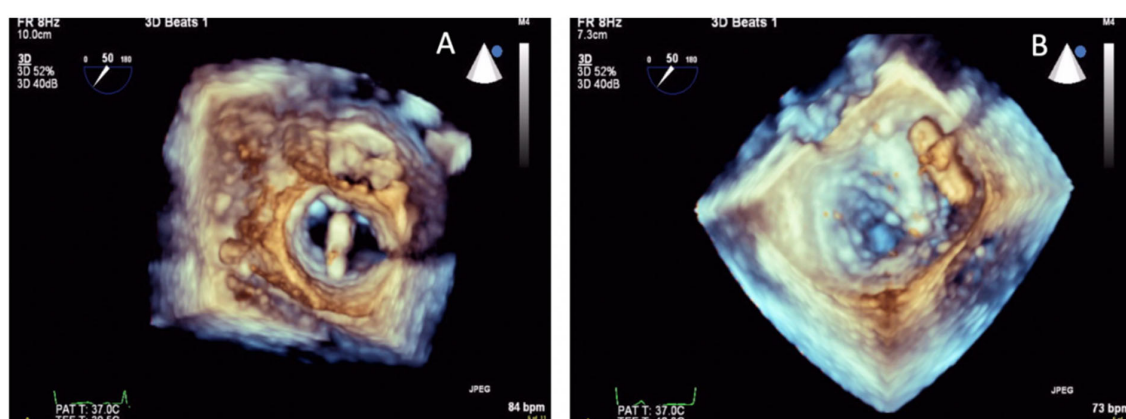


Figure 7. Real-time 3D transesophageal echo (RT 3D TEE) to guide placement of the MitraClip device by lining the device perpendicular to the line of coaptation (A); RT 3D TEE to guide placement of the second MitraClip device (B)

for intracardiac positioning of catheters only, it now also includes assisting with the actual deployment of devices. This is crucial for percutaneous mitral and tricuspid repair, paravalvular leak closures, atrial septal defects (ASDs) closure, left atrial appendage (LAA) closure, and emerging new devices such as the transcatheter MV repair and replacement devices (TMVR). In the specific case of TMVR, 3D TEE imaging is essential for the trans-septal puncture (position ideally slightly inferior and posterior from the middle of the interatrial septum) to allow for ability to maneuver the TMVR system. If the approach is apical, TEE is used to locate the optimal site for incision. TEE is then used with fluoroscopy for positioning of the TMVR device within the native annulus or surgical ring, usually with 3D imaging or biplane imaging (which requires 3D technology) to avoid malpositioning of the TMVR during deployment^[39].

3D TEE imaging's role is pivotal with MitraClip procedures^[40] [Figure 7], for depth perception as well as the spatial orientation not well appreciated during fluoroscopic imaging^[41].

Echo and fluoroscopic images are displayed simultaneously to facilitate orientation of catheters and device positioning. Given the great utility of 3D TEE in the assessment of patients with multiple valve orifices or complex or multiple regurgitant jets, its advantages over traditional 2D TEE are self-evident when evaluating patients after MitraClip [Figure 8]^[42].

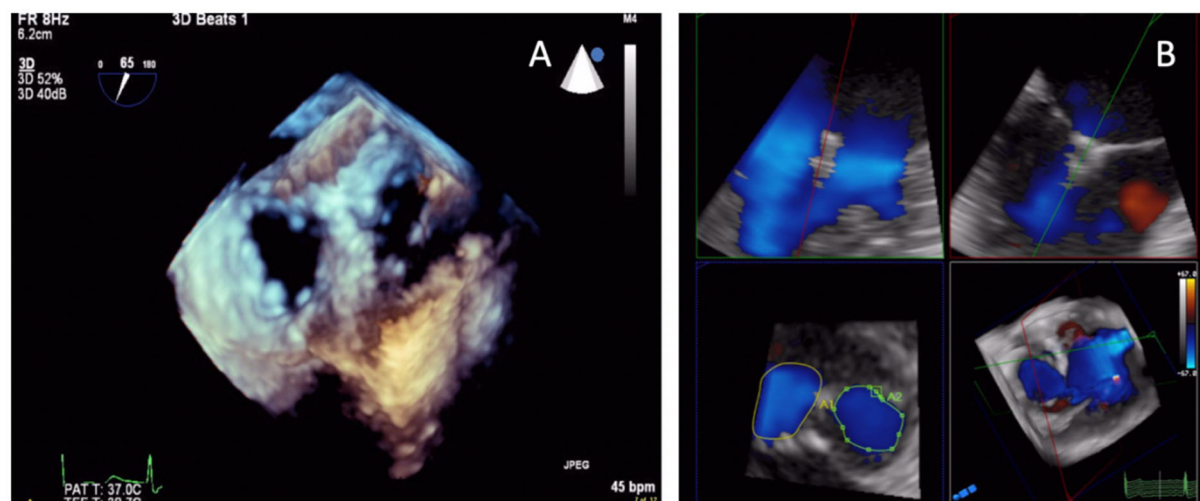


Figure 8. Transesophageal 3D view of the double orifice created by MitraClip, a percutaneous mitral valve repair device (A); quantitative assessment of valve area of the double orifice by MPR; Multi-planar reconstruction (B)

Assessment of severity of residual MR is central as it determines procedural success and patient prognosis after percutaneous edge-edge clip repair. Grading residual MR severity after percutaneous edge-edge clip repair is challenging and assessment has traditionally relied on integration of multiple 2D echo parameters as recommended by national and international societies^[43,44].

Notably, 3D color Doppler allows for direct measurement of the effective orifice area or VC area. In a recent retrospective study enrolling 155 patients, Avenatti and colleagues evaluated the feasibility and performance of summative VC area of multiple jets for residual MR after percutaneous MV repair against expert multiparametric appraisal of MR severity and invasive hemodynamics; the authors found that summative VC area correlated well with invasive hemodynamics and that a VC area threshold of 0.27 cm^2 had good diagnostic accuracy for identification of \geq moderate MR with an area under the curve of 0.81. Additionally, smaller VC area were associated with less clinical symptoms as measured by New York Heart Association functional class improvement. This study introduces total VC area after mitral valve edge-edge clip repair as a novel technique in quantification of residual MR severity and may have potential value in Guidelines recommendations^[45].

3D color Doppler has also been used to assess the location and amount of paravalvular regurgitation jets [Figure 9] both immediately and after repair, or later during paravalvular leak closure procedures^[46].

Valve area immediately after repair can also be accurately assessed by 3D planimetry^[42]. Multiplanar reformatting (MPR) is used for linear annular measurements of the tricuspid and mitral valves^[47,48] and aids in the periprocedural sizing of rings and valves, sizing of ASDs, sizing of LAA for LAA closure devices, and valve sizing for transcatheter valve replacement^[28,49,50].

3D TEE has been shown to have increased accuracy compared to 2D echo which in many cases is relevant for improved clinical outcomes. Johri *et al.*^[51] found that in complex ASDs (33% of a total of 24 ASDs studied), ASD area measured by 3D TEE was larger than 2D TEE (2.8 ± 1.3 vs. $1.7 \pm 1.4 \text{ cm}^2$; $P < 0.05$). This was clinically important because 3D TEE areas were also 27% larger in patients that had residual shunt after ASD closure suggesting that 2D TEE can underestimate the area of complex-shaped ASDs. Streb and colleagues compared real time 2D TEE with real time 3D TEE in 40 patients during place of a LAA occlusion device and found that there was better device size agreement (weighted Kappa 0.62 vs. 0.28,

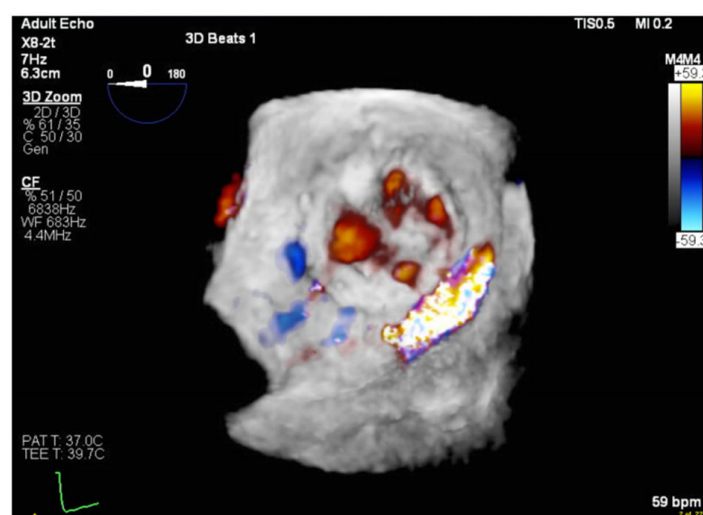


Figure 9. Transesophageal 3D-color assessment of a paravalvular leak

respectively) with 3D TEE vs. 2D TEE^[52]. Kronzon *et al.*^[53] used real time 3D TEE for evaluation of residual MR after mitral repair and replacement and found that 3D TEE provided more information than 2D echo including accurate evaluation of the origin of residual MR, the type of ring or prosthesis used, and location, size, shape, and area of the dehisced segment in cases of MV replacement. This increased detail could aid in perioperative decisions to address the defect.

FUTURE SCENARIOS

In the future, TEE may routinely be combined with fluoroscopy for multimodality real-time fusion guidance during interventional procedures. Indeed, overlay of markers and images has the potential to improve communication between the operator and the imager, increase procedural success, and decrease radiation exposure. Future prospective studies are necessary to evaluate the utility of live fusion imaging. In addition, the advent of 3D capabilities of intracardiac echocardiographic catheters suggests that this imaging modality should be revisited for potential guidance during catheter-based interventions^[54].

Until live fusion or “augmented reality” imaging is fully realized, 3D printing may be a useful option to translate CT and 3D TEE imaging. 3D printing allows for discussion and preoperative planning on patient-specific structural heart anatomy and pathology as well as preparing for 2D and 3D imaging best to elucidate the anatomy and device positioning. 3D printing may also help select device size in cases of unclear valve sizing. Currently, 3D printing is often obtained from CT or MRI modalities due to their high spatial resolution. However, 3D TEE printing is now possible, and may be applicable to future valve interventions after fine tuning work flow, 3D printing times, and software compatibility for conversion of 3D TEE to models^[55].

THE VALUE OF EXPERIENCE WITH 3D ECHO

Although 3D echo provides real-time images of the heart valves, training is required to differentiate normal anatomy from artifacts. The majority of studies demonstrating benefits in diagnosis of valve pathology have included expert readers with significant experience. Less experienced readers may not be able to accurately interpret 3D echo information. The utility and accuracy of 3D echo and MPR is dependent on user familiarity; multiple studies have shown that 3D echo measurements differ among expert vs. novice echocardiographers. Even among experts, there is a significant difference between 2D and 3D measurements

suggesting that 3D measurements are not interchangeable with 2D measurements. Bouchez *et al.*^[56] studied the difference in anterior MV leaflet length as measured by expert and novice imagers. The authors found there were systematic differences in anterior mitral leaflet length in 2D vs. 3D measurements and between beginner vs. expert imager measurements ($P < 0.001$ and $P = 0.005$, respectively). Tsang *et al.*^[57] found both novice and intermediate readers had greater difficulty interpreting 3D images of the MV than 2D. In contrast, expert readers were very proficient at identifying mitral valve anatomy in 2D and 3D. Hien's study looked at the diagnostic accuracy of 2D vs. 3D echo for MV prolapse and found that 3D TEE conferred an advantage to both expert and inexperienced echocardiographers^[24]. Therefore there is some debate as to the advantage of 3D echo interpretation and diagnosis among different level imagers. The heterogeneity of the literature suggests there is a significant learning curve for 3D echo and variation in interpretation regarding level of expertise. It is important to note that many of the studies conferring benefit of 3D and improved expert were performed by expert readers and may not be applicable in clinical practice.

CONCLUSION

3D echo use, both in the clinical cardiology and perioperative settings, has increased because of its ability to add important information to the standard 2D exam and evaluate structures without geometric assumptions. Both real time 3D TEE and offline quantitative measurements from 3D acquisitions have become integral for qualitative and quantitative analysis of structures and for surgical and procedural guidance. However, many of these studies were performed under optimal conditions with expert echocardiographers. The true advantage of 3D in the everyday clinical setting may be less than reported. The next generation of echo probes with high temporal resolution, improved parametric modeling, and the emergence of new structural heart devices will continue to facilitate the use of 3D echo perioperatively and increase diagnostic abilities in the clinical setting. It is imperative that imaging echocardiographers be familiar with 3D spatial orientation of intracardiac structures as well as how to perform quantitative analysis.

DECLARATIONS

Authors' contributions

Conception, design, data acquisition, drafting, critical revising, final approval: Rong LQ, Di Franco A

Availability of data and materials

Not applicable.

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Conflicts of interest

Both authors declared there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Review

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The link between hypertension and preeclampsia/eclampsia-life-long cardiovascular risk for women

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Abstract

Cardiovascular diseases remain the main cause of death and morbidity in women. Despite the active preventive measures and the reduction in the total number of morbidity and mortality rates, the rate of cardiovascular morbidity remains high in the population, moreover cardiovascular morbidity is increased in women of 35-54 years. Cardiovascular morbidity has several unique characteristics for women; pregnancy, gestational hypertension, preeclampsia/eclampsia are gender-specific risk-factors for further cardiovascular morbidity in women, it's possible to detect these risk-factors in younger age groups and start prevention as early as possible. Arterial hypertension, which is characterized by genetic polymorphism, is an important and a powerful risk factor for development of both acute and chronic cardiovascular diseases; association of arterial hypertension with different metabolic disorders such as metabolic syndrome, diabetes seems particularly dangerous in pregnancy in terms of peri-pregnancy and life-long morbidity. Preeclampsia shares some common features with atherogenesis and metabolic changes and atherogenesis and metabolic changes, so presence of hypertension during pregnancy increases the risk of cardiovascular diseases and diabetes later in the life. Is pregnancy revealing or predisposing factor of development cardiovascular diseases is not still clear and to answer these questions more and more studies are required.

Keywords: Arterial hypertension, women, pregnancy, cardiovascular risk

INTRODUCTION

Cardiovascular diseases remain the main cause of death and morbidity in women^[1,2]. Despite the active preventive measures and the reduction in the total number of morbidity and mortality rates, the rate of



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cardiovascular morbidity remains high in the population, moreover cardiovascular morbidity is increased in women at 35-54 years^[3]. Arterial hypertension is an important, powerful risk factor for cardiovascular acute episodes, is characterized by genetic polymorphism^[4]; association of arterial hypertension with different metabolic disorders - metabolic syndrome, diabetes is particularly dangerous^[5]. Moreover, it was found that haemorrhage and hypertensive disorders are major contributors to maternal deaths in developing countries^[2].

Preeclampsia/Eclampsia is a multiorgan syndrome associated with pregnancy, which occurs in 2%-8% of pregnant women^[6-9]. The main characteristics of preeclampsia/eclampsia are pregnancy and elevation of blood pressure. It is considered that the arterial hypertension in pregnancy is clearly associated with cardiovascular morbidity during lifetime^[1,6,7,10,11].

Preeclampsia/Eclampsia develops in 3%-5% of pregnancies in developed countries and 7.5%-8% of all over the world^[9,12-14]. Preeclampsia/eclampsia is clearly linked to gestational age, offspring baby weight, nulliparity, *etc.* The cohort research study in Denmark of 536419 female has shown that delivery at 32-36 weeks of pregnancy increases the risk of next early premature labor from 2.7% to 14.7%, and the risk of preeclampsia from 1.1% to 1.8%. This study also showed that the first childbirth up to 28 weeks of pregnancy increases the risk of premature labour at next pregnancy by 26% and the risk of development of preeclampsia by 3.2%. Preeclampsia during first pregnancy increases the risk of preeclampsia during next pregnancy from 14.1% to 25.3%. Other studies also confirm that there are 3 times rise of cardiovascular morbidity and 7 times the incidence of hypertension in this group of population^[15,16].

Excessive weight, hypertension before pregnancy, age, metabolic disorders before pregnancy increases the risk of development of preeclampsia^[6,17,18]. On the other hand, preeclampsia is clearly associated with the development of metabolic disorders in life, so it is not surprising that women with preeclampsia and eclampsia have a higher risk of cardiovascular morbidity.

DEFINITION OF HYPERTENSIVE DISORDERS DURING PREGNANCY

Hypertension in pregnancy comprises^[19]:

- Pre-existing hypertension: precedes pregnancy or develops before 20 weeks of gestation. It usually persists for more than 42 days post-partum and may be associated with proteinuria.
- Gestational hypertension: develops after 20 weeks of gestation and usually resolves within 42 days post-partum.
- Preeclampsia: gestational hypertension with significant proteinuria (> 0.3 g/24 h or Albumin/creatinine ratio ≥ 30 mg/mmol). It occurs more frequently during the first pregnancy, in multiple pregnancy, in hydatidiform mole, in antiphospholipid syndrome, or with pre-existing hypertension, renal disease, or diabetes. It is often associated with foetal growth restriction due to placental insufficiency and is a common cause of prematurity.
- Pre-existing hypertension plus superimposed gestational hypertension with proteinuria.
- Antenatally unclassifiable hypertension: this term is used when BP is first recorded after 20 weeks of gestation and hypertension is diagnosed; re-assessment is necessary after 42 days post-partum.

Preeclampsia and eclampsia develops before, during and after delivery. It may be firstly developed in the postpartum period, accompanied by manifestations of severe multiorgan impairment. Late postpartum eclampsia can be manifested with severe brain, heart, pulmonary artery impairment in postpartum period^[20].

European Society of Cardiology considers arterial blood pressure 140-159/90-109 mmHg as mild and 160/110 mmHg as severely elevated for pregnant women, what is in agreement with other societies as well^[6,19,20].

Risk - factors

The risk factors for preeclampsia and eclampsia are: nulliparity, family history of preeclampsia, multiple gestation, history of diabetes and hypertension, hypertension during pregnancy, rapidly growing hydatidiform mole, mother's age, antiphospholipid syndrome, impaired glucose tolerance, caesarean delivery, race^[6,8,21-23]. There is evidence that women who smoke are less likely to develop eclampsia, although the reason is not clear^[24]. Almost all these risk-factors are linked to cardiovascular diseases as well (hypertension, impaired glucose tolerance, age, inflammatory profile, race, *etc.*).

Systematic review and meta-analysis of cohort studies of 25,356,688 pregnancies among 92 studies out of 27 countries^[25] has shown, that the risk of development of preeclampsia is clearly linked to antiphospholipid antibody syndrome, prior preeclampsia, chronic hypertension, pregestational diabetes, assisted reproductive technology, and BMI > 30. These factors are strongly associated with a high rate of preeclampsia, and presence of any of them will help to reveal the woman with "high risk" of preeclampsia.

Clinical characteristics

Patients with preeclampsia/eclampsia may develop following complaints^[26,27]: headache, seizures, visual disturbances (blurred vision, migraine - blinking scotoma), changed mental status, blindness cortical or retinal, shortness of breath, dyspnea, edema, epigastric or pain in the upper right corner of the abdomen, weakness, inability, may be presented signs of haemolytic anemia. It is noteworthy that eclampsia may be developed without prodromal symptoms^[28,29].

For a long time it was considered that eclampsia follows the pregnancy, but in recent years information about late (postpartum) eclampsia is growing, and more and more cases of late eclampsia are described and presented. Different authors describe different frequencies of late eclampsia, although the number of complications is not small and varies from 0.3% to 27%^[30,31]. Almost half of eclampsia cases develop after childbirth^[13,32]. Seizure is predominantly developing in the first 48 hours after childbirth, although it may occur even at 60 day^[18].

Whether early and late preeclampsia/eclampsia have the same pathophysiological mechanisms is not clear. Since pregnancy contains cardiovascular and metabolic stress, response to this stress may represent a woman's personal risk during lifespan, such serious and dangerous complication as venous thromboembolism and pulmonary thromboembolism among others^[33].

Late onset postpartum preeclampsia differs clinically from antepartum eclampsia. Thus study conducted on 194 patients with eclampsia (92 antepartum and 92 postpartum) showed, that patients with postpartum preeclampsia were older, multiparous and of lower socio-economic status than patients with antepartum preeclampsia, additionally, patients with postpartum preeclampsia have more clinical symptoms like headache, elevated blood pressure, abnormal vision, nausea/vomiting, seizures, shortness of breath and pedal edema, they also show significantly higher laboratory markers, than patients with antepartum preeclampsia. And additionally, they more often require blood pressure treatment after discharge^[34].

It is known that eclampsia and preeclampsia increases the risk of cardiovascular morbidity at 2-4 times in lifetime and reaches the level of risk related to tobacco consumption^[3]. In this group of population is manifested life-long increase of incidence of arterial hypertension and metabolic disorders. Thus, after two years of observation of women with preeclampsia and eclampsia had been shown the increase the risk of cardiovascular disease^[11]; Hypertension during pregnancy is associated with rise of 10 year cardiovascular risk in women^[35], women with preeclampsia who remain having hypertension after delivery have a twofold rise of risk of developing CVD in the next 10-30 years^[36], particularly during their fifth decade^[37]. That's why

the American Heart Association is considering eclampsia and preeclampsia as gender-specific risk factors of CVD^[3].

Preeclampsia itself is the risk factor for the other complications, including life-threatening complications. A population based study conducted in Sweden of 1,003,489 deliveries showed that preeclampsia, multiple childbearing, caesarean deliveries are important risk factors for pulmonary embolism and stroke^[33,38]; On the background of preeclampsia increases the incidence of pulmonary embolism and stroke 3-12 times in late pregnancy, at delivery, and in the puerperium, and similar increases in risks were also observed for multiple pregnancies and caesarean delivery. At the time of pregnancy physiologically develops hypercoagulation state, rise of D-dimer; continuous rise of these markers can lead to or is associated with vein thrombosis and pulmonary thromboembolism^[39].

Hypertension can occur firstly in postpartum period as well, the pathophysiology of this condition is not clear. In retrospective study of 988 women showed that women with postpartum hypertension have clinical risk factors and an antepartum plasma angiogenic profile similar to those found in women with preeclampsia what could be sign of subclinical or unresolved preeclampsia^[40].

Development of hypertension later in the life seems is clearly linked to complications and hypertension during pregnancy; thus a population-based study of 146,748 women showed that hypertension during pregnancy was associated with an elevated risk of future CVD or hypertension during life-time, irrespective of time of development of hypertension^[41].

Physiology

Normal physiologic changes during pregnancy are expressed in rise of cardiac output (CO) up to 20%-50% starting by 6 week of gestation and reaching maximum by 16-28 weeks (usually around 24 week); all of this is followed by rise of heart rate and stroke volume. It remains near peak levels up to 30 week^[42]. CO is rising by another 30% during labor but then rapidly drops after delivery and reaches 15%-25% above normal level because of contraction of the uterus. Then continues gradual reduction (mostly over the next 3 to 4 weeks) and reaches the pre-pregnancy level at about 6 week postpartum. The rise of CO during pregnancy is mostly determined by increased requirements of the utero-placental circulation; Rise of cardiac output is determined also by the needs of skin to regulate the temperature and kidneys to excrete fetal wastes as well. Changes in cardiac function are associated with changes in renal function, thus glomerular filtration rate (GFR) rises by 30%-50%, reaching maximum again by 16-24 week gestation, and remains at same level almost until term^[42-44].

Gestational Hypertension and Preeclampsia/Eclampsia are associated with impairment of cardiac function longer than in normal pregnancy. A prospective longitudinal case-control study showed that in one year postpartum, preeclampsia is associated with diastolic dysfunction, asymptomatic left ventricular moderate-severe dysfunction/hypertrophy, functional/geometric abnormality, which is even more expressed in women with preterm preeclampsia (56%) than with term preeclampsia (14%) or matched controls (8%; $P < 0.001$)^[45]. This study showed that majority of preterm preeclamptic women have stage B asymptomatic heart failure postpartum, and 40% develop essential hypertension within 1 to 2 years after pregnancy.

Fibrinoid necrosis with a perivascular mononuclear cell infiltrate vessel wall in early phase of preeclampsia, later lipophages are found in vessels of these women^[46]. These changes are quite close to atherosclerotic process. Acute atherosclerosis is not found in normal pregnancies including normal pregnancies in diabetic women, but can develop in vessels of women with preeclampsia or in women with small-for-gestational-age infants, or both.

Pregnancy leads to response from endocrine glands as well, partly because the placenta produces hormones and partly because most hormones circulate in protein-bound forms and this protein binding increases

during pregnancy what affects response from endocrine glands. Thus, during pregnancy levels of estrogen, progesterone, thyroid hormones, aldosterone, cortisol are rising^[47], changes in insulin-resistance are developing. Each of these factors participate in atherogenesis and impairment of metabolic profile.

All above mentioned are well-known factors associated with normal pregnancy and impairment in one of them may have influence on others as well.

Preeclampsia (Eclampsia) is condition related to ischemia of placenta. There are studies that confirm the link between preeclampsia and gestational arterial hypertension with endocrine and metabolic diseases- preeclampsia is considered as a risk-factor of hypothyroidism, diabetes mellitus, and dyslipidaemia^[48-50]. Each of them independently increases the incidence of cardiovascular morbidity.

Healthy pregnancy is driven into a growing of pro-atherogenic metabolic state^[51,52]. Shortly after conception pregnant women develops a high cardiac output^[53], hypercoagulability^[54], and increased inflammatory activity^[55]. After 20 weeks there is insulin resistance^[52,56] and hyperlipidaemia^[57]. Healthy women responds adequately and tolerates well physiologic changes during pregnancy, but woman with inherited or acquired predisposition to different chronic diseases may not tolerate pregnancy induced hormonal or hemodynamic changes^[57]. These gestational changes are usually more pronounced in women who later develop preeclampsia. The effect of coexisting risk-factors are clearly confirmed in several studies. This is partly due to pre-existing, subclinical inflammatory and/or cardiovascular risk factors in “healthy” women who go on to develop preeclampsia^[58]. These women are more likely to be overweight^[59,60] have higher lipid levels, higher blood pressure, insulin resistance and are more likely to have a thrombophilia, compared with women who go on to have a normotensive pregnancy.

Angiogenic factors also contribute to development of preeclampsia. Seems that maternal diseases (or predisposition) is related to anti-angiogenic factors sFlt-1 and sEng, released by an affected placenta. Are these anti-angiogenic proteins involved in development of the maternal diseases later is not clear, however could indicate the predisposition of development of cardiovascular abnormalities^[22].

Preeclampsia is associated with impairment of vascular function, impaired endothelial function, share some common features of development of atherosclerosis. The 498 women from the Epidemiology of Coronary Artery Calcification Study were evaluated for presence of subclinical coronary atherosclerosis using logistic regression model, up to 10.4% had history of hypertension during pregnancy, what also was associated with impairment of kidney function and coronary artery calcium score later during lifespan^[22,61].

Insulin resistance is developing physiologically in healthy pregnant women, however may remain after delivery and even progress in certain women with predisposition (because of acquired or inherited factors). In a population-based, retrospective cohort study for 1,010,068 pregnant women was determined two-fold rise of risk of development of diabetes during up to 16.5 years after pregnancy, even in the absence of gestational diabetes. The presence of preeclampsia or gestational hypertension in women with gestational diabetes also significantly rises the lifelong risk of diabetes compared to gestational diabetes without preeclampsia or gestational diabetes^[21,62] which is independent risk for future cardiovascular events.

Vascular wall seems that is responding to preeclampsia. Thus study preformed showed that Carotid Artery Intima-media thickening is reliably higher in women with preeclampsia, is reducing after delivery, but remain significantly higher in a year after delivery in women with previous preeclampsia^[63].

CONCLUSION

Cardiovascular morbidity is multifactorial, preclinical stage is starting in early ages, is linked to multiple risk-factors. Cardiovascular morbidity has several unique characteristics for women; pregnancy, gestational

hypertension, preeclampsia/eclampsia are gender-specific risk-factors for further cardiovascular morbidity in women, which is possible to reveal in young ages and start prevention as early as possible. Seems abnormalities detected during pregnancy are linked to life-long cardiovascular morbidity, so pregnancy-related morbidity starts to exceed concerns of obstetricians and takes the important part of primary care physicians and cardiologists as well. Is pregnancy just revealing or predisposing factor of development cardiovascular diseases is not still clear and to answer these questions more and more studies are required.

DECLARATIONS

Authors' contributions

Study design: Vakhtangadze T

Data collection: Gakhokidze N

Manuscript writing: Vakhtangadze T, Gakhokidze N

Manuscript review: Vakhtangadze T

Serching and evaluating the references: Khutsishvili M, Mosidze S

Availability of data and materials

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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.

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Case report

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Type III coronary perforation during chronic total occlusion percutaneous coronary interventions treated with Cyanoacrylate glue embolization: case report and review of the technique

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Abstract

In recent times the outcome of chronic total occlusion (CTO) percutaneous coronary interventions (PCI) in dedicated centers has steadily gained high success rate (> 80%) and low rate of coronary complications. Nevertheless comparing with non-CTO PCI the complications rate is higher, due to the higher lesion and technical complexity. Among the complications Type III coronary perforations remain the most troublesome events of CTO PCI and still carry a significant risk of death for the patients. The management of Type III coronary perforations has been extensively described as a flow chart of interventions and techniques to obtain rapid cessation of the blood extravasation and sealing of the ruptured vessel. Several techniques have been described to obtain bleeding cessation also in small vessel (< 2 mm) perforations. In this paper we will describe two cases of CTO PCI with Type III small vessel coronary perforations treated with percutaneous Cyanoacrylate/(NBCA-MS)-based glue infusion through a conventional CTO microcatheter. This technique is fast and straightforward and can be applied to any conventional CTO microcatheter.

Keywords: Type III coronary perforations, coronary chronic total occlusion complication, cyanoacrylate/(NBCA-MS)-based glue



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INTRODUCTION

Coronary chronic total occlusion (CTO) is characterized by heavy atherosclerotic plaque burden within the artery, resulting in complete occlusion of the vessel. Although the duration of the occlusion is difficult to determine, a coronary occlusion is defined as true CTO when the duration is at least 3 months or undetermined^[1]. Patients with CTO usually develop collaterals, which can be visualized through coronary angiography, from ipsilateral or contralateral vessel. However, these collaterals often do not provide sufficient blood flow to prevent myocardial ischemia during exercise and therefore anginal symptoms may appear^[2].

The incidence of CTO among patients who have a clinical indication for coronary angiography has been reported to be as high as 15% to 30%^[3,4].

The first important step made in CTO revascularization was to recanalize the occluded vessel using coronary collaterals. The septal channel has historically been the first described solution. The progressive improvement in the technology of guidewires and microcatheters expanded the ability to cross different collateral channels even in very complex cases. Nowadays, examples of collaterals which can be used in CTO procedures, also include epicardial collaterals, even ipsilateral, and occluded saphenous grafts. Experienced operators are able to successfully treat very difficult CTO lesions using a combination of different pathways and techniques.

The results of older data registry (early CTO era) showed low success rate and higher MACE compared with non-CTO PCI, whereas CTO revascularization performed in experienced centers has now proven success rate up to 80%-90%. On the other hand, due to the higher lesion and technical complexity, CTO complications rate has shown to be higher than non-CTO PCI (1.6% vs. 0.8%; $P < 0.0001$)^[5].

Perforation is one of the most troublesome complications of CTO PCI. Despite the fact that coronary perforations are infrequent (0.33% of all cases) they are associated with poorer short- and long-term outcomes. In the British Cardiovascular Intervention Society Database CTO PCI was one of independent predictors of risk of perforation^[6].

Coronary artery perforations (CAP) are categorized according to Ellis classification as: Type I, extraluminal crater without extravasation; Type II, epicardial fat or myocardial blush without contrast jet extravasation; Type IIIa, extravasation through frank (> 1 mm) perforation; Type IIIb "cavity spilling" (CS), which refers to perforations with contrast spilling directly into either the left ventricle, coronary sinus or other anatomic circulatory chamber [Table 1]^[7]. Grade I or II perforation are usually managed conservatively since they have a more benign clinical course. On the other hand, type III CAP was associated with a worse outcome. In earlier registers CAP Type III was associated with a very high in-hospital mortality rate (44%), with the majority of patients requiring emergency surgery (60%)^[8], while more recent data shows lower mortality rate (15.2%) and lower rate of emergency surgery (16%)^[9].

Among interventional collaterals suitable for CTO procedures, epicardial channels are considered the trickiest ones and appear more prone to perforation or rupture. This is caused by the relative high frequency of tortuosity and their small size (CC1 in Werner classification)^[10]. Perforation of epicardial channels carries a higher risk of cardiac tamponade and death when the treatment is delayed. This is due to the spillage of blood directly into the pericardial space.

The current endovascular treatment for perforated coronary arteries involves the use of prolonged balloon inflation and/or the use of covered stents (CS). The use of CS is feasible only when CAP is located in a large vessel due to the availability of CS starting just from a diameter of 2.25-2.5 mm^[11]. Moreover, when collateral vessels emerge at a short distance from the CAP, the use of CS could lead to the occlusion of the collaterals and to the subsequent myocardial infarction. When CAP occurs in smaller vessels or collaterals (< 2 mm),

Table 1. Ellis classification of coronary artery perforations

ELLIS classification Types	Ellis 1	Ellis 2	Ellis 3a	Ellis 3b
Details	Extraluminal crater without extravasation	Epicardial fat or myocardial blush without contrast jet extravasation	Extravasation through frank (> 1 mm) perforation	Perforations with contrast spilling directly into either the left ventricle, coronary sinus or other anatomic circulatory chamber
Mortality	5.8%	5.2%	16.6%	0%

various materials, such as autologous clots or fat^[12,13], gel foam^[14], fibrin glue^[15], microcoils^[16] and polyvinyl alcohol form^[17-23] can be embolized to the site in order to provide haemostasis and bleeding cessation.

When percutaneous treatment of CAP is not effective, surgical repair represent the bail out strategy. Efficacy of surgical repair however is not very high, as reported by a register from Al-Lamee *et al.*^[9], where the rate of success was just 44.4%.

In this case report we used (NBCA-MS)-based glue embolization (GLUBRAN2, GEM s.r.l. ITALY) in order to seal the perforation of small vessels during retrograde and antegrade revascularization of two cases of CTO-PCI.

Sterile glue is available for medical use either as pure synthetic glue (Histoacryl), or as dual component fibrin glue (fibrin plus thrombin). Sterile glue has been described as an effective embolization material for neurointerventional indications^[24,25], closure of oesophageal varices^[26], femoral pseudoaneurysms^[27], septal ablation in Hypertrophic Obstructive Cardiomyopathy^[28] and as a surgical adjunctive tool to stick a patch over the myocardial wall after an acute myocardial infarction complicated with cardiac rupture^[29,30]. The use of sterile glue has already been described for the embolization of the right coronary artery's distal portion^[31].

The (NBCA-MS)-based glue can be injected pure or pre-mixed with Ethiodized oil (Lipiodol/Ethiodol). The mechanism of the (NBCA-MS)-based glue is related to his reaction to Na ions of tissue fluids. When (NBCA-MS)-based glue comes into contact with Na ions the glue solidifies in a variable amount of time which varies from a few seconds to one minute depending on the proportion of Ethiodized oil you pre-mix with [Table 2]. Ethiodized oil also works as a Contrast agent which produces radiopacity in the mixture and helps the physician to confirm the site of embolization. Na ions are also present in Heparin and in Contrast media therefore both sterile cup and the syringes used for mixing the components shouldn't have been in contact with blood and Heparin. Furthermore, the microcatheter should be carefully flushed with dextrose solution just before the injection of the (NBCA-MS)-based (pure or mixture) in order to clean the inner surface from blood or heparin residues. This flushing avoids the premature start of the glue polymerization process into the microcatheter, which could occlude it totally or partially and make the microcatheter useless for multiple injections.

The injection can be done in a "single shot" fashion or with multiple "sandwich" injections when the mixture is alternated with dextrose boluses.

Choosing the best proportion of ethiodized oil and the right amount of mixture is of paramount importance to achieve the best results. The proportion of (NBCA-MS)-based with ethiodized oil component in very small leakage of distal perforations can be 1:1 or 2:1 with a "single shot" 0.5-1 mL bolus. This strategy allows fast and effective sealing with the same microcatheter already in place. In case of a bigger leak when the perforation is more proximal or if there are other branches very close to the perforation site the multiple "sandwich" technique with (NBCA-MS)-based/ethiodized oil proportion of 1:2 to 1:4 with small boluses of 0.3-0.5 mL allows more precise embolization with smaller risk of back flow of the mixture which could cause side branch occlusion or thrombosis. After last embolization it is always advisable to perform a rapid

Table 2. Polymerization time based on the proportion of Lipidol into the mixture (Ethiodized oil: Cyanoakrylate glue)

	Ethiodized oil 1:1	Ethiodized oil 2:1	Ethiodized oil 3:1	Ethiodized oil 4:1	Ethiodized oil 5:1	Ethiodized oil 6:1
Start polymerization	5 sec	10 sec	10 sec	18 sec	20 sec	25 sec
End polymerization	40 sec	60 sec	75 sec	85 sec	110 sec	120 sec

pull-back of the microcatheter (“hit and run”) considering that the glue could solidify in the distal tip of the microcatheter or trap it into the coronary system.

CASE REPORT

The first patient is a 78-year old male with stable angina Canadian Class Society^[3]. He was admitted 1 month earlier for stable angina and the coronary angiography showed a severe coronary artery disease with critical stenosis of left anterior descending artery (LAD) and left circumflex artery (LCX) and a CTO of the right coronary artery (RCA) [Figure 1A]. The case was discussed in Heart Team and surgical revascularization was excluded by patient's preference.

After revascularization of the LAD and LCX, revascularization of the CTO of RCA was staged after 1 month.

JCTO score of the RCA CTO was 3 and the most appealing interventional collateral from LAD to RCA was a septal branch and a very tortuous epicardial vessel from distal LAD [Figure 1B]. After several unsuccessful attempts to advance the guidewire on the septal, we managed to cross the epicardial vessel with regular SION guidewire (Asahi Intecc). The guidewire successfully crossed the most tortuous section of the channel but it was unable to progress further for lack of support from the microcatheter which was stocked at the entry point of the epicardial channel. We tried to rotate and push the Corsair microcatheter (Asahi Intecc) in order to advance closer to the guidewire's tip [Figure 1-C], but during the manipulation the catheter suddenly stepped forward out of the vessel and a type 3 perforation occurred [Figure 1D]. After confirming the site of the coronary rupture, we placed the microcatheter 10-20 mm proximal to the perforation. Promptly a Ethiodized oil and Glubran mixture (1:1 ratio) was prepared. After flushing the microcatheter with a dextrose solution, the mixture was injected into the distal LAD [Figure 1E]. After 10-15 seconds cessation of the bleeding extravasation was observed [Figure 1F]. The patient remained stable and asymptomatic. No significant pericardial effusion was observed after several echocardiographic exams. The patient was discharged after 7 days. A new attempt of the recanalization is planned in the next few months.

The second patient is a 82-year old female affected by arterial hypertension, dyslipidaemia and mild carotid atherosclerosis. The patient had no cardiovascular history; she complained of effort dyspnoea and legs oedema. At the Echocardiography we observed a dilatation of the left ventricle with diffuse hypokinesia and moderate impairment of the ejection fraction (EF 40%) and moderate functional mitral regurgitation. Because of the new onset of heart failure, we planned a coronary angiography. The angiography showed a severe, calcific tri-vessel coronary disease, involving the left main and proximal LAD, and a total occlusion of the mid LAD and RCA [Figure 2A]. During the Heart Team discussion, the patient was refused from the cardiac surgeon because of advanced age and frailty and a complete percutaneous revascularization was planned. The CTO of the RCA was short and a visible microchannel seemed to give a good chance to cross with sliding technique. Thus, we decided to perform recanalization of RCA first, with antegrade approach avoiding double access due to absence of coronary collaterals from left coronary system and severe left main disease. We readily crossed the lesion with a soft polymeric guidewire Fielder XT-R (Asahi Intecc), [Figure 2B]. After reaching the distal vessel and confirming the position of the wire with multiple projections we performed several dilatations with small compliant balloons. After dilatation, we observed a severe perforation of the posterior-lateral branch, probably due to the guidewire positioned distally in a smaller branch [Figure 2C]. We planned to implant a covered stent crossing and covering the small branch

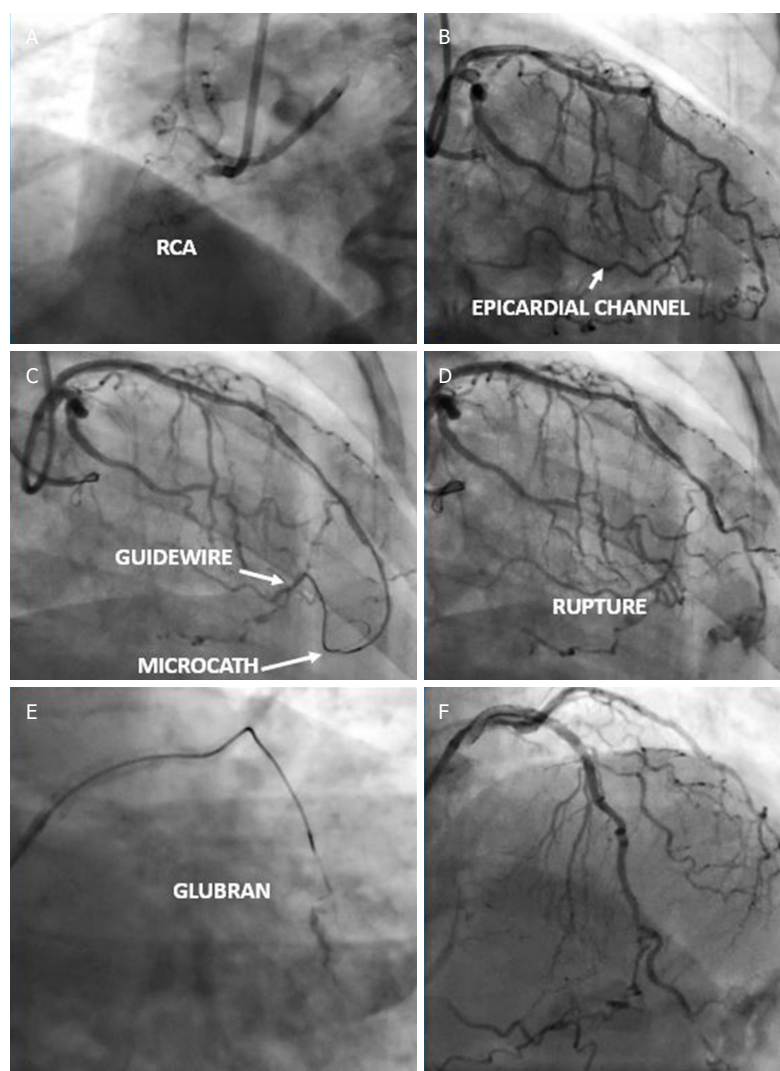


Figure 1. First case presented. A: lesion at RCA; occluded at proximal portion; B: epicardial channels from LAD to RCA; C: Guidewire into the epicardial channel; D: Evidence of extra-vascular bleeding from distal LAD; E: (NBCA-MS)-based glue injection; F: Final result

involved in the coronary rupture. Because the patient was stable and the vessel was diffusely calcified and narrowed, we decided to perform PTCA and stenting of the RCA proximally to the rupture in order to facilitate the CS progression. After 2 long DES implantation (3 mm × 28 mm and 3 mm × 40 mm), a low-profile CS (Aneugraft) it was not able to advance in the posterior descending artery (PDA) more than just at the origin of the vessel and we decided to deploy it there. Nevertheless the postero-lateral (PL) branch was still patent and at the angiography the bleeding extravasation was still present [Figure 2D]. We eventually decided to wire the small branch, advance a microcatheter and perform an embolization with (NBCA-MS)-based glue [Figure 2E]. After mixing Lipidol and (NBCA-MS)-based glue (3:1), the mixture was injected through a microcatheter Finecross (Terumo, Japan) using the “sandwich” technique for 3 overall “shots”. In the last angiographic control, the rupture appeared closed and the bleeding stopped [Figure 2F]. The RCA and posterior descending showed a final TIMI 3 flow. We concluded the procedure with a PCI and 2 drug eluting stent (DES) implantation on left main artery to LAD-LCX (LM-LAD-LCX) bifurcation with a double kissing (DK) crush technique and CTO-PCI of LAD and LCX was planned as a staged procedure.

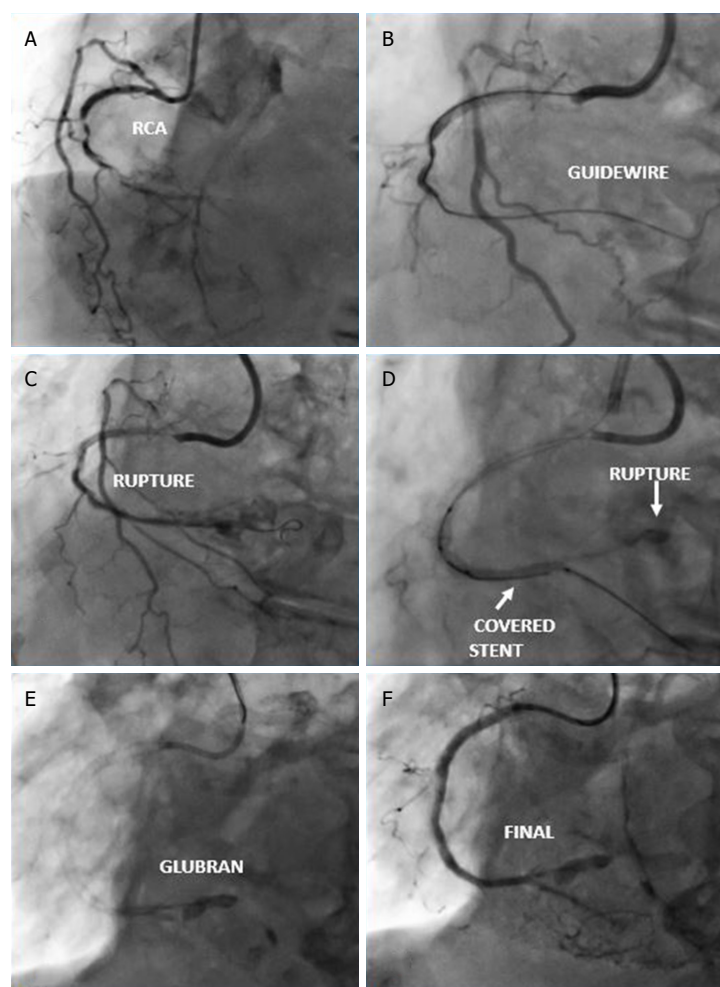


Figure 2. Second case presented. A: CTO of the RCA (Antegrade injection); B: Guidewire in the PL branch after crossing the CTO lesion; C: Evidence of extravasal bleeding at the level of the PL branch; D: persistence of extravasal bleeding at the distal edge of the covered stent; E: (NBCA-MS)-based glue injection; F: Final result. CTO: chronic total occlusion

DISCUSSION

Coil embolization for the treatment of small vessel Ellis type III perforations is considered the gold standard for emergent sealing. CTO operators always keep a set of different coils ready in the catheterization laboratory and should know the compatibility of the coils with different microcatheters used for CTO PCI^[32]. Ideally, coils should be delivered in every CTO microcatheter but this is not always true. Moreover, sealing perforations with coils can be time-consuming and significantly costly when multiple coils are needed. On the other hand, auxiliary embolization material such as subcutaneous fat tissue or clots or trombin, are troublesome and inconvenient to prepare and are not adequately precise and reliable to deliver. For these reasons we believe that (NBCA-MS)-based glue should be an effective and inexpensive tool to keep in every catheterization laboratory as an alternative to the embolization coils.

On the other hand, the use of (NBCA-MS)-based glue compared with coils requires some experience to be delivered in a precise and safe manner. The adverse events described after glue embolization are basically divided in three main categories: inadvertent vascular embolization, suboptimal agent polymerization time, and catheter retention^[33]. Both the suboptimal agent polymerization time and the catheter retention are related to the operator's inexperience. Correct proportion of the mixture (NBCA-MS)-based glue/ethiodized oil and use of small boluses or sandwich technique make the procedure safer and keep such complications infrequent.

On the other hand when the bleeding site is very close to major branches, coiling is always preferable because risk of inadvertent vascular embolization is not negligible, though coiling is definitely more onerous and time-consuming^[34,35].

Another point in favor of (NBCA-MS)-based glue is his ability to create a sort of wide patch of polymeric material around the rupture that can cover different size of coronary perforations. This could be of adjunctive help in epicardial perforations when coiling from one side of the collateral could be not enough to stop the bleeding, and coiling from the other side could be troublesome if the CTO is not recanalized. The mechanism of the sealing in this setting could be explained with formation of aggregates of chain growth polymers in the tissue around the spillage, along with the obstruction of the afferent vessel.

Interestingly no tissue adverse reactions was described after embolization despite the wide spectrum of medical use of (NBCA-MS)-based glue.

DECLARATIONS

Authors' contributions

All authors contributed to the manuscript.

Availability of data and materials

Not applicable.

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None.

Conflicts of interest

All authors declared that there are no conflicts of interests.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Case report

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Moderator band connections: an unusual route in retrograde chronic total occlusion procedure: moderator band connections as options to right coronary artery chronic total occlusion

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Abstract

We describe a right coronary artery (RCA) chronic total occlusion (CTO) percutaneous coronary intervention (PCI) procedure in a very high risk patient, in whom a complex PCI with the support of Impella CP device plus Rotablator rotational atherectomy was performed 6 months ago to revascularize a very calcified left anterior descending. This was an unusual approach because it was performed through a very rare connection by retrograde technique. It was performed through a third distal septal branch connecting with the moderator band artery. Reverse controlled antegrade and retrograde tracking technique was then successfully performed. After RCA CTO PCI, there was an improvement in the patient symptoms and quality of life. This case highlights the important role of this unusual and rare source of collateral circulation in RCA CTO.



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Keywords: Chronic total occlusion complex, percutaneous coronary intervention, right coronary artery, retrograde approach, moderator band

INTRODUCTION

The use of the retrograde approach^[1-9] has dramatically increased in the last years, not only through the septal collaterals and saphenous venous graft^[10], but also via epicardial connections^[11] to the chronic total occlusion (CTO) lesion.

We report the case of a right coronary artery (RCA) CTO recanalized using the retrograde approach through the contralateral channels of a third septal artery connecting with the moderator band artery [Figure 1] that was connected with the right ventricular branch of the RCA.

CASE REPORT

A 64 year-old male, with hypertension, dyslipidemia, left ventricle dysfunction with 35% ejection fraction, and known coronary artery disease. A previous percutaneous coronary intervention (PCI) was performed 6 months before revascularizing an extremely calcified left anterior descending artery with rotational atherectomy and Impella CP mechanical circulatory support. The patient remained symptomatic due to RCA CTO [Figure 2] with 24% ischemic burden involving the inferior wall in myocardial scintigraphy. Therefore, PCI of RCA CTO was indicated.

We started the procedure through double femoral access, trying to perform the antegrade approach. We tried to reach the intimal plaque and then the distal vessel, first using AL-1 guide catheter 7F in the RCA, Turnpike spiral microcatheter (Teleflex), Fielder FC guidewire (Asahi Intec.) and Conquest Pro 12 (Asahi Intec.), however without success. After 15 min, we decided to switch retrograde approach, using EBU 4.0 7F guide catheter in left coronary. We used a Turnpike spiral microcatheter (Teleflex) and a Whisper Light Support (Abbott) guidewire to access the third septal, where we could navigate into a connection through the moderator band artery into the right posterior atrioventricular branch. Then, we used the Turnpike Spiral microcatheter to replace the previous guidewire for a new Sion (Asahi Intec.) guidewire due to the fragility of this channel. After accessing the RCA [Figure 3], the reverse controlled antegrade and retrograde tracking technique [Figure 4] was performed and the retrograde guidewire was externalized [RG3 (Asahi Intec.)]. As we reached the RCA in the middle part of the vessel, we used a double lumen catheter TwinPass (Teleflex) to introduce a Fielder FC guidewire antegradely into the distal RCA, and then PCI with 3 DES Xience Alpine (Abbott) was performed with a good result [Figure 5].

DISCUSSION

The retrograde approach has dramatically increased as an alternative to recanalize complex CTO in case of antegrade failure. Data from the ERCTO registry showed that retrograde approach was only used in 15% of the CTO procedures^[12]. The septal collaterals are the safest and easiest to track to achieve retrograde access, but in our case we showed an unusual route of the septal channel that was connected probably with the moderator band artery^[13]. Sadek *et al.*^[14] described this forgotten access, showing that first, the moderator band is a muscle column that courses inferiorly from the right portion of the interventricular septum to the base of the anterior papillary muscle of the right ventricle. Second, the source of the moderator band artery lies in the first three septal arteries, most often in the second one; in our case we used the less common third one. Finally, this artery makes connections with various branches of the RCA, hence representing a real source of collateral circulation to RCA CTO as we practically demonstrated through this case.

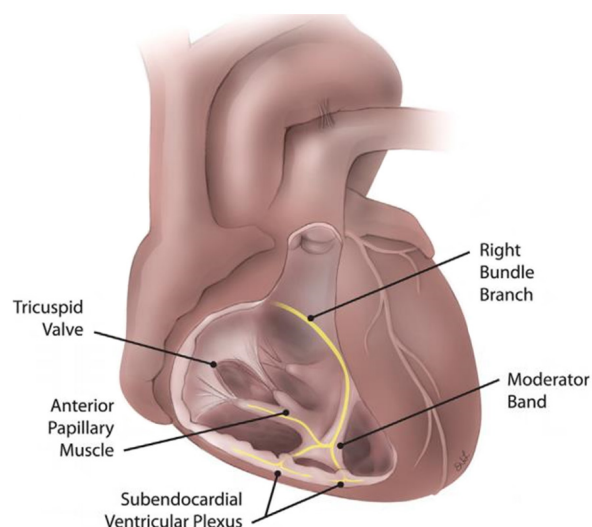


Figure 1. Anatomy of the moderator band artery, crossing from the septum to the free wall of the right ventricle and supporting the anterior papillary muscle of the tricuspid valve



Figure 2. Very calcified and long CTO segment in RCA. RCA: right coronary artery

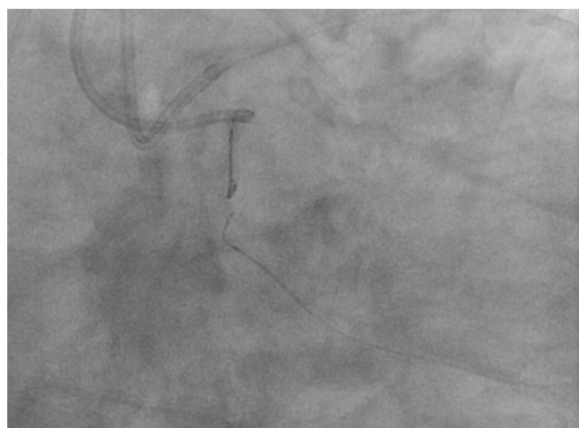


Figure 3. With a Whisper LS we crossed through the moderator band artery to a Right Ventricular Ramus reaching the RCA. RCA: right coronary artery

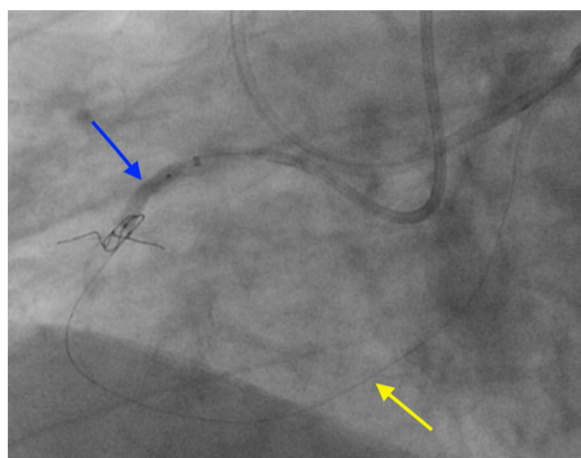


Figure 4. The yellow arrow shows the route through the moderator band and the blue arrow shows the reverse cart in the proximal RCA. RCA: right coronary artery



Figure 5. Pervious RCA with 3 Xience Alpine stents 3.0 mm × 38 mm. RCA: right coronary artery

To the best of our knowledge, this is the first case report of a retrograde approach using the moderator band artery as source of collaterals to open a RCA CTO.

In conclusion, the retrograde route described above could be useful for CTO RCA cases after antegrade failure and when standard approach of using the septal collaterals to connect with the posterior descending artery is not available.

DECLARATIONS

Authors' contributions

Contributed substantially in data interpretation and writing: Dallan LAP, Ribeiro MH, Boukhris M
Contributed substantially reviewing the manuscript for important intellectual content: da Silveira JAB, Campos CM, Galassi AR

Contributed substantially in the analysis and interpretation of data: Weilenmann D, da Silveira JAB
Contributed in data collection, interpretation and manuscript review for important intellectual content: Sumitsuji S

Availability of data and materials

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None.

Conflicts of interest

All authors declared that there are no conflicts of interests.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Review

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Del Nido cardioplegia: from an infant conceive to an adult life - a brief review of the current evidence in adult patients

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Abstract

The increasing number of minimally invasive procedures prompted the quest for a simple and effective single shot cardioplegia to allow the surgeons to focus on their workflow. The originally pediatric Del Nido solution was successfully tested in several centers and gradually extended to regular coronary and valvular cases. In the present review we report the current evidence on the use of the Del Nido solution in adult patients.

Keywords: Del Nido cardioplegia, adult cardioplegia, myocardial protection, blood cardioplegia, single shot cardioplegia

INTRODUCTION

Adult cardiac surgery has changed in the last decade. In the 2018 the STS^[1] database reports that about 75% of the patients submitted to myocardial revascularization had 3 or more grafts with an increasing number of non-elective procedures, diabetic and heart failure patients. At the same time 23% of the all the isolated



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mitral procedures performed in 2016 were minimally invasive and the isolated aortic valve procedures, the second most common cardiac operation, undergoing key-hole surgery have a steady increase. Clearly we are facing more complex procedures and worse clinical characteristics of our patients. This can imply longer cross clamp times which is a well-known risk factor in cardiac surgery. On the other hand the available cardioplegic solutions to protect the heart need to be repeated every 10 to 20 min or continuously infused in a retrograde fashion through the coronary sinus. Although the results with the current cardioplegias are consistently good, some surgeons, in particular those who have focused in minimally invasive procedures, are searching for a “*solution*” which could combine effective and consistent long lasting myocardial protection with easy of deliver.

In the last few years, when its original the patent expired, the paediatric del Nido cardioplegia (DNC) has been increasingly used in adult patients^[2]. This cardioplegia allows for an interval between infusions up to 90 min and has some unique features that appear to be promising to the adult cardiac surgeons.

The DNC is a 1:4 blood cardioplegia which can be classified as a modified depolarizing cardioplegia, containing Lidocaine and Magnesium. Clinically it has been validated in valve surgery^[3] and at the moment, in low risk coronary patients^[4]. We hereby are summarizing the basic concepts behind its formulation and use, along with the available evidence in the adult patients.

DEVELOPMENT OF DNC

For long time paediatric cardiac surgeons had to rely on the common adult cardioplegic solutions to operate on their patients. However, the crystalloid solutions in use in the 80's and early 90's had controversial results in young populations with, for instance, the St Thomas solution being reported either effective^[5] or ineffective^[6]. Although infant and paediatric hearts have some distinctive histologic and metabolic features, a “dedicated” cardioplegia was missing. Histologically the paediatric heart has a poorly developed sarcoplasmatic reticulum^[7], fewer mitochondria, a higher concentration of poly unsaturated fatty acids^[8] in the cell membrane and a deficient free radical scavenge system with less active superoxide dismutase, catalase and glutathione reductase^[9]. In addition these hearts depends more on the extracellular calcium for contraction. At Pittsburgh University Hospital the team led by Pedro J. del Nido focused on many of these aspects and developed a solution preventing the intracellular accumulation of Calcium, providing effective free radicals scavenge whit maintenance of the anaerobic glycolysis and assuring effective buffering during prolonged periods of cardiac arrest. A detailed description of the development of the cardioplegia is available in the literature^[10].

COMPONENTS

The DNC is a 1:4 Blood to Crystalloid solution with additional components to achieve depolarized arrest and mitigate the effects of temporary myocardial ischemia [Table 1].

Plasmalyte a solution

The Plasma Lyte A (Baxter Health Care Corp. Deerfield, IL USA) solution forms the crystalloid base of the DNC. It is an extracellular (Na^+ 140 mEq, K^+ 5 mEq/L) solution with a final pH of 7.4 and an osmolality of 294 mOsm/L. It is commonly used as a fluid volume replacement infusion in many clinical conditions. Noticeably it does not contain glucose.

Potassium

Similarly to other common depolarizing solutions, the final content of K^+ ions in the DNC is about 24 mEq/L which is obtained from the basal content of Plasma-Lyte (5 mEq) plus the added 26 mEq and an assumed 4.5 mEq/L from the patient's blood.

Table 1. Composition of the del Nido cardioplegia

Del Nido formulation	Plasma-Lyte a solution 1000 mL
Plasma Lyte A Solution 1 L	Sodium 140 mEq
Mannitol 20 % (16.3 mL)	Potassium 5 mEq
Magnesium Sulfate 50% (4 mL)	Magnesium 3 mEq
Sodium Bicarbonate 8.4% (13 mL)	Chloride 98 mEq
Potassium Chloride 2 mEq/L (13 mL)	Gluconate 23 mEq
Lidocaine 1% (13 mL)	Acetate 27 mEq
Blood : Crystalloid 1:4	

As known Potassium increases the resting potential of myocytes to about -46 mV, well above the depolarization threshold of - 65 mV. In doing so it leaves the cells in a state of arrest. Hence, indirectly potassium blocks the inward current of Na^+ during the phase 0 of the myocardial action potential

Lidocaine

Lidocaine is a class I antiarrhythmic drug that directly blocks the Na^+ channels in phase 0. Its half-life is relatively long and is obviously increased by the absence of coronary circulation. It also blocks the so called “window” channels which remain open during the depolarized arrest and allow some Na^+ and Ca^{2+} inward current in the cell. Lidocaine therefore allows for prolonged periods of cardiac arrest and participates in the control of intracellular accumulation of calcium during the ischemic period.

Magnesium

Magnesium is a natural Calcium channels blocker. Contrary to the skeletal muscle, the cardiac myocyte is largely dependent from extracellular calcium for its contraction. Calcium ions enter the cardiac myocyte during phase 2 plateau of the action potential through L-Type channels which are blocked by Magnesium ions. In doing so Magnesium prevents the contraction of the myocytes and accumulation of Calcium in the cell. Interestingly both paediatric and “aged” cardiomyocytes have an altered homeostasis of Calcium which can be modulated by Magnesium

Mannitol

Mannitol is a common additive to cardioplegia solutions. Its usage prevents cellular oedema and scavenges free radicals. The cell membrane of immature myocardium has high concentration of poly unsaturated fatty acids providing more sites for oxidative damage, on the other hand oxidative stress is believed to be potent promoter of myocardial aging.

1:4 Blood ratio

The addition of blood to crystalloid cardioplegia is far beyond the simple concept of substrates and oxygen deliver to the arrested heart. As the haemoglobin dissociation curves are altered during hypothermia, the oxygen deliver is minimal and dependent from the gas dissolved in the solution. However, blood proteins and the other components have several potential benefits which include buffering from proteins and carbonic anhydrase contained in red cells, free radicals scavenge and more favourable rheological properties. In addition, as a result of the lower haematocrit compared to the classic solution with a 4:1 ratio, the DNC has a very low Calcium content which enhances the effects of Lidocaine and Magnesium.

DELIVER AND TECHNICAL ASPECTS

With the widespread use of blood cardioplegias the perfusionist can easily arrange a circuit to deliver the DNC basic crystalloids components in a 4:1 ration with the patient's derived oxygenated blood (> 150 mmH pO_2). Sample circuits drawings are available in the literature from the original Boston Children Hospital and the Cleaveland Clinic^[11]. Table 1 depicts the current setting in use in our Centre. The DNC is usually

Table 2. Literature summary

Reference	Population (n)	Study design	Significant results in DNC group	No differences
Yerebakan <i>et al.</i> ^[24]	Acute MI CABG DNC = 48 WCBC = 40	Retrospective	↓ CPB ↓ X- Clamp	Enzyme release EF% Postoperative support Mortality
Sorabella <i>et al.</i> ^[18]	Reoperative AVR DNC = 52 Blood = 61	DNC vs. blood Retrospective	↓Cardioplegia volume	CPB, X-clamp time Complication rate
Mick <i>et al.</i> ^[3]	Isolated Valve Aortic = 85/85 Mitral = 110/110	Retrospective 1:1 Propensity score Matched	Aortic ↓ CPB, ↓ XC lamp, ↓Glucose ↓ Insuline Mitral ↓ Insuline ↓ Glucose	Enzyme Release EF% Clinical results
Ota <i>et al.</i> ^[17]	AVR (240) DNC = 178 Blood = 62	DNC vs. blood Retrospective Propensity matched 54 pairs	↓CPB, ↓ X-clamp ↓Use of retrograde	Inotropic support
Mishra <i>et al.</i> ^[31]	CABG or double valve DNC = 50 Blood = 50	DNC vs. blood Retrospective	↓CPB, ↓ X-clamp ↓Redosing ↓Ejection fraction	Complication rates
Timek <i>et al.</i> ^[23]	CABG DNC = 82 CB = 82	DNC vs. CB Retrospective Propensity score matched pairs	↓ Glucose	Cross Clamp Inotropes Enzyme Release EF%
Guajardo <i>et al.</i> ^[4]	CABG (408) DNC = 159 Blood = 249	DNC vs. blood Retrospective	↓ Need defibrillation ↓Transfusion ($P < 0.08$)	CPB, X-clamp time Length of stay Mortality
Vistarini <i>et al.</i> ^[27]	Min. invasive AVR DNC = 25 Blood = 21	DNC vs. blood Retrospective	↓ Need defibrillation ↓CK-MB ↓Insulin use	Complication rate Mortality
Kim <i>et al.</i> ^[21]	Valve DNC = 149 Blood = 892	DNC vs. blood Retrospective Propensity matched 111 pairs	↓CPB, X-clamp ↓Troponin ↓Transfusion	Inotropic support Mortality Complication rates
Hamad <i>et al.</i> ^[28]	AVR/CABG DNC = 25 Blood = 25	DNC vs. blood Retrospective	↓CK-MB, troponin T ↓CPB, X-clamp	Inotropic support Operative time Length of stay Complication rates
Ziazadeh <i>et al.</i> ^[29]	Min invasive AVR DNC = 77 Blood = 101	DNC vs. blood Retrospective Propensity matched 63 pairs	↓CPB, X-clamp ↓Glucose levels	Troponin T Ejection fraction Complication rates
Koeckert <i>et al.</i> ^[30]	Min. invasive AVR DNC = 59 Blood = 122	DNC vs. blood Retrospective Propensity matched 59 pairs	↓Redosing ↓Cardioplegia volume ↓Use of retrograde	CPB, X-clamp time Inotropic support Transfusion Length of stay Complication rates
Ad <i>et al.</i> ^[26]	CABG ± valve DNC = 48 Blood = 41	Randomized, controlled	↓ A.Fib Postop (*) ↓ Troponine (*)	CPB, X-clamp time Complication rates Inotropic support Need defibrillation
UCAK <i>et al.</i> ^[25]	CABG elective DNC = 112 IWBC = 185	DNC vs. IWBC Randomized Controlled	↓ CPB ↓ X-clamp ↓ Glucose	Enzyme release Clinical events
O'Donnel <i>et al.</i> ^[20]	CABG DNC=54 BC = 27	DNC vs. BC Retrospective	↓ CPB ↓ X-clamp ↓ Defibrillations	No difference in clinical outcomes
Pragliola <i>et al.</i> ^[33]	All kinds of adult surgery including emergencies DNC = 102 IWBC = 102	DNC vs. IWBC Retrospective Propensity score matched pairs	↓Ejection fraction in low EF subgroup	No differences overall

AVR: aortic valve replacement; CABG: coronary artery bypass graft; CPB: cardiopulmonary bypass time; DNC: del Nido cardioplegia; X-clamp: cross-clamp time; IWBC Intermittent warm blood cardioplegia CC Cold Cardioplegia BC Blood Cardioplegia. *(see text for details) Note that alpha value for statistical significance was $P < 0.001$, thus nonsignificant trends exist.

delivered in the aortic root at a dose of 20 mL/kg with a maximum dose of 1000 mL. Rate of infusion is usually between 150 mL/min to 300 mL/min for a pressure of 100 mmHg in 2 to 4 minutes. The cardioplegia's circuits include a heat exchanger to deliver the solution at 4 °C for a final myocardial temperature of less of 15 °C. As known the myocardium Oxygen consumption decreases of 50% for any 10 °C reduction of

temperature, at 10 °C the oxygen requirements should be in the 15% to 20% range of the baseline. Hence ice slush for local temperature control is added by the surgeon in the pericardium at the aortic cross clamp time. However, continuous myocardial temperature is not routinely used.

The cardioplegia can also be infused directly into the coronary arteries in case of severe Aortic regurgitation, as can be infused retrogradely at the same doses used in the aortic root although this is not common practice according to the literature. Unlikely other blood cardioplegias, we strongly advise not to use a continuous infusion. This can result either in an excess of volume or Lidocaine and magnesium. Najjar *et al.*^[12] in a series of 14 patients undergoing re-operative surgery and continuous infusion reported a mean total volume of 4367 mL \pm 751 mL for an aortic cross clamp time of 81 min \pm 35 min. With retrograde continuous infusion in patients submitted to aortic valve reimplantation, Jiang *et al.*^[13] reported a 26% incidence of postoperative heart block resulting in 6.7% incidence of permanent heart block. Due to the limited number of patients and the inherent surgery they were submitted to, it is not possible to reach a definite conclusion, but caution is advised.

EXPERIMENTAL STUDIES

The conflicting evidences on the premature myocardium metabolism which were evident at the time the DNC was developed at Boston Children Hospital have been stressed by Matte in his report. In brief the Del Nido was conceived as a hyperpolarizing (K^+), extracellular (Na^+) glucose free (Plasmalyte), hyperosmolar (Mannitol), buffered (Bicarbonate, blood proteins) solution controlling the calcium influx into the cells (Magnesium and Lidocaine). The presence of lidocaine in an unperfused coronary bed (slowly wiped off by the collateral coronary flow) allows for long intervals between the infusion of the solution. This is as important as the maintenance of a low myocardial temperature and the use of the cold cardioplegic solution when manually testing the anastomosis during CABG surgery. These details are collateral, but not less important parts of the technique in adults^[14].

However, there are at least two experimental studies supporting the use of the DNC in aged hearts. During cardioplegic arrest induced by DNC in an isolated cells model from senescent rats, the intracellular Calcium content was lower and the cells were not reactive to electric field stimulation as well as they did not develop hypercontraction at reperfusion contrary to the same model treated with conventional cardioplegias. The Authors concluded that according to these results, the DNC had the potential to better protect senescent hearts preventing electromechanical activity during the arrest and hypercontraction at the time of reperfusion^[15]. Similarly, in an isolated working model of senescent hearts, the treatment group that underwent 60 min of cardiac arrest induced by DNC had better contractility and lower enzyme release compared to the group treated with conventional cardioplegia^[16].

EXPERIENCES IN ADULT PATIENTS

Interestingly, although it is now clear that major cardiothoracic units are regularly using the DNC solution, available studies deal only with limited subpopulations.

Matte *et al.*^[10], describing the development of the DNC reports the regular use in Adult Congenital cases at Boston Children Hospital. Ota *et al.*^[17] and Sorabella *et al.*^[18] published their experiences with first time and re-operative Aortic Valve surgery, all with safe and comparable results. Mongero^[19] state that the DN cardioplegia is the only solution in use in their Centre, the Columbia University Presbyterian Hospital NY, since 2011 and call for a broader use of it in adults. O'Donnell *et al.*^[20] reports that the DNC is the cardioplegia of choice in CABG since 2015.

In many institutions, including ours, the del Nido was initially used in minimally invasive Mitral cases and then gradually extended to cover all procedures. This path, though not openly stated, was probably started at the Columbia University and followed in Cleveland^[21] where the Del Nido was propensity matched with good results to the Buckberg solution in minimally invasive or robotic valvular cases and showed better glucose control, reduced cross-clamp and operative times.

As the field of minimally invasive and robotic surgery is rapidly expanding, teams dealing with these techniques are looking for a simple and effective cardioplegia. Amongst the available alternative solutions, the Buckberg^[19] entails a staged deliver in different phases and shorter intervals of ischemia (15 m to 20 m) which can slow down the surgical workflow. Besides, the retrograde infusion in minimally invasive surgery is a sophisticated and sometime difficult technique to control in a limited surgical field. The Custodiol[®]^[22] solution for long time has been the only single shot cardioplegia. Initially introduced for the donor hearts that usually are exposed to long ischemic times during organ procurements, it achieves a long-lasting myocardial protection. However, this solution requires the infusion of a large volume of hyponatremic crystalloid which is usually drained during the donor heart harvest but can be problematic in patients operated with a minimally invasive approach. Although ultrafiltration can help solve this problem, the volume overload and the hyponatremia can complicate the postoperative period.

There are also several studies testing the DNC in coronary revascularization. Timek *et al.*^[23] reported on a group of CABG patients receiving the DNC, propensity score matched to a population operated with usual Cold Blood Cardioplegia infused at 15-20 min intervals. Not surprisingly the DNC resulted in a lower volume infused and a lower peak glucose level during cardiopulmonary bypass compared to the matched population. No clinical differences were noted in the outcomes.

The DNC has also been tested in high risk coronary cases with Acute Myocardial infarction by Yerebakan *et al.*^[24] in 2014 with excellent clinical results. Two recently published Randomized Controlled Trials (RCT) compared the use of the DNC to the intermittent whole blood cardioplegia in CABG or CABG plus valve surgery. Ucak *et al.*^[25] could observe shorter aortic cross-clamp and CPBP times and better glucose control in the DNC group, without meaningful clinical differences in a population with an average Euroscore of 4.1. Similarly, Ad *et al.*^[26] conducted the single registered RCT (NCT02442050) for the DNC in adults. The randomized patients had an average STS score of 1.3. Initially designed as a non-inferiority study to include 500 patients, it was prematurely interrupted because the DNC patients had a better rhythm recovery after surgery. The study was then turned into a superiority study with a required level of evidence of $P < 0.001$. With these new parameters there were no clinical differences in the outcome although the peak T troponin level was lesser in the DNC group at $P < 0.04$ without sufficient power to achieve statistical differences. Table 1 summarizes the available studies. Many of them were also included in an extensive metanalysis which favoured the del Nido in reducing the volume of cardioplegia infused, shortening the cardio-pulmonary bypass and cross clamp times and had comparable results in terms of troponine and CKmb release. All the studies had comparable clinical results^[27,32].

In the available literature, the common criticisms to the use of the DNC in adults are two: the limited number of patients included in the studies, usually with a single pathology and the low risk of these groups of patients.

In his elegant statistical study, Kim *et al.*^[21] matched two similar groups of 104 patients treated with the DNC or Blood Cardioplegia out of 1041 consecutive patients. Again the DNC showed an advantage in the postoperative peak troponin release and shorter cross-clamp times. Noticeably all kind of procedures were done in these groups, including multiple complex valves and aortic arch surgery. However, the logistic Euroscore II for the DNC group was 2.9 ± 3.3 .

Comments and Conclusion

Understandably Lazar^[14] put forward a few questions about the use of the DNC in high-risk cases with low EF%, high Euroscore or high Pulmonary Artery Pressures; adult patients in whom the solution has not been extensively tested.

We do not have a definite answer to these questions. In our Centre the DNC quickly took over the IWBC to be the standard solution in use^[33]. Over the last 1000 consecutive cases in 2017-2018 we could propensity score match 102 pairs including two groups with a high Euroscore II (mean ESII 10) and one with low EF (EF 30%) in whom the DNC provided sufficient protection without major differences with the IWBC and allowed the surgeon to focus on the his surgical workflow (data in press). There are not clear guidelines on cardioplegic solutions and the debate whether it should be warm or cold, blood or asanguineous, antegrade or retrograde, intermittent, single shot or continuous flooded the surgical literature. Clearly the perfect myocardial protection is the result of a complex interaction of the surgical team with the procedure performed, the patient characteristics, the cardioplegic solution and the technique of delivery. This is coming from practice, consistence and excellence as certainly occurred in all the centres where the DNC was adopted routinely in adult cases.

A large randomized superiority trial enrolling only high risk cases will be difficult to complete and poses several potential problems : some ethical and some very practical. To date, as a result, there are not similar trials registered in the Clinical Trials website.

In conclusion, there is sufficient evidence to engage with the single shot DNC in all the routine cases either valvular or coronary, especially in minimally invasive procedures. Whether this will expand into the moderate and high risk cases will depend from the surgical team preferences.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study: Pragliola C, Hassan E

Performed data analysis and interpretation: Pragliola C, Hassan E, Al Gharni KD

Performed data acquisition, as well as provided administrative, technical, and material support: Alfonso JJT, Al Hossan A, Al Otaibi K, Al Khalaf A.

Availability of data and materials

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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.

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Review

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Total arterial coronary grafting: outcomes, concerns and controversies

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Abstract

Choice of conduit remains the Achilles heel of coronary artery bypass grafting. Conduit choice is crucial as it is deemed to influence the long-term outcomes. While the important survival advantage of a left internal mammary artery graft over vein grafts is universally accepted, controversy reigns supreme regarding the next best conduit. There is plenty of evidence to suggest that arterial grafts are not only superior in terms of patency and survival, but they also protect the native coronary arteries against further progression of atherosclerotic disease. Total arterial coronary grafting, utilizing various configurations of bilateral internal mammary arteries, radial artery and occasionally right gastroepiploic artery is a safe and reproducible strategy. However, concerns about additional operative time, enhanced technical complexity, graft spasm with hypoperfusion, competitive flow, increased risk of bleeding, deep sternal wound infection, and most importantly lack of randomized trial data have prevented the universal adoption of total arterial coronary grafting. This review evaluates the current outcomes of total arterial coronary grafting and summarizes the concerns and controversies associated with this strategy.

Keywords: Coronary artery bypass grafting, bilateral internal mammary artery grafting, multiple arterial grafting, total arterial grafting, total arterial revascularization

INTRODUCTION

Coronary artery bypass grafting (CABG) remains, more than five decades after its introduction into clinical practice, the most scrutinized surgical procedure and a therapeutic intervention of paramount



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importance for patients with coronary artery disease^[1]. The choice of the graft conduit for CABG has significant impact both on the short- and long-term outcomes. The patency of a coronary conduit is fundamentally related with a smooth postoperative course, improved long-term patient survival and enhanced freedom from re-intervention^[2]. Long saphenous vein has been the most commonly used conduit in CABG. However, progressive saphenous vein graft (SVG) failure remains a major impediment to the long-term success of CABG^[3]. Total arterial coronary grafting also known as total arterial revascularization (TAR) is a logical solution to deal with late vein graft atherosclerosis, and occlusion.

RATIONALE

Arterial coronary grafts are relatively resistant to atheromatous changes and have better patency rates, resulting in less recurrent angina, fewer myocardial infarctions and reoperations and better survival than with SVGs^[4]. Hence it is logical to use arterial grafts instead of SVGs. Multiple large studies have documented better long-term outcomes for CABG with two internal mammary arteries (IMAs) over one^[5-7]. Arterial grafts (unlike SVGs) also synthesize and release nitric oxide and other favorable vasoactive agents that protect the coronary artery downstream from development of further atheromatous changes^[8].

CURRENT UTILIZATION RATES

Utilization rates of TAR are variable. It is estimated that about 20% CABG procedures in Europe utilize TAR while utilization rates are up to 80% at some centers in Australia. On the other hand, in North America almost 5% of patients undergoing CABG receive TAR^[9-11]. This large variation in practice can be partially attributed to the paucity of evidence from adequately powered randomized controlled trials (RCTs) with long-term follow-up. Furthermore, increasingly complex patient profiles and enhanced scrutiny facing the cardiac surgeons in an era of public reporting of surgeon-specific mortality data may also impact adoption rates of TAR.

CONFIGURATIONS

The deployment of arterial grafts and their configuration is generally dictated by the availability of conduits, the degree of stenosis in the native coronary arteries and the technical expertise of the surgeon. There are numerous potential configurations that can be achieved during TAR highlighting the fact that that there is no single operation that is suitable for every patient - it is not a case of “one size fits all” as would be the scenario for the use of a single internal mammary artery and supplemental vein grafts^[12].

Bilateral internal mammary arteries

Several configurations have been used to accomplish TAR of left-sided coronary system with bilateral internal mammary arteries (BIMA) only^[13]. These include *in situ* right internal mammary artery (RIMA) to the left anterior descending (LAD) artery and the left internal mammary artery (LIMA) to circumflex marginal branches^[14] [Figure 1], routing the RIMA through the transverse sinus in a retroaortic course^[15] [Figure 2], and free RIMA grafts anastomosed proximally either to the LIMA^[16] [Figure 3] or to the ascending aorta^[17]. Table 1 summarizes the pros and cons of these configurations.

Radial artery

The radial artery can be combined with BIMA to achieve TAR. The radial artery from the aorta to the posterior descending artery (PDA) is an attractive approach in the presence of 80% or more stenosis in the right coronary artery (RCA) or ideally if the RCA is completely blocked thereby reducing competitive flow [Figure 4]. An alternative strategy, especially if a no touch aortic technique is indicated, is to use the main body of the RIMA to construct a composite left-sided graft while anastomosing the radial artery to the proximal *in situ* RIMA^[12]. The RIMA will frequently fail to reach the PDA even after full

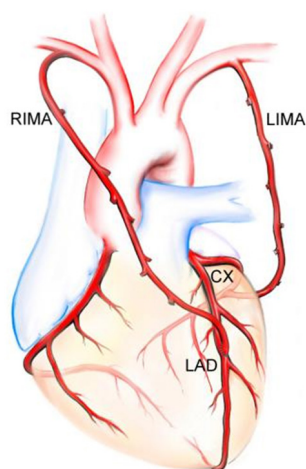


Figure 1. *In situ* right internal mammary artery (RIMA) to the left anterior descending (LAD) artery and the left internal mammary artery (LIMA) to circumflex (Cx) marginal branches. (Figure courtesy Marcie Bunalade)

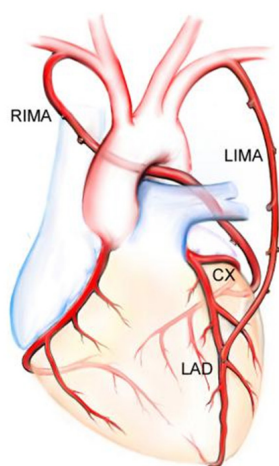


Figure 2. *In situ* left internal mammary artery (LIMA) to the left anterior descending (LAD) artery and the *in situ* right internal mammary artery (RIMA) through the transverse sinus in a retroaortic course to the circumflex (Cx) marginal branches. (Figure courtesy Marcie Bunalade)

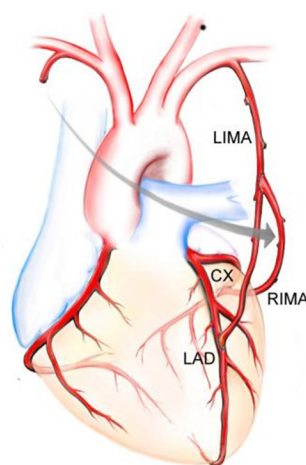


Figure 3. Composite Y graft with free right internal mammary artery (RIMA) connected proximally to the left internal mammary artery (LIMA) with LIMA anastomosed to the left anterior descending (LAD) artery and RIMA anastomosed to the circumflex (Cx) marginal branches. (Figure courtesy Marcie Bunalade)

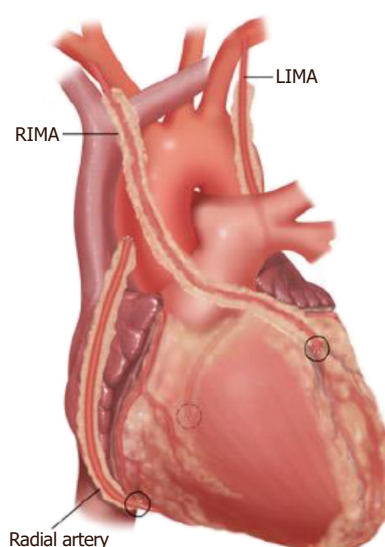


Figure 4. Radial artery from the aorta to the posterior descending artery with *in situ* right internal mammary artery (RIMA) anastomosed to the left anterior descending artery and *in situ* left internal mammary artery (LIMA) anastomosed to the circumflex marginal branches. (Figure courtesy Marcie Bunalade)

Table 1. Configurations of bilateral internal mammary arteries

Configuration	Pros	Cons
Retroaortic <i>in situ</i> RIMA via transverse sinus to circumflex marginal branches with <i>in situ</i> LIMA to LAD	<p>The LAD is revascularized by the <i>in situ</i> LIMA, which is well accepted as a gold standard technique</p> <p>The left coronary system is perfused by 2 <i>in situ</i> IMAs</p> <p>It avoids the difficulties of anastomosing a thin-walled vessel, such as the free RIMA, to a thick-walled vessel, such as the aorta</p> <p>There are no grafts crossing the midline behind the sternum, and both IMAs are in a safe position, which decreases the risks in case of mediastinal revision or reoperation</p> <p>It offers the possibility to easily apply the no-touch principle by using different composite graft configurations.</p>	<p>The inability to control bleeding from retroaortic RIMA branches</p> <p>Aortic compression of the <i>in situ</i> RIMA, and compromised graft patency because of undetected kinks, graft overstretching, rotation, and spasm of distal RIMA</p>
Retrosternal crossover <i>in situ</i> RIMA to LAD with <i>in situ</i> LIMA to circumflex marginal branches	<p>This strategy is easily reproducible and technically less demanding</p> <p>The LAD is grafted by an intact <i>in situ</i> IMA, complete left-sided IMA grafting is readily achieved, and the principle of multiple-origin blood supply is maintained</p> <p>The additional length obtained by harvesting the IMA as a skeletonised vessel enables better selection of the LAD anastomotic site and precludes the use of the more distal vasospastic RIMA segments</p>	<p>The potential risk of damage to the artery during repeat sternotomy</p>
Composite LIMA-RIMA T or Y grafting	<p>The composite anastomosis is ideally matched and avoids the problems of proximal anastomoses to the aorta</p> <p>The aortic “no touch” technique reduces the risk of stroke and is particularly useful in off pump surgery</p> <p>A greater length of RIMA is available for more extensive myocardial revascularization, perhaps avoiding the use of a third conduit</p>	<p>Single source blood supply with steal phenomenon, competitive flow, and hypoperfusion syndrome as potential disadvantages</p>
Right internal mammary artery for grafting the right coronary system	<p>The aortic “no touch” technique reduces the risk of stroke and is particularly useful in off pump surgery</p>	<p>Gross mismatch between RCA and RIMA sizes</p> <p>Usage of the distal part of the pedicled RIMA to graft PDA increases the risk of vasospasm</p>

LIMA: left internal mammary artery; PDA: posterior descending artery; RCA: right coronary artery; RIMA: right internal mammary artery

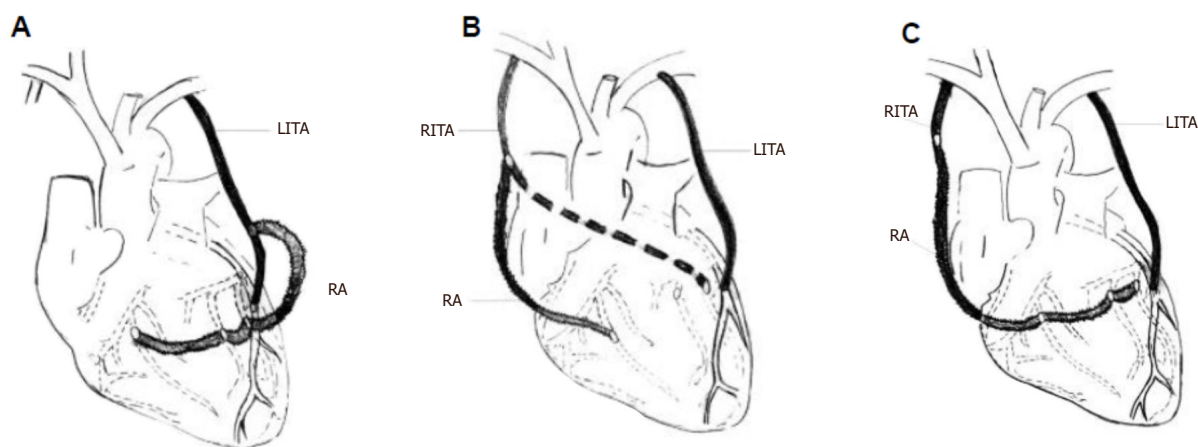


Figure 5. Composite configurations of radial artery. A: Radial artery (RA) Y or T graft from the in situ left internal mammary artery (LITA) anastomosed to the circumflex marginal branches and distal branches of right coronary artery; B: RA Y or T graft from the in situ right internal thoracic artery (RITA) anastomosed to the distal branches of right coronary artery; C: Extension of RITA with RA. (Figure courtesy Marcie Bunalade)

skeletonization and can be lengthened with recycled LIMA or radial artery [Figure 5]. The RIMA should not be anastomosed to the main RCA because of the possibility of competitive flow due to size disparity and ultimately evolution of progressive disease at the crux^[12].

Right gastroepiploic artery

The right gastroepiploic artery can be combined with *in situ* RIMA to the LAD and LIMA to the obtuse marginal. Right gastroepiploic artery is particularly useful for grafting an occluded dominant ungrafted RCA or one with a failed graft in the presence of patent grafts to the left side. Grafting of the PDA can be achieved off-pump through a reasonably small incision via the lower sternum.

OUTCOMES

Perioperative outcomes

The perioperative outcomes of TAR are similar to those of conventional CABG. Majority of the studies report 1% mortality and a 1%-3% rate for stroke, intra-aortic balloon pump use and myocardial infarction^[18-21]. There is increasing acceptance that TAR should be offered only to younger patients (usually now perceived as less than 70 years old), predominantly with preserved ventricular function and absence of significant co-morbidity, as they are more likely to benefit from the superior long-term patency of the arterial grafts^[12]. However, TAR in combination with off-pump CABG can also be offered to the elderly to allow a true “no touch aortic technique” where there is robust evidence for a reduction in the risk of most major complications and, in particular, stroke^[22]. There is evidence from RCTs that TAR with composite grafts is a safe and useful procedure in the elderly^[23-25].

Long-term outcomes

The added value of TAR in CABG becomes particularly apparent when assessing long-term results. Tavilla *et al.*^[26] recently reported 20-year outcomes of TAR using BIMA and gastroepiploic artery as *in situ* grafts in patients with 3-vessel disease. The Kaplan-Meier estimated survival probabilities were 73.9% (95%CI: 67.2%-79.5%) and 63.5% (95%CI: 55.7%-70.4%) for overall survival and 57.9% (95%CI: 50.7%-64.5%) and 47.9% (95%CI: 40.1%-55.3%) for freedom from major adverse cardiac events at 15 and 20 years respectively. The respective estimated cumulative incidences at 15 and 20 years were 7.0% (95%CI: 3.5%-10.6%) and 7.8% (95%CI: 4.0%-11.6%) for myocardial infarction, 8.6% (95%CI: 4.7%-12.5%) and 9.3% (95%CI: 5.2%-13.3%) for percutaneous reintervention, 7.0% (95%CI: 3.5%-10.5%) and 7.0% (95%CI: 3.5%-10.5%) for

reoperation, 8.6% (95% CI, 4.7%-12.6%) and 12.9% (95% CI, 7.6%-18.2%) for cardiac death, and 10.8% (95% CI, 6.5%-15.2%) and 15.2% (95% CI, 9.8%-20.6%) for death from other causes.

Tatoulis and colleagues^[27] in a multicentre analysis compared outcomes in patients who underwent TAR ($n = 12,271$) with outcomes in those who did not ($n = 21,910$). They determined the impact of TAR on 10-year all-cause late mortality by propensity score analyses in 6,232 matched pairs. The 30-day mortality was 0.8% (96/12,271) for TAR patients and 1.8% (398/21,910) for non-TAR patients ($P < 0.001$). Late mortality was 7.5% (918/12,271) for TAR patients and 8.9% (1,952/21,910) for non-TAR patients ($P < 0.001$). The mean follow-up time was 4.9 years. In the propensity-matched cohort, the perioperative mortality was 0.9% (53/6,232) for TAR patients versus 1.2% (76/6,232) for non-TAR patients ($P < 0.001$). Kaplan-Meier survival in the matched cohort at 1, 5, and 10 years was 97.2%, 91.3%, and 85.4% for TAR patients and 96.5%, 90.1%, and 81.2% for non-TAR patients ($P < 0.001$). Late mortality was 8.0% ($n = 500$) for TAR patients and 10.0% ($n = 622$) for non-TAR patients ($P < 0.001$). Stratified Cox proportional hazards models showed lower risk for all-cause late mortality in the TAR group (TAR:HR 0.80, 95% confidence interval 0.71 to 0.90, $P < 0.001$).

A systematic review and meta-analysis of 130,305 patients from 4 smaller shorter follow-up RCTs, plus 15 matched/adjusted and 6 unmatched/unadjusted larger longer follow-up observational studies suggested that TAR may improve long-term survival compared with conventional CABG by 15%-20% even when compared with two arterial grafts^[28].

CONCERNS

Single blood source

The composite grafting technique has the disadvantage of complete reliance of the coronary bypass flow on the flow of the proximal IMA. Multiple clinical and experimental studies have assessed the adequacy of the IMA as the sole blood source in composite arterial grafting^[29,30]. Sakaguchi and colleagues^[30], utilizing positron emission tomography, demonstrated that the composite Y graft was not as efficient as independent grafts for increasing the coronary flow reserve soon after bypass grafting. However, most investigations have reported that the flow reserve of the proximal IMA is adequate as a blood source of composite grafts in TAR. Affleck *et al.*^[31], in an effort to determine the constraint posed by a single source inflow recorded intraoperative flow in each limb of the T graft before and after distal anastomoses in 204 patients. They also compared flow capacity with completion coronary flow. Free flow for the radial arterial limb was reported as 161 ± 81 mL/min, the IMA limb as 137 ± 57 mL/min (combined 298 ± 101 mL/min) compared with simultaneous limb flow of 226 ± 84 mL/min resulting in a flow restriction of $24\% \pm 14\%$. Completion coronary flow was 88 ± 49 mL/min for the radial artery, 60 ± 45 mL/min for the IMA, and 140 ± 70 mL/min for both limbs simultaneously to give a flow reserve (vs. simultaneous free flow) of 160% or 1.6. This flow reserve of 1.6 compares favorably with an IMA flow reserve of 1.8 at 1-month postoperatively and 1.8 for both the IMA T graft and the IMA/radial artery T graft at 1-week postoperatively as reported by Wendler and associates^[32].

Graft spasm and hypoperfusion

Hypoperfusion syndrome, associated with a high mortality, is a recognized sequela of vasospasm of arterial grafts. Spasm of the proximal IMA in case of composite grafting may result in hypoperfusion of the whole left coronary system and may lead to calamitous consequences^[30]. Similarly, the radial artery and gastroepiploic artery with an enhanced spasmodic tendency, owing to preponderance of smooth muscle, predispose to a risk of hypoperfusion due to spasm of these vessels if used to construct a composite graft^[33].

In practice however, 1% to 2% of the patients undergoing composite arterial grafting experience perioperative hypoperfusion resulting in myocardial ischemia, infarction, low output states, or even extreme hypotension^[33,34]. Injury to the conduit during harvest, technical errors in the anastomosis, linear

tension on the conduit, angulation at anastomotic site, and unresolved harvest spasm are recognized reasons for hypoperfusion syndrome^[33,34]. Preoperative angiographic evaluation of the quality of the IMA conduit and the subclavian artery, careful conduit harvesting and meticulous construction of anastomoses, insertion of 1.5-mm flexible probe into the IMA and the radial artery after harvesting, and flow measurement using transit time Doppler flow meter after completion of anastomosis are some of the strategies which can mitigate the risk of perioperative hypoperfusion^[33,35].

Competitive flow

Another concern is the augmented risk of competitive flow in the composite graft in comparison with the individual bypass graft. Competitive flow reduces the antegrade flow especially in the diastole, and the phasic delay in pressure wave in the IMA causes a retrograde flow in the early systole^[33]. This oscillating flow pattern in the competitive scenario influences the endothelium. The release of nitric oxide and prostacyclins is affected leading to string sign, which is considered a physiologic vasoconstriction of the arterial graft. String sign is associated with moderate stenosis in the target coronary artery^[29,35] and results in failure of the arterial graft^[35,36].

In the composite graft, the mechanism of competitive flow is more intricate than that in the individual graft. In addition to the relation between the graft and its target coronary branch where competitive flow occurs, the interactions of all anastomosed branches within the composite graft, the phasic delay between the *in situ* grafts, and the whole graft arrangement in the patient contribute to this phenomenon. Therefore, avoidance of competitive flow and graft occlusion relies on both adequate surgical strategy and maneuver^[33,35]. It is perhaps wise to avoid using composite grafts on moderately stenotic coronary arteries particularly moderately stenotic branch in the RCA territory which is the most important predictor of competitive flow and graft occlusion^[33,35].

Deep sternal wound infection

Deep sternal wound infection (DSWI) is a dreadful complication of TAR, especially when BIMA is part of the revascularization strategy. The Arterial Revascularization Trial reported a 1.3% increase in the incidence of sternal wound reconstruction associated with the BIMA^[37]. Different techniques of harvesting the IMA may influence these results. DSWI can be reduced to less than 1% by avoiding BIMA usage in morbidly obese patients (body mass index above 35), insulin-dependent diabetic patients, and those with severe chronic obstructive airways disease, and by appropriate timing of prophylactic antibiotics, including redosing after 4 h, tight blood glucose control intraoperatively and for 48 h, alcohol-based antibacterial preparation, and Vancomycin paste to the sternal edges^[27].

Skeletonized technique of IMA harvesting has been shown to conserve considerable collateral flow to the sternum by sparing some of the sternal and intercostal branches that originate from the IMA as a common trunk^[38,39]. This technique is claimed to reduce the risk of sternal wound complications by improving wound healing, especially when both left and right IMAs are harvested, due to preservation of sternal blood supply^[40].

Other concerns

Harvesting additional arterial conduits takes an additional 20 to 30 min. However, the avoidance of a proximal anastomosis (*in situ* RIMA), and the use of sequential anastomoses and “Y” grafts, result in shorter aortic clamp and bypass times, which may benefit myocardial protection and blood element preservation^[27].

Another concern is the potential risk of increased bleeding. A trend towards a higher rate of re-exploration for bleeding in the TAR patients is reported^[41], suggesting the need for extra attention during hemostasis when using 3 arterial conduits.

The single most important and perhaps greatest issue in encouraging adoption of TAR has to be a consideration of data quality. As it has occurred in other areas to which changes to long-standing and previously well-established practices have been recommended, skepticism may well override reason in the absence of “gold standard” prospective RCTs^[42]. The arena of TAR unfortunately has not enjoyed the benefit of great amounts of such data. As a consequence, even cursory reviews of the available retrospective data readily identify “easy targets” of dissonant data typical of retrospective studies that offer a ready opportunity for disproving the conclusions of these studies^[42].

CONTROVERSIES

Are three arterial grafts better than two?

Whether the addition of a third arterial conduit (mainly radial artery) to BIMA is associated with better survival than BIMA plus SVGs remains a controversial area, with published literature reporting conflicting results^[41,43-51] [Table 2]. Luthra *et al.*^[50] in a retrospective, single-center, propensity-matched study compared the impact of a third arterial or venous conduit to the right circulation on early and intermediate survival after CABG in patients with at least two arterial grafts to the left circulation. They failed to demonstrate a significant difference in early or intermediate survival in the propensity-matched groups (venous *vs.* arterial, 99.2% *vs.* 99.2%; $P = 1.000$ at 1 year; 85.2% *vs.* 88.8%; $P = 0.248$ at 5 years and 69.2% *vs.* 88.8%; $P = 0.297$ at 7 years). Similarly, Formica and associates^[51] comparing the use of radial artery as a third arterial conduit versus SVG failed to show long-term survival benefit of addition of third arterial graft to BIMA. One possible explanation for these contradictory findings is that the survival benefit provided by the use of a third arterial graft is lower when compared with the use of the first or second arterial conduit as most of these single-institutional studies, with small sample sizes, were underpowered to detect moderate differences in survival^[52]. Interestingly, a meta-analysis of these studies reported that the use of a third arterial graft is not associated with an increase in the operative risk but rather with a 24% survival benefit at a mean follow-up of 77.9 months^[52]. Clearly, there is a need for an RCT, preferably multi-institutional, with a large sample size to address this controversy.

Are all configurations of total arterial grafting equal?

The optimal conduit choice and configuration in achieving TAR remains controversial, with uncertainty regarding the individual prognostic impact of IMAs and supplemental arteries. Shi and associates^[53], in a multicentre propensity matched study showed that all configurations of TAR are not equivalent. They compared long-term survival after TAR using single IMA and BIMA supplemented with radial arteries and reported that the use of BIMA as *in situ* or free conduits is associated with greater survival and seems to offer a prognostic advantage over the use of only a single IMA supplemented by radial arteries. Similar findings were reported by Navia and colleagues^[54].

The recently published 10-year final analysis of the Arterial Revascularization Trial (ART), comparing single IMA with BIMA, failed to show significant between-group difference in the rate of death from any cause in the intention-to-treat analysis^[55]. One plausible explanation offered by the authors for this outcome was that 14% of the patients who had been randomly assigned to the BIMA group actually underwent single IMA grafting, and 22% of those who had been randomly assigned to the single IMA group also received a second arterial graft in the form of a radial artery graft. The use of radial artery grafts in ART may be a key confounder, because it is likely to preferentially benefit the single IMA group by the addition of an arterial graft to the second most important coronary artery. When data from patients were analyzed according to the actual receipt of two or more arterial grafts, as compared with a single arterial graft (the as-treated analysis), there appeared to be a meaningful difference in mortality in favor of multiple arterial grafts^[53]. It is anticipated that the Randomized Comparison of the Clinical Outcome of Single versus Multiple Arterial Grafts (ROMA) trial^[56] will address this controversy.

Table 2. Key studies comparing impact of three arterial grafts versus two arterial grafts on long-term survival

Author	Year	Study type	PSM Numbers		Follow-up Duration (months)	Improved survival
			2-Art	3-Art		
Benedetto <i>et al.</i> ^[41]	2016	PSM	275	275	2-Art = 126 ± 58.8 3-Art = 126 ± 54	No
Di Mauro <i>et al.</i> ^[43]	2008	PSM	590	295	2-Art = 88 3-Art = 128	No [^]
Glineur ^[44]	2013	PSM	203	93	2-Art = 196.8 ± 74.4 3-Art = 192 ± 64.8	Yes
Grau <i>et al.</i> ^[45]	2015	PSM	183	183	NR (max 14 y)	Yes
Locker <i>et al.</i> ^[46]	2012	PSM	NR	NR	Mean: 91.2 ± 55.2 Median: 87.6	Yes
Mohammadi <i>et al.</i> ^[47]	2016	PSM	249	249	2-Art = 97.8 (IQR, 0.03-22.6) 3-Art = 97.2 (IQR, 0.02-17)	No
Nasso <i>et al.</i> ^[48]	2012	PSM	3584	3584	Mean: 37.2	No
Shi <i>et al.</i> ^[49]	2016	PSM	262	262	2-Art = 144 ± 60 3-Art = 144 ± 60	Yes
Luthra <i>et al.</i> ^[50]	2018	PSM	167	167	Max: 7 y	No
Formica <i>et al.</i> ^[51]	2019	PSM	190	190	Max: 18.5 y (IQR, 5.6-13)	No

*Non-propensity matched cohort; [^]Increased mortality and cardiac death with addition of third arterial conduit (gastroepiploic artery)

Abbreviations: 2-Art: 2 arterial grafts; 3-Art: 3 arterial grafts; IQR: interquartile range; NR: not reported; PSM: propensity score matched

CONCLUSION

TAR, with its well-recognized benefits of enhanced long-term survival and freedom from re-intervention and cardiac events, is an attractive revascularization option for patients with multi-vessel coronary artery disease. However, the universal adoption remains extremely low due to lack of evidence from RCTs, relatively greater technical complexity and length of the procedure, the perceived increased risk of DSWI and other complications, and the prolonged interval before survival benefits are derived from this strategy. If TAR is to gain popularity then compelling data from RCTs is the single most important strategy to improve uptake of this technique.

DECLARATIONS

Authors' contributions

The author contributed solely to the article

Availability of data and materials

Not applicable.

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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.

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Review

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Fractional flow reserve guided coronary artery bypass grafting - new developments and future perspectives

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Abstract

The potential role of fractional flow reserve (FFR) in coronary artery bypass grafting (CABG) planning and post-CABG patency assessment are currently under intense investigation to determine whether the favourable outcomes reported with FFR-guided percutaneous coronary intervention can be translated to surgical practice. This review provides an overview of the principles that guide FFR measurement, the clinical evolution of FFR in CABG practice, the much anticipated outcomes of recent investigations that compare FFR-guided and angiography guided CABG and outlines the potential of alternative technology that may assist in ensuring ongoing improvement in surgical revascularization outcomes.

Keywords: Coronary artery bypass grafting, percutaneous coronary intervention, fractional flow reserve, outcomes

INTRODUCTION

We are currently witnessing rapid evolution in diagnostic and interventional technology for coronary artery disease (CAD). It is now well recognized that the visual assessment of coronary artery stenosis by angiography^[1] or intravascular ultrasound^[2,3] do not accurately reflect its physiological impact on myocardial territory. Fractional flow reserve (FFR) emerged as a transcatheter tool to potentially quantify the physiological significance of coronary artery stenosis^[4,5] and various reports over the last 2 decades repeatedly confirmed the favorable impact of FFR on percutaneous coronary intervention (PCI) decision-



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making and clinical outcomes^[6-13]. These studies also suggested that less than 50% of angiographic significant lesions were functionally significant and that up to 25% of post-angiography guided-coronary artery bypass grafting (CABG) targets had no impact of physiological myocardial perfusion, potentially risking early graft failure, accelerate native artery disease and late graft failure exacerbation^[14]. This review provides an outline of the principles that guide FFR measurement, the clinical evolution of FFR in CABG practice, the outcomes of recent investigations that compare FFR-guided and angiography guided CABG and describe potential alternative technology that may assist in ensuring ongoing improvement in surgical revascularization outcomes.

FRACTIONAL FLOW RESERVE AS A PHYSIOLOGICAL MEASURE OF CORONARY ARTERY STENOSIS

FFR measurements usually form part of routine diagnostic radial or femoral access cardiac catheterization under local anaesthesia^[6-13]. A pressure transducer within a fluoroscopically visible guide wire record the coronary arterial pressure distal to the identified lesion and once in position, facilitate the administration of a hyperemic stimulus by intracoronary or intravenous vasodilator (usually adenosine) injection. The mean arterial pressures from the pressure wire transducer and from the guide catheter are then used to calculate FFR, which is defined as the ratio of the average distal coronary pressure to average aortic pressure at maximal steady state hyperemia (normal value = 1.0). This equates to expressing the maximum achievable blood flow across an epicardial coronary stenosis to the maximum achievable blood flow in the absence of any stenosis. Various anatomical and clinical scenarios^[15-24], which are outlined in Table 1, potentially influence FFR measurement accuracy that may result in deferral- or over-treatment of physiologically significant culprit lesions (FFR measurement ratio of 0.8 or less).

THE EVOLUTION OF FFR IN CORONARY ARTERY BYPASS GRAFTING

The indications and procedural recommendation for CABG, which now include minimally invasive- and hybrid surgical procedures are well described in contemporary guidelines^[25]. CABG of target lesions more than 50% stenosis were historically performed with the intention of restoring distal perfusion and to provide distal protection against native disease progression^[26]. Subsets of early FFR-PCI measurement trials identified that up to 25% of target lesions had no myocardial perfusion improvement post-CABG with early graft failure, accelerate native artery disease and late graft failure exacerbation considered to be unfortunate consequences^[14].

The well-defined benefits of CABG compared with angiography-guided PCI as reported in the ASCERT^[27], SYNTAX^[28,29], FREEDOM^[30] and BEST trials^[31] became subjected to intense scrutiny following the introduction of FFR and newer generation drug-eluting stent technology. Following the ground-breaking DEFER study^[6], which established the basis for FFR-guided PCI investigation, the FAME^[7,8] and FAME II trials^[9,10] introduced the concept of physiological revascularization and reinforced the positive impact of FFR in PCI of multi-vessel disease, which included decreased repeat revascularization (4.3% in the FFR-guided group, 17.2% in angiography-guided group, $P < 0.001$), number of stents (mean 1.9 in FFR-guided group, mean 2.7 in angiography-guided group) and equivalence in 3-year comparative PCI-procedural costs (\$16,792 for FFR-guided group, \$16,737 for angiography guided group, $P = 0.94$). However, after 5 years, the authors reported no statistically significant difference in the incidence of major adverse cardiac events (MACE) between FFR-guided and angiography-guided PCI (31% in the angiography-guided group, 28% in the FFR-guided group, $P = 0.31$). A functional SYNTAX score of lesions with FFR less than 0.8 reclassified up to 32% of CABG candidates to lower risk groups treatable with both FFR-guided PCI and CABG as opposed to CABG alone^[13]. Whether the favorable impact of FFR on PCI outcomes could be translated to surgical practice, became a subject of intense investigation.

Table 1. Potential contributing factors to FFR measurement inaccuracies

Anatomical factor	Mechanism and Impact on FFR-measurement
Diffuse sequential lesions ^[5]	Multiple isolated sequential stenoses independently decrease coronary pressure and hyperaemic blood flow
Short left main stenosis ^[15,16]	Pressure damping and limited hyperaemic flow with optimal vasodilatation. FFR potentially overestimated
Acute coronary syndrome ^[17,18]	Cascade of coronary vascular receptors down-regulation, endothelial impairment and vasoconstriction. Potentially overestimate FFR/deleterious culprit-vessel deferral
Right heart failure ^[19]	Decrease coronary arterio-venous pressure gradients secondary to increases coronary venous- and microvascular pressures. FFR measurements potentially underestimated
Left heart failure ^[19]	Increased left ventricle end-diastolic pressure impedes myocardial perfusion. FFR increases 0.008 to 0.01/1 mmHg
Chronic multi-vessel disease collateralization ^[20,21]	Decrease in coronary artery-myocardial flow distribution. Microvascular disease is resistant to vasodilator hyperemia. FFR measurement potentially overestimated
Left ventricle outflow tract obstruction ^[22]	Left ventricle hypertrophy, elevated left ventricle end-diastolic pressure, increase microvascular resistance. FFR measurement potentially overestimated
Post-CABG conduit failure ^[23,24]	Competing flow, veno-arterial conduit resistance differences, arterial conduit autocrine activity, culprit-vessel pressure, collateral networks and sequential grafting techniques. FFR measurement inaccuracies due to technical challenges

FFR: fractional flow reserve

The impact of FFR on CABG graft patency was investigated by Botman and coworkers^[33], who reported a statistically significant 1 year graft occlusion incidence of 8.9% in FFR-guided vs. 21.4% of the angiography-guided CABG patients for both arterial (13.7% FFR-guided vs. 21.9% angiography-guided; $P < 0.2$) and venous (5.9% FFR-guided vs. 20.0% angiography-guided; $P < 0.03$) grafts. In those patients with angiographic stenosis of 50% to 70%, the graft patency was higher if the FFR was less than 0.75 and vessels diameter more than 2.0 mm.

A comparative study of angiography-guided- and FFR-guided CABG at the Cardiovascular Centre, Aalst (Belgium)^[33] observed that FFR measurement resulted in a significant downgrade of multi-vessel disease functional severity, a subsequent decrease in the number of CABG grafts applied and no difference in the incidence of MACE between the 2 groups after 3-year follow-up. The incidence of severe recurrent angina was significantly lower in the FFR-guided CABG group (31% vs. 4%; $P < 0.001$). In a subgroup of 155 patients (25%) who underwent repeated coronary angiography for clinical indications, freedom from graft occlusion was higher in the FFR-guided group (21% in angiography-guided group, 5% in FFR-guided group, $P = 0.031$). The extended 6-year results were recently reported by Fournier and colleagues^[34] and included 627 consecutive patients between 2006 and 2010. Both the rate of composite death or myocardial infarction (16% for FFR-guided group, 25% for angiography-guided group, $P = 0.020$) as well as death alone (11% for FFR-guided group, 18% for angiography-guided group, $P = 0.013$) were significantly lower in the FFR-guided CABG group. By Cox multivariate regression analysis, FFR-guidance was an independent predictor of reduced death or MI ($P = 0.008$). The Kaplan-Meier event rates diverged after 3 years to favour the FFR-guided CABG group. A propensity-matched cohort identified fewer MACE in the FFR-guided group (16% in FFR-guided group, 25% in angiographic-guided group, $P < 0.02$), which implies no increased risk of MACE by deferring FFR insignificant lesions.

The association of preoperative FFR on isolated total arterial CABG functionality 6 months postoperatively in patients with triple vessel disease were recently reported in the interim results of the IMPAG trial^[35] as a 2-centre, single-arm, blinded study. The interim results of 63 patients (54 bilateral internal thoracic Y-graft configurations), included the evaluation of 199 arterial anastomoses, of which 135 were sequential anastomoses. Overall, 85% of the left internal thoracic artery (ITA) and 69% of the right ITA were functional and patent, which was statistically significantly associated with preoperative FFR values of 0.78. As arterial grafts are physiologically active and risk atrophy if subjected to competitive flow, the authors suggested that sequential anastomosis that provide continuous antegrade flow to multiple targets

may provide graft protection for lesions in which FFR values were greater than 0.78. Antegrade flow in angiographic stenosed target vessels with large diameters, especially the right coronary artery (RCA), may still be adequate to cause significant competitive flow in arterial grafts and it is therefore suggested that the most appropriate FFR value for optimal RCA graft outcome was less than 0.71. The right ITA is often used for non-left anterior descending artery target vessels with poor distal run-off and apart from technical aspects, potentially explain the inferior graft patency.

NEW DEVELOPMENT AND CONCERNS OF ROUTINELY APPLYING FRACTIONAL FLOW RESERVE-GUIDED CORONARY ARTERY BYPASS GRAFTING

The recently published FARGO trial^[36] evaluated graft patency and clinical outcome of 100 patients referred for CABG by a heart team after randomly being assigned to either FFR- or angiography-guided CABG. In FFR-guided CABG, coronary lesions with FFR > 0.80 were deferred, and a new graft plan was designed accordingly, whereas the surgeon was blinded to the FFR values in patients who underwent angiography-guided CABG. Angiographic follow-up at 6 months were available for 39 and 33 patients in the FFR- and angiography-guided groups respectively. Graft failures of all grafts, death, myocardial infarction, stroke and repeat revascularization were similar in both groups (16% *vs.* 12%; $P = 0.97$). After 6 months, deferred lesions ($n = 24$) showed a significant reduction in mean FFR from index to follow-up (0.89 ± 0.05 *vs.* 0.81 ± 0.11 ; $P = 0.002$). The authors concluded that FFR-guided CABG had similar graft failure rates and clinical outcomes as angiography-guided CABG. However, FFR was reduced significantly after 6 months in deferred lesions and may potentially result in adverse events over longer follow-up.

The GRAFFITI trial^[37], of which the 12 month outcomes were presented at Euro-PCR in 2018 (Paris, France), was a prospective randomized trial that investigated the potential clinical benefits of FFR-guided *vs.* angiography-guided CABG in patients with left anterior descending or left mainstem disease and at least one other major coronary artery with angiographic intermediate stenosis (30%-90% diameter stenosis). The study design is described in [Figure 1](#). The intended CABG strategy was based solely on coronary angiography after which patients underwent FFR- or angiography-guided randomization. In the FFR group, the surgical planning was revised according to the functional significance of each coronary stenosis after the FFR values were disclosed to the surgeons. After 12 months follow-up, the rate of graft occlusion (20% and 19% in angiography- and FFR-guided groups respectively, $P = 0.885$, 64.5% complete), rate of death (2% and 3% in angiography- and FFR-guided groups respectively, $P = 0.65$), myocardial infarction (2% and 0% in angiography- and FFR-guided groups respectively, $P = 0.15$), stroke (0% and 2% in angiography- and FFR-guided groups respectively, $P = 0.16$) and repeat revascularization (5% and 2% in angiography- and FFR-guided groups respectively, $P = 0.35$) were reported to be similar for both groups. Lesions with FFR measurement less than 0.8 were deferred in 53% and 29% in the angiography-guided and FFR-guided groups respectively, which suggested that FFR-guidance was associated with higher functional appropriateness (69% and 79% in angiography- and FFR-guided groups respectively). CABG was performed on 44% of stenotic lesions with preserved FFR and deferred on 53% of lesions with abnormal FFR, which translated to a significant reduced number of grafts.

Despite the paradigm shift toward physiological revascularization with all the benefits described, the value of complete anatomical revascularization remains relevant. Mulukutla and colleagues^[38] recently reported a propensity-matched retrospective, observational analysis of patients with multi-vessel CAD who underwent angiography-guided CABG or FFR-PCI with second generation drug eluting stents between 2010 and 2018 and for whom data were available through the National Cardiovascular Data Registry or The Society of Thoracic Surgeons Adult Cardiac Surgery Database. Of the initial 6163 patients identified, the propensity-matched cohort included 844 in each group. The estimated 1-year mortality was 11.5% and 7.2% ($P < 0.001$) in the PCI and CABG groups respectively and overall MACE and individual outcomes of

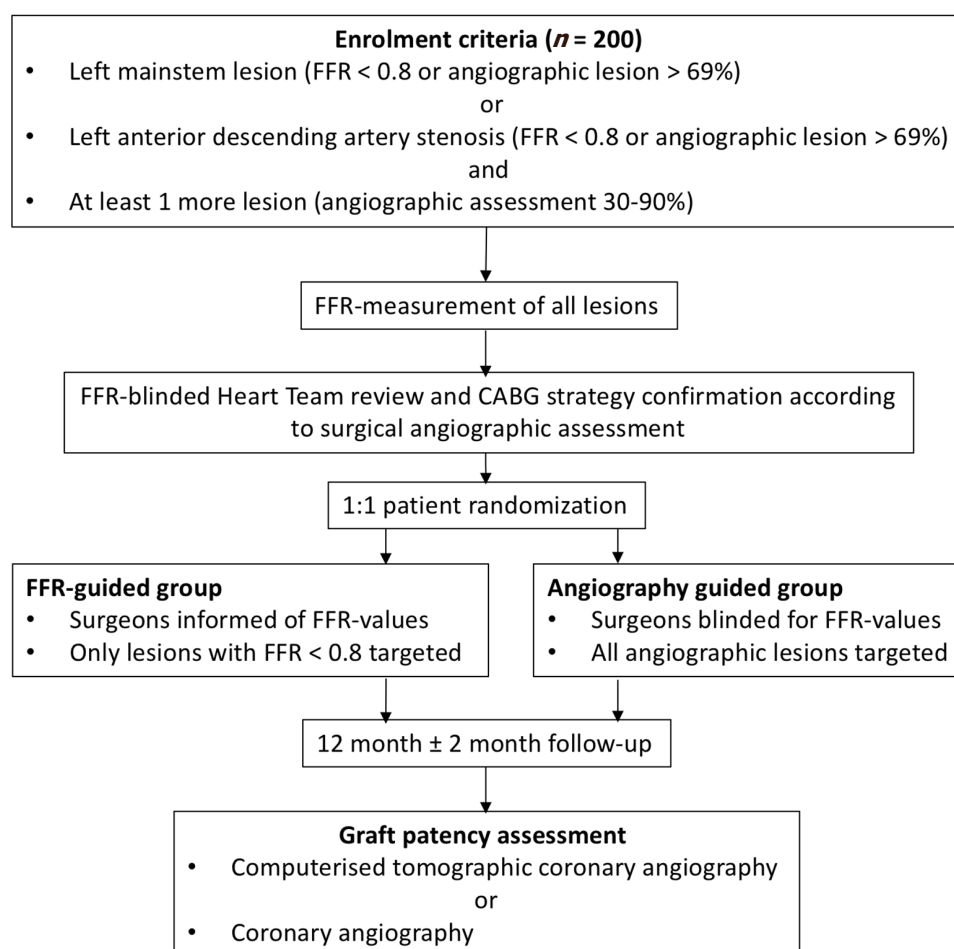


Figure 1. Graffiti trial design. FFR: fractional flow reserve

mortality, readmission, and repeat revascularization all favored CABG across all major clinical subgroups. CABG was angiography-guided and suggest that excellent outcomes that outperform PCI are achievable without FFR guidance.

The FUTURE trial, which was presented by Rioufol and colleagues at European Society of Cardiology Congress in Munich (Germany, 2018), was designed to explore the impact of FFR guided treatment strategies in patients with angiographic multi-vessel coronary disease in 31 French centers. In the FFR-guided group, FFR was performed on all target lesions, with FFR less than 0.80 regarded as eligible for PCI or CABG. There planned enrolment of 1,728 patients was halted due to an observed difference in all-cause mortality after 938 patients were randomized. The presenters highlighted three factors that could have played a role in the higher rate of mortality in the FFR-guided group: the lower-than-expected rate of CABG considering that all patients had multi-vessel disease, the higher rate of PCI in severe patients with a SYNTAX score over 32, and the high rate of ad hoc PCI (about 90% in both groups).

POTENTIAL ALTERNATIVE MODALITIES THAT MAY IMPROVE CORONARY ARTERY BYPASS GRAFTING PLANNING AND DECISION-MAKING

The rapid development in non-invasive imaging technology offers exciting potential alternatives to conventional invasive coronary angiography. Computerised tomographic coronary angiography (CTCA) is

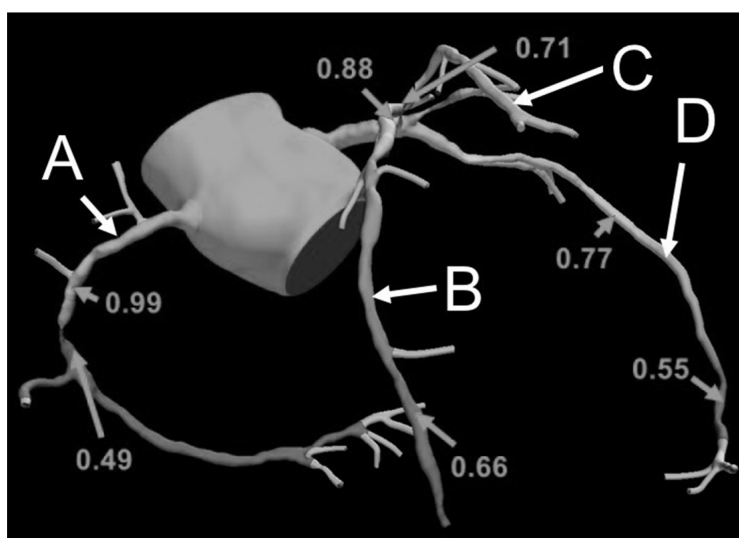


Figure 2. Computerised tomographic fraction flow reserve measurements of the right coronary (A), circumflex (B), intermediate (C), and left anterior descending artery (D)

a non-invasive, repeatable, safe and efficient primary investigation in stable CAD that reduces the incidence of “negative” invasive angiography where no intervention is indicated or required^[39]. Significant progress has been achieved regarding radiation exposure, with the introduction of dose-sparing protocols for ECG synchronization, the widespread use of lower tube voltages, and the development of iterative reconstruction algorithms. It has the major benefit of documented correlation with invasive FFR [Figure 2] and may result in a significant paradigm shift towards non-invasive coronary stenosis assessment and appropriate referral for treatment by CABG or PCI^[40,41]. Nogaard and colleagues reported that 185 (98%) of 189 patients (mean age 59 years, 59% male) that underwent FFR-CTCA had conclusive results, with FFR-CTCA < 0.8 correlating with invasive coronary angiography in 73% of patients and 70% of vessels. In patients with FFR-CTCA lesions > 0.80, invasive coronary angiography was deferred with no adverse cardiac events observed during a median follow-up period of 12 months (range 6 to 18 months). They calculated per-patient sensitivity and specificity (95% CI) to identify myocardial ischemia as 86% and 79% for FFR-CTCA, 94% and 34% for standard CTCA and 64% and 83% for invasive coronary angiography respectively.

Two non-hyperemic, invasive measures of inducing pressure might be useful for assessing the severity of coronary stenosis^[42]. The resting distal coronary artery pressure/aortic pressure (Pd/Pa) is the ratio of distal coronary artery pressure to aortic pressure over the entire cardiac cycle. Instantaneous wave-free ratio (iFR) measures coronary pressure during a specific period of diastole when the resting resistance is the lowest^[42]. Shiode and colleagues investigated the correlation between FFR-angiography, iFR and resting Pd/Pa by continuously measuring each component in 123 lesions in 103 patients with stable CAD by an intracoronary injection of papaverine. A receiver operator curve analysis revealed that the optimal iFR cut-off value for predicting an angiographic FFR of < 0.80 was 0.89 (sensitivity 84.1%, specificity 80.0%, diagnostic accuracy 81.3%), while the optimal resting Pd/Pa cut-off value was 0.92 (sensitivity 90.9%, specificity 78.5%, diagnostic accuracy 82.9%). Lesions with an iFR value < 0.89 and a Pd/Pa value < 0.92 were defined as double-positive lesions, while the lesions with an iFR value of > 0.89 and a Pd/Pa value of > 0.92 were defined as double-negative lesions. The ADVISE^[43], ADVISE-e^[44] and RESOLVE trials^[45] also suggested that iFR compares favourably with FFR-angiography, may defer up to 16% of FFR significant lesions, may reduce procedural time, lower procedural cost, improve patient comfort and avoid side effects of adenosine, especially for patients at risk. The clinical application of iFR and Pd/Pa will soon be defined.

CONCLUSION

Various studies now confirm that physiological revascularization by FFR-guided CABG result in fewer target lesions and improved conduit patency in the short term. Even though intermediate follow-up results suggest no significant difference in clinical outcomes compared to angiography-guided CABG, the risk that angiographic significant/FFR insignificant lesions may progress to detrimental clinical events are of greatest concern. Current evidence therefor does not support the routine use of FFR in CABG planning. The current CABG procedure recommendation of complete angiographic- and total arterial revascularization with minimal aorta manipulation should remain the standard until future studies clarify the role of FFR in long term CABG outcomes^[25]. Non-invasive CAD diagnostic modalities are rapidly developing and may offer exciting alternatives to FFR in planning CABG target lesions. The diagnostic-, shared decision-making-, informed consent- and therapeutic practices related to CAD treatment are sure to evolve with CABG to remain an invaluable- and excellent option.

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Authors' contributions

Read and agreed to the manuscript as written: Van der Merwe J, Casselman F

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Consent for publication

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Review

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Complete surgical myocardial revascularization: shift of paradigm of the gold standard in the current era

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Abstract

Coronary artery bypass surgery is recommended for the patients with symptomatic coronary artery disease or patients having critical left main trunk stenosis. From the inception of the procedure it has undergone several modifications during different time periods evolving into the safe, durable and effective procedure that is to-day. Complete myocardial revascularization (CR) restores blood supply to all the territories in the myocardium that are ischemic because of narrowed blood vessels. Earlier clinical studies from the 1980s showed that patients who had CR had better quality of life free of angina with less major adverse coronary events and had survival benefit when followed at 5 years and 10 years compared to the patients who had incomplete myocardial revascularization. According to the coronary artery surgery study registry the benefit is more pronounced when patients had severe triple vessel disease, class III-IV angina and decreased ejection fraction.

Keywords: Coronary artery disease, complete myocardial revascularization, off-pump surgery, on-pump surgery, percutaneous coronary intervention

INTRODUCTION

Both medical and surgical therapeutic interventions of coronary artery disease (CAD) are extensively studied by the patients, physicians, hospital administrators, epidemiologists, and the insurance companies because of the risks involved to the patient on one side and the cost of care on the other side. CAD still



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remains as one of the major healthcare entities that drains Billions of dollars and the treatment at best is only a temporizing situation without a permanent cure for the disease entity. The patient's genetic background, dietary habits, stress, lack of exercise, diabetes, smoking, other socioeconomic conditions and many more factors contribute towards varying outcomes. As physicians became comfortable with the existing methods of treatments, newer drugs and surgical techniques, percutaneous catheters and varying stents came into the field that have provided the impetus for physicians to constantly learn while providing care. This has resulted in improvements on one side and ongoing sporadic catastrophic events on the other side^[1,2]. This paper will outline the changes that have occurred over the years on the overall treatment of the disease.

EVOLUTION OF THERAPEUTIC OPTIONS

It was believed that the occlusion of coronary arteries led to myocardial ischemia or infarction from the pathological specimens. But it was not until Sones and Shirely^[3] performed several hundred coronary cine angiograms and showed the lesions in the coronary artery branches and their anatomical variations, surgeons could not plan a method of treatment to tackle the occlusions. Several advances occurred simultaneously that incorporated cardiopulmonary bypass routinely into cardiac surgery and surgeons gained experience to tackle peripheral vascular disease as well. Development of microvascular instruments, fine suture materials, wearing surgical loupes and a head light for better visualization have helped to improve technical proficiency for coronary artery bypass surgery (CABG). Improvements in myocardial protection starting from intermittent aortic cross clamping and a fibrillating heart to a completely quiescent heart with antegrade and retrograde blood cardioplegia gave an enormous leap forward for CABG surgery.

Saphenous vein was the preferred conduit until late 1970s in CABG surgery. At that time, it was shown to have a better patency when used to bypass left (Lt) anterior descending (LAD) artery, though there are graft failures to the other branches of coronary arteries. LAD being the most important blood vessel, was bypassed with the best quality of the available vein. The graft being straight and short, carried high blood flow into the large myocardial segment. These factors contributed to the high patency rate. A vein which lies normally in a low-pressure vascular system, once subjected to arterial pressure and blood flow, showed evidence of complete endothelial cell damage of the intima and subsequent development of rapid atherosclerosis. Though use of Lt internal thoracic artery (LITA/LIMA) in CABG was performed much earlier by Kolesov, universal acceptance by surgeons did not happen until Floyd Loop's^[4] landmark article about the long-term benefits of arterial graft to LAD using LITA. Subsequently as more and more data became available about vein graft atherosclerosis, surgeons have slowly adopted the use of more arterial grafts using right internal thoracic, radial (RA) and gastro epiploic arteries.

COMPLETE SURGICAL MYOCARDIAL REVASCULARIZATION

Before percutaneous coronary intervention (PCI) became a generally acceptable procedure to treat coronary artery stenosis, surgical revascularization with bypass grafts had been the standard of care to treat patients with significant CAD. During the peak of surgical myocardial revascularization in late 80s, CABG was only performed in large Medical centers in the hands of highly trained surgeons who were able to tackle even small blood vessels the size of 1 mm. Anatomically right (Rt.) Coronary artery has a rt. ventricular branch, posterior descending (PDA) and posterolateral (PLB). Lt coronary artery has LAD, 1 or 2 diagonal branches and main circumflex (CX) branch giving several marginal branches on the posterior wall of the Lt. ventricle. Between the LAD and CX a large ramus branch can come on to the lateral wall. Every suitable vessel with 70% or above stenosis was bypassed. Surgeons were able to perform 3 to 6 bypasses on an average and even the number increased to 7 or 8 depending on the coronary anatomy and diffuse nature of the disease. Complete surgical revascularization is accomplished when all the stenotic blood vessels are bypassed. While this is considered as an anatomical classification, a functional

classification is used when the blood vessels in the ischemic zones of the myocardium are only bypassed ignoring the areas that already had myocardial infarction and myocardial scarring. Number of distal anastomosis are counted and matched to the number of stenosis in the blood vessels that are technically by passable to determine whether a CR or incomplete myocardial revascularization (IR) is performed.

In anatomical classification, a completely occluded blood vessel proximally is bypassed as long as it is open distally and is suitable for bypass. It is considered that next to the scar tissue there is a zone of ischemic myocardium that can get benefit from blood flow through the bypass graft. It can also provide retrograde collateral flow to the other connecting vessels. The typical example is bypassing an open distal Rt. Coronary artery after its complete proximal occlusion. Most of the patients had at least one arterial graft to LAD and the remaining grafts were performed with saphenous vein. LAD revascularization is the key part of CR when ever there is evidence of 70% or more stenosis.

LAD atherosclerosis presents in different forms: (1) Proximal severe stenosis with excellent distal vessel and a bypass graft can be sutured anywhere distal to the stenosis; (2) Proximal stenosis followed by an open LAD along with diffuse atherosclerosis in the middle and the distal LAD is open at the apex of the Lt ventricle requiring bypass to both proximal and distal segments to protect the entire septum as well the anterior and apical segments of the myocardium; and (3) completely occluded LAD with atherosclerotic process and will need extensive endarterectomy to revascularize the vessel. Such large endarterectomy not only removes the atherosclerotic plaque from the LAD but also removes the plaque from the opening of the septal branches and can improve the blood flow to the interventricular septum. Bypasses requiring to diagonal, ramus and marginal branches on the Lt side will add up to 7 or 8 distal anastomosis very easily. Apart from LAD, bypassing another major vessel in the Lt coronary system has also increased the survival benefit.

Use of all arterial grafts to the Lt coronary system was popularized by creating a LIMA+RIMA as a “T” graft and performing multiple sequential grafts to the marginal branches with free RIMA graft and LIMA being anastomosed to the LAD as an *in situ* graft. Prior to “T” grafting became popular, my preferred operation was an *in situ* RIMA graft across the midline to LAD and LIMA graft to an OM branch whenever applicable. In aortic arch disease there is higher chance of Lt subclavian artery to get occluded than innominate artery. The next arterial graft that got popularized is the RA graft taken from the nondominant hand. This is also used as a free graft from the aorta or as “T” graft from the LIMA graft^[5-7].

The patients that were discharged from the hospital with-out any perioperative morbidity had performed well, free of anginal symptoms and without repeat hospital readmissions for myocardial infarction or heart failure. Zimarino *et al.*^[8] reviewed 28 studies that included 8,3695 patients that were treated with surgery as well as PCI. Patients who had CR with multi vessel coronary artery disease (MVCAD), at 4.5 years follow up showed less mortality and less repeat further interventions. CR performed as an elective surgical procedure has better outcomes over medical therapy or PCI in diabetic patients with MVCAD. Takagi *et al.*^[9] did metanalysis of patients from fourteen studies and compared 3,0389 patients and found that patients who had complete surgical revascularization did have 37% less mortality when followed over a period of time compared to similar group of patients that had IR.

REASONS FOR SHIFT OF PARADIGM

Changes in the trends of bypass grafting using multiple arterial grafts, introduction of “off-pump” CABG and operating on several coronary artery branches with multiple stents and advances in PCI procedures, complicated the CABG. Emergency surgical revascularization on patients who are loaded with antiplatelet agents also precluded prolonged surgical procedures for fear of perioperative bleeding complications. Collaborative effort between cardiologists and cardiac surgeons to address CAD, led to hybrid procedures

where a durable LITA graft to LAD is created through a Lt anterior small thoracotomy^[10] and remaining stenotic vessels were dilated and stented by the cardiologists. In addition, the mean age of patients having surgery became much higher and several comorbidities that increased overall surgical risk. Pre-existing cerebrovascular disease, chronic hemodialysis, advanced malignancy and severe chronic obstructive lung disease are few conditions to name that influenced plan of surgical treatment. More females are having surgery who are in general are higher surgical risk compared to men with similar risk factors. The difficulties encountered in constructing a satisfactory bypass graft through a small thoracotomy incision resulted in off-pump bypass surgery through median sternotomy. This approach also broadened the access to bypass other vessels like Rt. and Cx. coronary artery branches.

With rapid expansion of cardiac surgery into the community hospital setting, the total number of CABG surgeries performed by an individual surgeon has significantly decreased and simultaneously the experience to tackle difficult coronary surgical cases. They have adopted the general notion that bypassing one vessel in each territory of myocardium is enough to relieve the patient of his anginal symptoms and multiple bypass grafts will only prolong the operation and subsequently increase the immediate post op complications like perioperative bleeding, low cardiac output syndrome and need for prolonged ventilatory support. At one time majority of the surgeons had the comfort zone and experience to expose deep intramuscular LAD embedded in the interventricular septum, perform extensive endarterectomies in the vessels with diffuse atherosclerosis and bypass even the main CX coronary artery in the atrioventricular (AV) groove when the marginal branches were too many and too small for bypass. Generally main CX in the AV groove is free of atherosclerosis and is of larger caliber. Though coronary vessels are epicardial, some-times the proximal LAD and ramus or the first marginal branches are deeply embedded in the cardiac muscle that needed patience to identify and dissect out these vessels for bypass. These intramuscular vessels are again free of atherosclerosis that they are best suitable for bypass. These anatomical variations are the cause of IR in the hands of less experienced Surgeons. The difficulty is compounded in obese patients where the heart is covered with lot of fatty tissue and during redo coronary artery bypass graft surgery. PCI also became an established modality to treat CAD and experienced interventional cardiologist had shown excellent results in stenting multiple stenotic coronary arteries. Vein graft atherosclerosis resulted in stenotic bypass grafts which required redo-CABG. In late 90s, 15%-20% of the coronary artery surgical volume in all major surgical centers happened to be redo bypass surgery. With the advances in medical therapy and PCI, redo bypass surgery has significantly decreased. Perioperative use of statins helped to stabilize vein graft atherosclerosis so that cardiologists are able to address vein graft stenosis. As the surgical volume decreased, the peripheral cardiac surgical centers which at one time had two cardiac surgeons, are able to hold on to only one cardiac surgeon who doesn't want to take excessive responsibility of tackling difficulty cases. Public awareness of individual surgeon's data including surgical volume, mortality, morbidity and hospital readmissions following CABG surgery by the local state governments did not help the matters either. The number of grafts they are performing had come down as evidenced by the society of thoracic surgeons (STS) data base published in 2018. As per the STS the published data in 2016 showed that in spite of increase in CABG procedures per the year by 6.1% to a total of 156,931, the number of 4 and 5 distal anastomosis are much fewer than the previous years^[11]. As the isolated CABG procedures have gone down there has been a significant increase of valve with CABG procedures like aortic valve replacement or mitral valve/replacement. This has changed the complexity of the surgery as such surgeons focused on the main coronary artery branches for revascularization and proceeded with valve surgery. This is evidenced by the higher pre-operative risk assessment both by euroscore and STS risk calculator.

Off-pump coronary artery bypass surgery

While majority of cardiac surgeons are able to perform a satisfactory bypass to the blood vessels that are in the front of the heart while the heart is beating, only a few surgeons could master the technique

of bypassing all the blood vessels both in the back and on the lateral wall of the heart with-out much hemodynamic compromise. Avoiding the heart lung machine eliminated the complications related to it, and also decreased the cost of the procedure which in turn contributed to the exponential growth of CABG surgery in the third world countries. In the hands of less skilled surgeons the procedure instead of becoming a “beating heart surgery” became a “beat the heart” surgery. Intra operative conversion from off-pump to on-pump surgery showed increased perioperative morbidity and mortality. Off-pump surgery became an accepted approach when the ascending aorta appeared calcified and the chances of atherosclerotic emboli to the brain from the aorta are considered high^[7]. Benedetto *et al.*^[12] reviewed CABG patients that had surgery at Bristol Heart Institute, in England both off-pump 7427 pts and on-pump 7128 pts and showed that patients who had multiple bypass grafts lived longer when compared to the patients that had fewer number of grafts. Afilalo *et al.*^[13] published meta-analysis and meta-regression of 8961 patients and found that there is 30% stroke reduction in off-pump coronary artery bypass while mortality and myocardial infarction rates are the same in both groups. The decrease in stroke rate is attributed to avoidance of aortic manipulation. The benefit is apparent when aorta is not at all touched, but if the aorta is partially occluded to perform proximal anastomosis the risk of stroke is reintroduced whether the procedure performed is on-pump or off-pump. Because of the improvements in myocardial protection, now most procedures are performed on cardiopulmonary bypass with single aortic cross clamping.

The ROOBY trial studied 2203 patients randomized to either off or on pump CABG from 2002-2007. The 5-year mortality was 11.9% in on-pump *vs.* 15.2% in the off-pump group^[14]. The on-pump patients required fewer coronary interventions in the subsequent years. Now off-pump CABG is falling out of favor for triple vessel disease and can still be used for isolated one or two vessel disease involving anterior targets.

Minimally invasive procedures such as mini-thoracotomy along with endoscopic and robot assisted CABG procedures evolved but could not gain popularity as the cost and time involved to learn the required minimal skills is high and protracted.

SURGERY *VS.* PCI

In randomized studies comparing PCI *vs.* surgery, patients who underwent complete revascularization performed better when compared to patients with incomplete revascularization. When PCI is guided by the fractional flow reserve (FFR) evaluation, there may be fewer number of stent placements when compared to the angiographic observation of stenosis in the coronary arteries. It is still considered as CR as there is substantial evidence that FFR above 0.8 can be treated medically and does not need therapeutic intervention^[15]. Tackling the culprit vessel fared well when patient came to the hospital with acute ST segment elevation myocardial infarction. While some cardiologists performed multiple vessel PCI at the same time, majority preferred multiple staged procedures requiring repeated interventions and hospital admissions. In arterial revascularization therapy study 1143 patients that were randomized for either surgery or angioplasty, the patients that had complete revascularization performed better when compared to the patient that had incomplete revascularization with angioplasty^[16].

BYPASS CONDUITS

Saphenous vein quality is influenced by patient's age, sex, past history of phlebitis, pregnancy, and occupation. The technique of saphenous vein harvest also undergone several surgical modifications starting from one large skin incision from groin to the ankle to multiple skip incisions and finally endoscopic removal. There is no uniform standard for vein preservation solution. Perioperative use of antiplatelet agents and statin therapy have increased the vein graft patency as well the longevity. Endoscopic removal of the saphenous vein has become the standard practice for both cosmetic reasons as well as avoiding wound healing complications in the leg with open surgical removal. Saphenous vein is more adoptable and

much easier to use for multiple sequential grafting than mammary arteries or RA artery. LIMA graft has varying size and blood flow depending upon the body size and patient's age. Lt subclavian artery stenosis used to be a problem in the pre-angioplasty era but now even a critical stenosis in the subclavian artery is successfully dilated and stented, restoring blood flow in the mammary graft. Initially the mammary artery was removed from underneath sternum as a pedicle graft but skeletonizing the internal mammary became the preferred method, leaving capillary collaterals behind to feed the sternal edges. This technique is highly recommended in diabetics, females and whenever bilateral mammary arteries are used for bypass surgery. However skeletonizing the artery had challenges like injury to the arterial wall, development of intramural hematoma and intimal dissection leading to failure to use the graft. Various vasodilator pharmacological agents can be instilled over the mammary pedicle in-order to dilate the vessel and increase the blood flow. While the LIMA graft is used preferentially as an *in situ* graft, RIMA is used as an *in situ* or as a free graft depending upon the planned surgery. RA artery became the alternate arterial conduit after mammary arteries but developed arterial spasm because of the thick muscular wall and also evidence of calcification in elderly patients. Perioperative use of Calcium channel blockers has increased the patency of the RA artery graft but the limitation is that it's use is recommended to bypass the vessels with 90% or more stenosis to avoid any competitive blood flow. It showed better patency on the Lt coronary artery system than on the right side. Though RA artery showed better long-term patency as compared to the saphenous vein graft, because of the limitations it had, it is not as frequently used as recommended in the literature. The use of RIMA as a second arterial graft to the Lt coronary system obviated the need for a third arterial graft. Removal of both RIMA and RA artery became a time-consuming operation with added complications, and surgeons didn't want to use both unless there is definite benefit to the patient. The gastroepiploic artery also is prone for spasm and showed inconsistent blood flow. Constant research is going to improve the patency of the vein grafts by external stenting or other methods or to find a synthetic graft that can be used when patient doesn't have suitable conduit.

There was a push by Taggart in UK, Puskas in USA^[7] for performing CABG with only arterial grafts. Using both internal thoracic arteries for CABG surgery was promoted as a must to do thing. It did not become popular among American surgeons as majority of the patients that came for surgery are not only old but have several comorbidities that increased their over-all risk. After following for 10 years other than a LIMA graft to the LAD using all arterial grafts when compared to a single arterial graft and remaining venous grafts, didn't increase the survival benefit in elderly patients but the younger patients may benefit from multiple arterial grafts if their natural life expectancy is 10 years or beyond. The survival benefit of any procedure depends upon the biological and anatomical age of the patient. That is also true with CR as younger patients tend to live longer and symptom free while older patients with low ejection fraction tend to have heart failure symptoms^[17].

CONCLUSION

As the saying goes "There was never a successful incomplete operation". The gold standard of CABG still remains as the CR performed by an experienced operator so that all the blood vessels with critical stenosis can be tackled. The goal can be achieved by off-pump or on-pump surgery or by PCI. Complete revascularization rewards patients with symptom free or risk free postprocedural period. There were several clinical trials that studied CAD and therapeutic interventions over the years. There may be minor differences in the conclusions but majority of the studies do concur that CR gives better survival and symptom free life.

SPECIAL NOTE

This article reflects the surgical practice that is popular in USA and may vary from the common practice globally.

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Authors' contributions

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Review

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Contemporary indications for percutaneous revascularization of coronary chronic total occlusions

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Abstract

Chronic total occlusions (CTO) are frequently encountered during coronary angiography, and are generally regarded as the most challenging coronary lesions for percutaneous coronary intervention (PCI). Despite great technical advancements and greatly improved reported procedural success rates during previous years, data on clinical benefit of these procedures still remain scarce and controversial. Data from observational trials suggested that PCI for CTO could be linked to improvements both in symptoms and hard cardiovascular outcomes, while randomized controlled trials showed symptomatic improvement only, without improvement in patient's prognosis. This is in parallel with findings for non-CTO PCI in patients with stable angina. Having in mind complexity of these interventions, high costs, greater volume of contrast, and radiation exposure, appropriate patient selection is crucial for optimizing treatment effectiveness. There are few important factors that should be taken into consideration before planning and attempting PCI for CTO. These are: severity of patient's symptoms despite optimal medical therapy, presence of inducible myocardial ischemia and/or viability in the territory of occluded coronary artery.

Keywords: Chronic total occlusion, percutaneous coronary intervention, indications for intervention

INTRODUCTION

Chronic total occlusions (CTO) of coronary artery is defined as an occluded coronary vessel with thrombolysis in myocardial infarction flow 0, that lasts for 3 months or more^[1,2]. They are often found



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during coronary angiography, with a prevalence rate of 18%-52%^[3-5].

CTO remain the most challenging lesion subset in patients undergoing percutaneous coronary interventions (PCI). It was early recognized as one of the most important obstacle in achieving complete revascularization in patients with multivessel coronary artery disease. Even the father of interventional cardiology Andreas Gruentzig noted in an interview in 1985 (two weeks before his tragic passing in an airplane crash): “The total closure is a real problem, if we cannot solve the total closure problem, we probably will never really address the question of multivessel disease dilatation”^[6].

Myocardial territory distal to CTO is usually supplied by collateral flow, which is sometimes sufficient to preserve viability and contractile function in resting conditions. On the contrary, collaterals are most often not sufficient to provide adequate blood flow during increased demands, providing adequate protection against ischemia in only 5% of patients^[7]. This means that collateral vessels cannot fully substitute the function of open epicardial artery, which constitutes the rationale for performing PCI for CTO.

Regarding the prognostic impact of coronary artery bypass grafting (CABG) for CTO revascularization, this issue is lacking high-quality randomized data. Isolated CABG for an isolated CTO of the LAD (or other coronary arteries) cannot be justified on the basis of preventing future events compared with either medical therapy or PCI. CABG for CTOs will often be part of a strategy of offering complete revascularization^[8].

Technical aspects of the PCI for CTO

Although PCI for CTO remains most challenging for many interventionalists, we are witnessing gradual improvement in all procedural aspects during last decade. For experienced operators, procedural success rates could reach > 90%, which is the result of improvements in instrumentaria, better training, increasing operator expertise with complex techniques, and spreading the knowledge through the work of dedicated CTO organizations, such as EuroCTO club and others^[9-12].

Many technical factors should be taken into consideration while planning PCI for CTO^[2]. In general, ad hoc PCI for CTO is not encouraged; instead, staged, elective, and carefully planned approach is preferred. When dealing with selection of arterial access site, most dedicated CTO operators prefer to use femoral artery for targeting occluded artery, since it allows them to use larger size catheters (7 or 8 French) which offers better passive support and more space for simultaneous insertion of devices. Radial artery is most often used to cannulate non-CTO artery in order to visualize occluded artery distal to the place of occlusion via collateral circulation. Dual injection should be used whenever possible, since it allows operator to appropriately assess morphologic characteristics of the occluded segment. Selection of coronary guidewires is critical step for the successful PCI of CTO. Many characteristics of the guidewires should be taken into account when selecting appropriate guidewire like: polymer cover, wire coating, core material, and tip stiffness. Operators may choose between wires depending of what they need most in every phase of intervention: more torque control, more maneuverability, more penetration power, less potential to damage collateral channels, reentry etc. Microcatheters are devices that are almost always used in PCI for CTO. They offer the operator possibility to exchange guidewires rapidly, provides additional support for the guidewire, provides protection of collateral vessel, provide route to inject small amount of contrast (“tip injection”) to visualize distal vessel or collaterals. Many improvements in manufacturing technology give us wide array of available devices that meet different needs of operators in various situations. Contralateral contrast injection is frequently needed to precisely and safely navigate guidewire toward vessel distal to occlusion, and we strongly recommend using it whenever distal vessel cannot be adequately visualized via ipsilateral dye injection.

Traditionally, antegrade approach is used as a first strategy of CTO recanalization. With the development of new guidewires, microcatheters, the single wire techniques, parallel-wire technique, techniques with subintimal tracking, and antegrade dissection and reentry technique, it remained the most common first choice strategy. Retrograde techniques were developed over long period of time, and made major breakthrough in mid 2000s with the pioneering work of Dr Osamu Katoh who introduced the Controlled Antegrade and Retrograde subintimal Tracking technique of retrograde CTO recanalization. Detailed consideration of choosing between antegrade and retrograde strategies is beyond the scope of this review, and may be found elsewhere^[13]. It is worth mentioning few more aspects specific to PCI for CTO. In a recently published report from OPEN-CTO registry, major complication rates still remain significant and higher than in non-CTO PCI: in-hospital/one-month mortality was 0.9% and 1.3%, respectively, while coronary perforations requiring treatment occurred in 4.8% of patients^[14]. Operators should be aware of contrast toxicity and should limit the contrast volume to minimum needed and adjusted to renal function of the patient. Radiation safety is an important issue and physicians should make every effort to reduce radiation exposure, and to document radiation exposure during a PCI procedure.

Symptomatic and prognostic impact of the PCI for CTO

Despite these truly amazing technical improvements in achieving proficiency of CTO recanalization, many aspects of clinical efficacy of this demanding procedure remain controversial. Several non-randomized (observational) trials have shown that successful CTO revascularization could be linked to improved cardiovascular outcomes^[15-17] and better quality of life (QOL)^[18,19]. We have recently published a long term (66 months) follow-up of cohort of 283 patients in whom recanalization of CTO was attempted^[20]. Patients with successfully recanalized CTO had lower rate of MACE (defined as composite of cardiac mortality, myocardial infarction, and target vessel revascularization) than patients with failed procedure; the difference that remained statistically significant after adjustment for baseline between-group differences using propensity scores (adjusted HR 0.402; 95%CI: 0.196-0.824; $P = 0.013$). Christakopoulos *et al.*^[21] published a review of 25 observational trials and showed improved outcomes with successful PCI of the CTO (which included survival, angina severity, and the need for coronary artery bypass surgery). Having in mind all limitation of these kind of trials, no causal inferences could be drawn between PCI for CTO and positive cardiac outcomes. These conclusions may be regarded only as a hypothesis generating, and must be checked in appropriately designed randomized controlled clinical trials (RCTs). Only RCTs could answer questions related to causality between the procedure and outcomes, and we have data from few of them recently published.

The EXPLORE (Evaluating Xience and Left Ventricular Function in PCI on Occlusions After ST-Elevation Myocardial Infarction) study randomized 304 patients presented as STEMI which also had a CTO lesion in a non-infarct artery to additional PCI of CTO soon after primary PCI or optimal medical therapy only^[22]. Primary endpoints were left ventricular ejection fraction (LVEF) and left ventricular end diastolic volume (LVEDV) on cardiac MRI after 4 months. Authors did not report any benefit for CTO PCI in terms of LVEF ($44.1\% \pm 12.2\%$ vs. $44.8\% \pm 11.9\%$; $P = 0.60$) or LVEDV (215.6 ± 62.5 mL vs. 212.8 ± 60.3 mL; $P = 0.70$). Furthermore, they did not find the difference in terms of major adverse coronary events (5.4% vs. 2.6% ; $P = 0.25$). It was suggested that PCI of the CTO in LAD artery could be related to improved LVEF ($47.2\% \pm 12.3\%$ vs. $40.4\% \pm 11.9\%$; $P = 0.02$), but this finding remained hypothesis-generating. Even during prolonged follow-up (median of 3.9 years) of this trial, MACE did not differ between arms (13.5% vs. 12.3% , HR 1.03, 95%CI: 0.54-1.98; $P = 0.93$). Interestingly, reported cardiac mortality was higher in the CTO-PCI arm (6.0% vs. 1.0% , $P = 0.02$), while there was no difference in all-cause mortality (12.9% vs. 6.2% , HR 2.07, 95%CI: 0.84-5.14; $P = 0.11$)^[23].

Another randomized trial (the REVASC trial) assessed the effect of CTO recanalization on segmental wall thickening (SWT) (the primary endpoint) and improvement of regional wall motion and changes in left

ventricular volumes and ejection fraction (secondary endpoint) in the CTO territory^[24]. The change in SWT did not differ between the CTO PCI [4.1 (-14.6 to 19.3)] and non CTO PCI [6.0 (-8.6 to 6.0)] groups ($P = 0.57$). Similar findings were described for secondary endpoints^[24].

EuroCTO (Randomized Multicentre Trial to Compare Revascularization With Optimal Medical Therapy for the Treatment of CTO) trial was the first randomized clinical trial that demonstrated some measurable clinical benefit of PCI for CTO^[25]. This prospective randomized controlled clinical trial enrolled 396 patients to compare PCI of CTO with optimal medical therapy, with a 2:1 randomization ratio. This trial showed that at 12 months, there was a greater improvement of Seattle angina questionnaire subscales with PCI vs. OMT for angina frequency (5.23, 95%CI: 1.75-8.71; $P=0.003$), and QOL (6.62, 95%CI: 1.78-11.46; $P=0.007$). Physical limitation ($P=0.02$) also showed improvement in the PCI group. Complete freedom from angina was encountered more frequently with PCI (71.6%) than with optimal medical therapy (57.8%) ($P=0.008$). Nevertheless, this study did not show improvement in hard clinical end point event rates in PCI group (although not statistically significant, number of deaths and myocardial infarctions were numerically higher in PCI group). This led investigators to conclude that PCI for CTO leads to a significant improvement of the “health status” in patients with CTO as compared with optimal medical therapy alone^[25].

On the contrary, recently presented DECISION-CTO trial^[26] showed no difference between PCI and optimal medical treatment in SAQ subscales. This discrepancy with EuroCTO trial results could be explained by study design: in EuroCTO trial there was no influence of non-CTO lesions on endpoints (these lesions were treated before randomization), while in DECISION-CTO trial non-CTO lesions were treated after randomization, which could potentially have some influence on study endpoints^[25].

Overall, the amount of high-quality data from randomized clinical trials on positive effects of CTO recanalization is relatively small. Effects are probably limited to improvement in QOL, while it was not possible to demonstrate positive effects on hard clinical outcomes such as mortality and myocardial infarction. However, in modern era of treatment of coronary artery disease, it is becoming more and more difficult to make further improvements in outcomes in the treatment of coronary artery disease, because contemporary medical treatment and overall treatment strategies have already yielded excellent results. It was not possible to demonstrate the clinical benefit of standard PCI (and revascularization in general) over optimal medical therapy alone, in stable coronary artery disease patients^[27,28]. This means that task for proving the clinical benefit of PCI for CTO is even more difficult, having in mind inherent lower procedural success rate, higher rate of complications, and lower amount of viable myocardium in the territory of vessel irrigation which usually have developed collateral circulation.

Clinical indications for PCI for CTO

Most important factors that must be taken into consideration before proceeding to CTO-PCI are the presence of symptoms and objective evidence of ischemia, while in cases of left ventricular regional wall motion abnormalities in the CTO territory, objective evidence of viability should be sought. The decision to attempt PCI for CTO should be weighted against the risk of larger amount of administered contrast, longer time of fluoroscopy, and higher rates of MACE compared with PCI for non-CTO lesion^[11]. These factors taken together could be shown in the algorithm proposed by the EuroCTO club (Figure 1, modified from^[12]). We must emphasize the importance of optimal medical therapy for the control of stable coronary artery disease, which must be the first line of intervention in patients having CTO. In recent 2018 ESC/EACTS Guidelines on myocardial revascularization, it is recommended that percutaneous revascularization of CTOs should be considered in patients with angina that is resistant to medical therapy alone or with a documented large area of ischemia in the territory of CTO (class of recommendation IIa, level of evidence B)^[29]. The class of recommendation/level of evidence for PCI of CTO was not changed from the previous version of the Guidelines^[30].

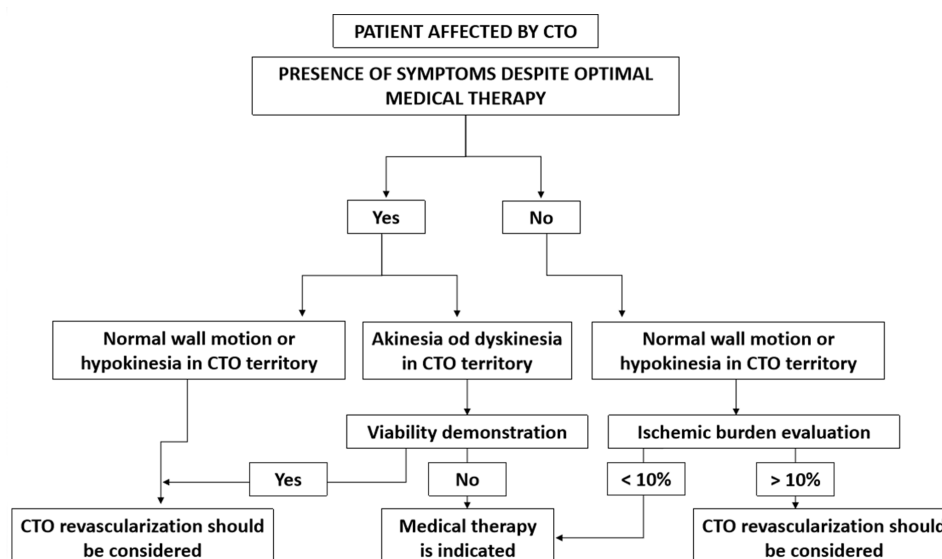


Figure 1. Indications of CTO revascularization according to symptoms, ischemia and viability. CTO: chronic total occlusions

The role of SYNTAX score is less suitable for evaluating patients with CTO than for non-CTO patients. CTO lesions are weighted higher (x5) than non-CTO lesions (x2). Therefore, patients with CTOs typically have much higher scores than those with non-CTO lesions for similar disease distribution. Consequently, the high SYNTAX scores strengthen the decision to send CTO patients more often to surgery. Since technical aspects of PCI for CTO have been significantly improved, it might be possible that impact of CTO (vs. non-CTO) lesions for SYNTAX score calculation might be overestimated^[31].

CONCLUSION

Finally, we believe that CTO recanalization performed by experienced operator still has place in interventional practice. The reasonable approach in patient selection would be to take into consideration the following aspects: the burden of symptoms, the extent of ischemia, and the amount of viable myocardium in CTO territory.

DECLARATIONS

Authors' contributions

Conception and design of the study, review of the available literature and drafting of the manuscript: Dobric M, Stojkovic S

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Consent for publication

Not applicable.

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Meta-Analysis

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Transthoracic vs. transesophageal echocardiography in transcatheter aortic valve implantation: a systematic review and meta-analysis

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Abstract

Aim: Traditionally, transcatheter aortic valve implantation (TAVI) was performed under general anesthesia (GA) accompanied by intraprocedural transesophageal echocardiography (TEE). Recently, minimalist TAVI with monitored anesthesia care (MAC) and transthoracic echocardiography (TTE) has gained popularity. However, TTE imaging quality may be suboptimal compared to TEE and may increase the risks of paravalvular leak (PVL). We sought to compare TTE to TEE for PVL (primary outcome) and secondary safety outcomes in a study-level meta-analysis.

Methods: Ovid versions of Medline and Embase were searched from 1946 to 2018 for studies comparing TTE to TEE in TAVI directly or MAC to GA in TAVI (must also specify echocardiography usage) and meta-analyzed in a random effects model.

Results: Sixteen studies ($n = 3,510$) were included in the meta-analysis. The rate of any PVL was not significantly different between TTE-TAVI and TEE-TAVI groups (18.4% vs. 21.4%, risk ratio: 1.01, 95%CI: 0.83 to 1.23, $P = 0.92$, $I^2 = 36\%$). Similarly, there were no significant differences in secondary safety outcomes. Resource utilization was lower with TTE-TAVI; hospital LOS [mean difference (MD): -1.55 days, 95%CI: -2.27 to -0.83, $P < 0.01$], contrast volume (MD: -24.75 mL, 95%CI: -49.48 to -0.03, $P = 0.05$) and procedure time (MD: -31.09 min, 95%CI: -54.77 to -7.40, $P < 0.01$) were significantly lower.



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Conclusion: The use of TTE-TAVI in conjunction with MAC was not associated with an increased risk of PVL and was associated with lower hospital resource utilization. However, other factors, such as mode of anesthesia, may have influenced these findings.

Keywords: Transcatheter aortic valve implantation, transthoracic echocardiography, transesophageal echocardiography

INTRODUCTION

The volume of transcatheter aortic valve implantation (TAVI) has surpassed surgical aortic valve replacement in Europe and North America^[1-3]. Traditionally, TAVI uses general anesthesia (GA) accompanied by intraprocedural transesophageal echocardiography (TEE) to assess root geometry and guide valve placement during the procedure^[4]. While rare, TEE can also lead to serious complications such as esophageal perforation, aspiration and oropharyngeal damage^[5].

Recently, minimalist TAVI conducted under monitored anesthesia care (MAC) has gained popularity. Studies suggest that it is less invasive and allows for earlier mobilization and patient discharge without compromising early outcomes^[6]. Since TEE is not usually conducted under MAC, minimalist TAVI often uses transthoracic echocardiography (TTE) instead^[5]. Image quality with TTE may be suboptimal compared to TEE and may have a lower sensitivity in detecting paravalvular leaks (PVL)^[5,7-8]. Studies have shown that PVL after TAVI is often poorly tolerated and associated with excess late mortality^[8,9]. Thus, understanding whether PVL increases in patients undergoing TAVI with TTE compared to TEE is paramount. There are few studies directly comparing TTE and TEE in TAVI and the sample size in these studies are limited. However, there are many studies that compare usage of MAC and GA in TAVI^[6]. Since TTE and TEE usage is closely associated with the mode of anesthesia, MAC and GA can be used as surrogate variables for TTE and TEE respectively.

This quantitative meta-analysis aims to compare early echocardiography parameters and safety outcomes in TTE-TAVI and TEE-TAVI. The primary outcome is PVL and secondary outcomes include 30-day mortality, renal failure, stroke, major bleeds, hospital length of stay (LOS), ICU LOS, fluoroscopy time, contrast volume and procedure time.

METHODS

Systematic literature review

Ovid versions of Medline and Embase were searched from 1946 to June 18, 2018 for the following key terms: “Transcatheter aortic valve implantation,” “echocardiography” and “anesthesia”. The full search strategy is shown in Supplementary Appendix S1. Inclusion criteria included the following: English, comparison of TTE to TEE or MAC to GA in TAVI, reported at least 1 chosen outcome. MAC vs GA papers must also specify the frequency of TTE and TEE usage. Exclusion criteria included the following: non-English studies, conference abstracts, proceedings, case reports and other non-comparative study designs. The citations from the literature search were compared to papers in a meta-analysis comparing MAC to GA in TAVI to ensure papers were not missed^[6].

All titles and abstracts were reviewed. Full papers of citations that could potentially be included in the study were further analyzed. The literature search results were reviewed independently by 2 investigators (T.L. and A.D.). Disagreements were discussed by investigators until an agreement was reached.

Quality assessment and data abstraction

Quality of studies were assessed with the Grading of Recommendations Assessment, Development and Evaluation approach^[10]. The following outcomes were abstracted: usage of TTE, usage of TEE, mild PVL,

moderate PVL, severe PVL, any PVL, 30-day mortality, renal failure, stroke, major bleeds, hospital LOS, ICU LOS, fluoroscopic time, contrast volume, and procedure time.

Analysis

A random effects meta-analysis was performed with R packages meta and metafor (R version 3.5.0; R Foundation for Statistical Computing, Vienna, Austria). If only the median and quartiles were provided in a study, mean and standard deviation were estimated based on protocol found in the literature^[11,12]. Risk ratios (RRs) weighted based on number of events and sample size were calculated using the Mantel-Haenszel method for perioperative dichotomous outcomes. The event rates and percentages for all subgroups were calculated based on raw data for perioperative dichotomous outcomes. Mean differences (MD) were used to analyze continuous variables as calculated by the inverse variance method. Means were also calculated based on raw data for continuous variables. Heterogeneity was reported as low ($I^2 = 0\%-25\%$), moderate ($I^2 = 26\%-50\%$), or high ($I^2 > 50\%$), as reported previously in the literature^[13]. Data was analyzed as a pooled group then as 2 sub-groups: matched and unmatched observational studies.

One additional post hoc analysis was conducted. It compared studies that only used TTE in the MAC group to studies that had mixed TTE and TEE usage in the MAC group on the outcome of PVL.

RESULTS

After searching in Ovid and Embase, 1,133 citations were screened. Sixteen citations ($n = 3510$ patients) fulfilled the inclusion and exclusion criteria. There were 10 unmatched ($n = 2037$ patients)^[7,14-22] and 6 matched ($n = 1473$ patients)^[4,23-27] retrospective observational studies [Supplementary Figure 1].

The studies were rated at low or moderate risk of bias [Supplementary Table 1]. All studies had concurrent controls. Twelve studies^[4,14-18,20,21,24-27] did not report follow up rates. One study lost more than 10% of patients in the 1 year follow-up^[7]. TTE-TAVI and TEE-TAVI shared similar baseline characteristic, including predicted mortality risk scores [Supplementary Tables 2 and 3].

Primary outcome: PVL

Event rates and relative risks for the primary outcome and periprocedural events are summarized in Table 1. Corresponding forest plots are shown in Figure 1 and Supplementary Figures 2-8. The incidence rate in pooled results for mild PVL was not significantly different when TTE-TAVI was compared to TEE-TAVI (32.1% vs. 34.0%, RR: 0.95, 95%CI: 0.72 to 1.25, $P = 0.71$, $I^2 = 54\%$). Similar non-significant results were found for moderate PVL (6.6% vs. 6.7%, RR: 0.87, 95%CI: 0.55 to 1.38, $P = 0.56$, $I^2 = 0\%$), severe PVL (0.9% vs. 0.3%, RR: 1.39, 95%CI: 0.06 to 33.21, $P = 0.84$, $I^2 = 53\%$), and any PVL³ mild (18.4% vs. 21.4%, RR: 1.01, 95%CI: 0.83 to 1.23, $P = 0.92$, $I^2 = 36\%$). All subgroups also did not show any significant difference between TTE-TAVI and TEE-TAVI.

Periprocedural events

When comparing patients that had TTE and TEE at the time of TAVI, there was no significant difference in pooled event rates for 30-day mortality (4.0% vs. 5.0%, RR: 0.69, 95%CI: 0.49 to 1.13, $P = 0.14$, $I^2 = 0\%$) [Table 1]. Similarly, when TTE-TAVI was compared to TEE-TAVI, no significant differences were found for renal failure (5.4% vs. 4.0%, RR: 0.86, 95%CI: 0.58 to 1.26, $P = 0.43$, $I^2 = 0\%$), stroke (2.8% vs. 2.7%, RR: 0.85, 95%CI: 0.52 to 1.39, $P = 0.52$, $I^2 = 0\%$), and major bleed (2.8% vs. 5.1%, RR: 0.58, 95%CI: 0.28 to 1.22, $P = 0.15$, $I^2 = 45\%$) [Table 1]. All subgroups also did not show a significant difference when TTE-TAVI was compared to TEE-TAVI.

Hospital resources

The weighted means and MDs for the continuous outcomes are summarized in Table 2. Corresponding forest plots are depicted in Figure 2 and Supplementary Figures 9-12. Hospital LOS was significantly

Table 1. Pooled event rates and relative risks for dichotomous events from meta-analysis

	TTE event rate	TEE event rate	RR (95%CI)	P value
Mild PVL				
Observational adjusted	33/93 (35.5%)	33/106 (31.1%)	1.09 (0.4 to 2.95)	0.87
Observational unadjusted	132/421 (31.4%)	158/456 (34.6%)	0.89 (0.73 to 1.08)	0.23
Pooled	165/514 (32.1%)	191/562 (34.0%)	0.95 (0.72 to 1.25)	0.71
Moderate PVL				
Observational adjusted	3/47 (6.4%)	0/64 (0.0%)	9.48 (0.5 to 179.24)	0.13
Observational unadjusted	32/482 (6.6%)	32/417 (7.7%)	0.82 (0.52 to 1.31)	0.41
Pooled	35/529 (6.6%)	32/481 (6.7%)	0.87 (0.55 to 1.38)	0.56
Severe PVL				
Observational adjusted	3/350 (0.9%)	1/311 (0.3%)	1.39 (0.06 to 33.21)	0.84
Observational unadjusted				N/A
Pooled	3/350 (0.9%)	1/311 (0.3%)	1.39 (0.06 to 33.21)	0.84
Any PVL				
Observational adjusted	118/710 (16.6%)	95/675 (14.1%)	1.34 (0.81 to 2.22)	0.25
Observational unadjusted	189/954 (19.8%)	199/698 (28.5%)	0.89 (0.76 to 1.05)	0.16
Pooled	307/1,664 (18.4%)	294/1,373 (21.4%)	1.01 (0.83 to 1.23)	0.92
30-day mortality				
Observational adjusted	5/90 (5.6%)	5/86 (5.8%)	0.96 (0.28 to 3.27)	0.94
Observational unadjusted	37/955 (3.9%)	31/628 (4.9%)	0.65 (0.37 to 1.11)	0.11
Pooled	42/1,045 (4.0%)	36/714 (5.0%)	0.69 (0.49 to 1.13)	0.14
Renal Failure				
Observational adjusted	20/707 (2.8%)	10/655 (1.5%)	1.42 (0.57 to 3.55)	0.45
Observational unadjusted	59/760 (7.8%)	36/486 (7.4%)	1.61 (0.34 to 7.7)	0.18
Pooled	79/1,467 (5.4%)	46/1,141 (4.0%)	0.86 (0.58 to 1.26)	0.43
Stroke				
Observational adjusted	15/614 (2.4%)	15/627 (2.4%)	1.01 (0.49 to 2.07)	0.99
Observational unadjusted	26/834 (3.1%)	17/555 (3.1%)	0.73 (0.37 to 1.44)	0.36
Pooled	41/1,448 (2.8%)	32/1,182 (2.7%)	0.85 (0.52 to 1.39)	0.52
Major bleed				
Observational adjusted	23/567 (4.1%)	39/563 (6.9%)	0.48 (0.13 to 1.7)	0.25
Observational unadjusted	12/689 (1.7%)	9/382 (2.4%)	0.79 (0.31 to 2.05)	0.63
Pooled	35/1,256 (2.8%)	48/945 (5.1%)	0.58 (0.28 to 1.22)	0.15

Rate of characteristics are depicted as crude counts and percentages. Using the Mantel-Haenszel method, the weighted RR ratios were calculated. TTE: transthoracic echocardiography; TEE: transesophageal echocardiography; RR: relative risk; CI: confidence interval; PVL: paravalvular leak

shorter in the TTE-TAVI group compared to the TEE-TAVI group (MD: -1.55 days, 95%CI: -2.27 to -0.83, $P \leq 0.01$). However, hospital LOS was significantly lower in unmatched observational studies (MD: -1.89 days, 95%CI: -2.6 to -1.17, $P \leq 0.01$) but equivalent in matched observational studies (MD: -0.91 days, 95%CI: -2.27 to 0.44, $P = 0.19$).

ICU LOS was not significantly different in the TTE group in comparison to the TEE group (MD: -5.6 h, 95%CI: -13.66 to 2.45, $P = 0.17$). ICU LOS was significantly lower in unmatched observational studies (MD: -12.68 h, 95%CI: -22.28 to -3.09, $P \leq 0.01$) but equivalent in matched observational studies (MD: 6.22 h, 95%CI: -16.34 to 28.78, $P = 0.59$).

Fluoroscopic time was not significantly different in pooled results when TTE-TAVI was compared to TEE-TAVI (MD: -1.34 min, 95%CI: -2.84 to 0.16, $P = 0.08$). Likewise, fluoroscopic time was significantly reduced in unmatched observational studies (MD: -1.79 min, 95%CI: -3.36 to -0.22, $P = 0.03$) but equivalent in matched observational studies (MD: -0.71 min, 95%CI: 3.91 to 2.5, $P = 0.67$).

Analysis of the data showed that contrast volume was significantly higher in the TTE group (MD: -24.75 mL, 95%CI: -49.48 to -0.03, $P = 0.05$). However, this trend was driven by the matched observational subgroup,

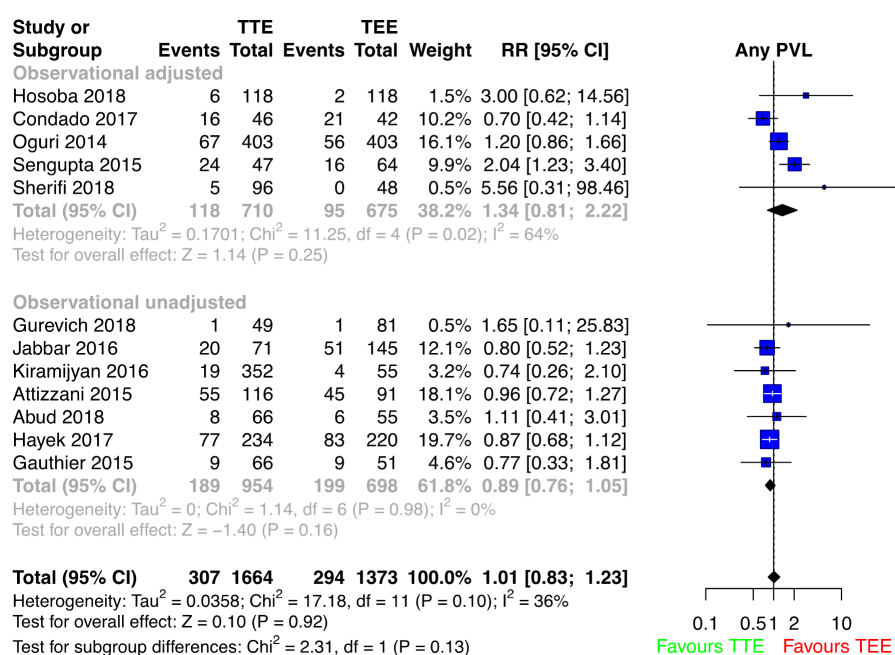


Figure 1. Forest plot for relative risk ratios of any PVL for TTE-TAVI in comparison to TEE-TAVI with subgroups based on study type. TTE; transthoracic echocardiography; TEE: transesophageal echocardiography; RR: relative risk; CI: confidence interval; PVL: paravalvular leak; TAVI: transcatheter aortic valve implantation

Table 2. Pooled event means and weighted differences for continuous events from meta-analysis

	TTE mean	TEE mean	MD (95%CI)	P value
Length of stay (days)				
Observational adjusted	6.3	7.2	-0.91 (-2.27 to 0.44)	0.19
Observational unadjusted	5.3	7.2	-1.89 (-2.6 to -1.17)	< 0.01
Pooled	5.6	7.2	-1.55 (-2.27 to -0.83)	< 0.01
ICU time (h)				
Observational adjusted	59.7	54.2	6.22 (-16.34 to 28.78)	0.59
Observational unadjusted	39.6	59.5	-12.68 (-22.28 to -3.09)	< 0.01
Pooled	47.4	49.9	-5.6 (-13.66 to 2.45)	0.17
Fluoroscopic time (min)				
Observational adjusted	20.9	21.7	-0.71 (3.91 to 2.5)	0.67
Observational unadjusted	21.0	22.8	-1.79 (-3.36 to -0.22)	0.03
Pooled	21.0	22.3	-1.34 (-2.84 to 0.16)	0.08
Contrast volume (mL)				
Observational adjusted	126.7	161.7	-35.0 (-56.89 to -13.11)	< 0.01
Observational unadjusted	96.2	119.0	-22.83 (-51.14 to 5.48)	0.11
Pooled	101.2	126.0	-24.75 (-49.48 to -0.03)	0.05
Procedure time (min)				
Observational adjusted	98.6	118.1	-44.29 (-90.96 to 2.38)	< 0.06
Observational unadjusted	123.1	142.6	-19.44 (-33.01 to -5.87)	< 0.01
Pooled	110.5	130	-31.09 (-54.77 to -7.40)	0.01

TTE: transthoracic echocardiography; TEE: transesophageal echocardiography; MD: mean difference; CI: confidence interval

which was significant (MD: -35.00 mL, 95%CI: -56.89 to -13.11, $P \leq 0.01$), and not significant in the unmatched observational subgroup (MD: -22.83 mL, 95%CI: -51.14 to 5.48, $P = 0.11$).

The procedure time was significantly shorter in the TTE group compared to the TEE group (MD: -31.09 min, 95%CI: -54.77 to -7.40, $P \leq 0.01$). The subgroups also showed similar patterns.

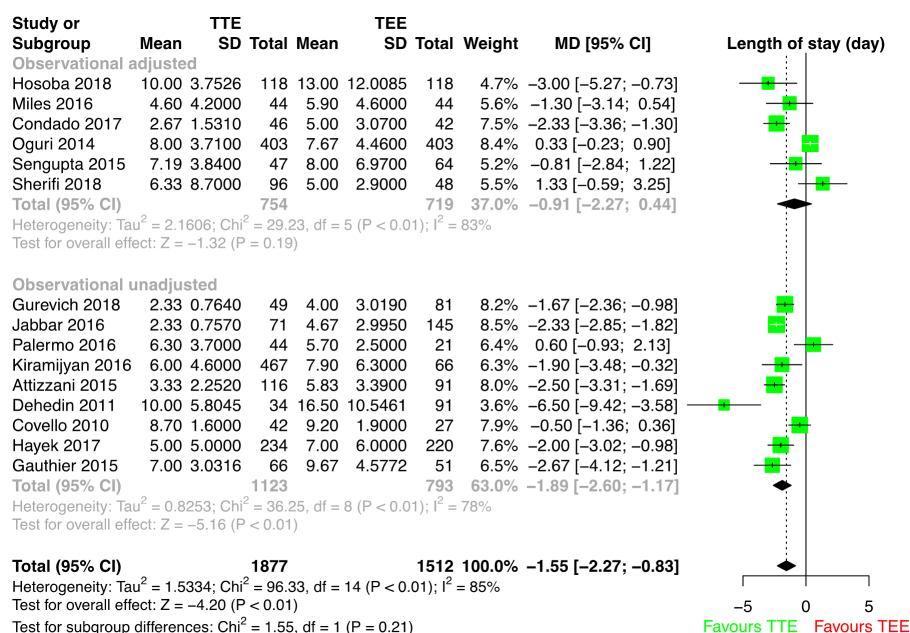


Figure 2. Forest plot for weighted mean differences (days) of length of stay for TTE-TAVI in comparison to TEE-TAVI with subgroups based on study type. TTE: transthoracic echocardiography; TEE: transesophageal echocardiography; MD: mean difference; CI: confidence interval; TAVI: transcatheter aortic valve implantation

Post hoc analyses

Any PVL rates between TTE-TAVI and TEE-TAVI were compared in a subgroup analysis of MAC groups that only used TTE vs. MAC groups that had mixed TTE and TEE usage. There were 909 patients in the 100% TTE group and 755 patients in the mixed group. In the mixed echocardiography usage group, the mean use of TTE in the TAVI with MAC group was 50.0%. There were similar crude rates of PVL between the 100% TTE group compared to the mixed echocardiography usage group (18.4% vs. 21.4%). There were no significant differences of PVL between the 100% TTE group (RR: 1.00, 95%CI: 0.79 to 1.26, $P = 0.98$) and mixed echocardiography group (RR: 1.15, 95%CI: 0.84 to 1.57, $P = 0.39$, interaction $P = 0.49$) [Supplementary Figure 13].

DISCUSSION

This is the first meta-analysis using method of anesthesia to infer TTE and TEE status in conjunction with papers that directly compare TTE-TAVI with MAC and TEE-TAVI with GA. The rate of any PVL was not significantly different between TTE-TAVI with MAC and TEE-TAVI with GA. There was also no significant difference found for mild, moderate and severe PVL between the two groups. Importantly, there were no significant differences in safety outcomes such as 30-day mortality and complication rates. Resource utilization was lower with TTE use in conjunction with MAC; hospital LOS, contrast volume and procedure time were reduced. These results are important because the acute nature of post-TAVI PVL is poorly tolerated by patients; even mild PVL leads to higher early and late mortality^[9,28]. Existing post-TAVI PVL treatments have not been widely validated^[9]. For this reason, prophylactic measures to prevent PVL is paramount to safe TAVI procedures.

Of the 16 papers included in this meta-analysis, only 2 directly compared TTE and TEE^[7,27]. Our meta-analysis narrowed the gap in the literature by using a surrogate variable, which enabled us to use an additional 5 matched and 9 unmatched observational studies for a total of 3150 patients. Similar to the previous 2 studies investigating echocardiography in TAVI, the Hayek et al.^[7] and Sherifi et al.^[27] (propensity matched) papers did not find significant differences in PVL complications between TTE-TAVI

and TEE-TAVI groups^[7,27]. However, this meta-analysis provides additional insight because it combines all available studies on this topic, allowing us to increase the sample size and power to examine for any differences.

Since TTE-TAVI with MAC was associated with shorter hospital LOS and procedure time, TTE-TAVI with MAC appeared to utilize less hospital resources without any increase in peri-operative morbidity and mortality. However, other factors may affect cost of procedure. Hayek *et al.*^[7] found that TTE-TAVI was associated with significantly higher rates of second valve implantations (odds ratio = 1.58, 95%CI: 1.01 to 2.46), which may negate the beneficial effects of shorter LOS and procedure time on cost. Hence, a more detailed cost analysis will need to be conducted to determine the economic benefits of anesthesia and echocardiography type at the time of TAVI.

Limitations

This meta-analysis should be interpreted in the context of its limitations. While this is the largest meta-analysis to date to compare TTE to TEE for any PVL, we recognize that the sample size is still limited. Thus, our sample sizes for examining the outcomes of PVL grade may be underpowered to detect differences in outcomes. Only observational studies were found in the literature after a systematic literature review. Although there were no significant differences in baseline characteristics, including surgical risk scores, observational studies inherently include a risk of confounding variables. For example, PVL is influenced by many factors such as the type of prosthesis used, differences in procedural technique and center experience^[29]. However, mode of anesthesia is the most concerning confounding variable. We acknowledge that differences in resource utilization may be the result of using MAC rather than substituting TTE for TEE alone. It is difficult to separate effects of anesthesia from the type of echocardiography as often the mode of anesthesia influences the choice of echocardiography performed. Furthermore, this study only analyzed early outcomes but did not assess late outcomes. Randomized clinical trials are needed to further investigate differences between TTE and TEE in TAVI.

In summary, the use of TTE-TAVI with MAC does not appear to adversely increase PVL rates compared to TEE-TAVI with GA. Furthermore, early mortality was similar between the two imaging modalities while resource utilization appeared to be lower with TTE, although this may have been associated with other factors such as mode of anesthesia.

DECLARATIONS

Authors' contributions

Data collection: Lam TK, Dixit AR

Data analysis/interpretation, drafting article: Lam TK

Approval of article: Lam TK, Tam DY, Dixit AR, Froles SE

Concept/design: Tam DY, Froles SE

Critical revision of article: Tam DY, Dixit AR, Froles SE

Availability of data materials

Not applicable.

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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.

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Review

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Respite for hybrid coronary revascularization

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Abstract

Hybrid coronary revascularization incorporates a surgical anastomosis of the left internal mammary artery to the left anterior descending coronary artery through a thoracotomy and percutaneous implantation of drug eluting stents in diseased non-left anterior descending coronary arteries. Hybrid coronary artery revascularization can be performed as a 1-stage procedure in a hybrid operating room or as a tightly scheduled 2-stage procedure. Hybrid coronary artery revascularization is seldom the selected modality for coronary revascularization due to the lack of a hybrid operating room in many hospitals, the recommended thoracotomy approach for bypass, or the rigid schedule of surgical and endovascular revascularization. A 2-stage approach, using a sternotomy as compared to standard thoracotomy, and a flexible schedule between surgical and endovascular procedures may facilitate the adoption of hybrid coronary revascularization with non-complex multi-vessel stable coronary artery disease.

Keywords: Hybrid, coronary artery bypass, revascularization, multi-vessel, saphenous vein graft, left anterior mammary artery, percutaneous coronary intervention

INTRODUCTION

Medical therapy aiming at the stabilization and possibly the reversal of atherosclerosis in patients with stable coronary artery disease (CAD) has steadily progressed over the past 20 years^[1-4]. However, patients with CAD who remain symptomatic despite optimal anti-anginal therapy or with limited life expectancy due to multi-vessel CAD (mCAD) are candidates for coronary artery revascularization (CAR)^[5,6]. CAR no longer requires open heart surgery for the implantation of a coronary artery bypass graft (CABG); it



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now can be achieved by percutaneous coronary intervention (PCI) with endovascular implantation of drug eluting stents (DES), which target coronary obstructions that hamper myocardial perfusion during exercise^[7-12]. The indications, benefits and shortcomings of surgical and endovascular CAR are evolving and a constant source of debate^[6,13]. However, two aspects of CAR are overwhelmingly agreed on: the left internal mammary artery (LIMA) is the longest-lasting and most event free conduit for CABG and the completeness of CAR is an important determinant of its long-term outcome^[14-16].

Due in part to LIMA long-lasting patency, CABG surgery is considered to be the CAR procedure of choice in patients with mCAD that encompasses the left anterior descending artery (LAD)^[17]. However while revascularization of non-LAD coronaries is commonly achieved with implantation of saphenous vein grafts (SVG) alongside the LIMA, another treatment option is a staged procedure, referred to as hybrid coronary revascularization (HCR), where revascularization of non-LAD coronaries is achieved by percutaneous implantation of 2nd generation DES^[18,19]. Although it was advocated for ≥ 2 decades, HCR only represents < 0.5% of the total of CABG procedures performed^[20].

The rationale for HCR and the barriers to its widespread adoption are first discussed. A pragmatic approach to HCR, that may facilitate its adoption by the cardiovascular community, is then advocated.

SAPHENOUS VEIN GRAFT PATENCY

Autologous saphenous vein was the conduit used by Favaloro in his landmark CABG operation 50 years ago^[21]. SVG still account for > 80% of conduits used in CABG surgery^[22,23]. The rate of SVG occlusions has been estimated be 15% 1-year post implantation, 1%-2% per year from year 1 to 6 and 4% per year from year 6 to 10 resulting in a patency rate of 60% at 10 years^[24]. More recent data suggests 42.8% of SVG failure (defined by $\geq 75\%$ stenosis) at 12-18 months after CABG surgery in 1828 patients who underwent angiography for clinical reasons or per protocol^[25]. The rate of SVG failure was similar 12-18 months implantation in 926 patients enrolled in the Project of EX-Vivo Vein Graft Engineering via Transfection (PREVENT) IV trial^[26]. The bulk of SVG patency rate data was collected in large volume University or Veterans Administration medical centers when 400,000 CABG operations were performed annually in the United States^[27]. Nowadays with PCI being the most common procedure for CAR^[6], CABG surgery is less performed and the current patency rate of SVG is unknown.

Rapid development of SVG atherosclerosis is the primary reason for the low SVG patency rate 5-10 years after implantation^[28]. Systemic arterial pressure and harvesting-related trauma cause endothelial damage and intimal hyperplasia. An inflammatory response follows with recruitment of immune cells, activation of pro-thrombotic factors, vascular smooth muscle cells migration and extra cellular matrix degradation^[29]. Macrophages and foam cells promote the development of necrotic cores that expand and eventually rupture leading to intra vascular thrombosis and clinically full-blown atherosclerosis^[24]. Due to its diffuse and friable nature, SVG atherosclerosis progresses rapidly and is not mitigated by implantation of DES^[28]. Further, mobilization of embolic debris and serotonin-induced vasospasm during PCI may result in a no-reflow phenomenon and in-stent restenosis^[30,31]. Targeted therapy at all the steps of the atherosclerosis cascade has failed to alter the progression of atherosclerosis in SVG. Atherosclerosis is diffuse and often concentric involving 90% to 100% of the graft circumference^[30,32].

Redo revascularization is currently the only therapeutic option for diseased SVG^[31,33], and is seldom achieved by repeat CABG surgery due to technical difficulties and a mortality that is 5-fold greater than that of the initial operation^[34-36]. Redo revascularization through PCI with implantation of bare metal stents or DES is also problematic^[37-39]. Percutaneous interventions on diseased SVG are associated with a high-rate of in-stent restenosis, target vessel revascularization, peri-procedural myocardial infarction and in-hospital mortality^[40,41]. Current wisdom advocates to focus revascularization efforts on the native vessel lesions and not on the diseased SVG^[40,41].

LIMA PATENCY AND OUTCOME

The LIMA is the conduit used for bypass of a diseased LAD in 95% of patients who undergo CABG surgery^[42]. In patients with a LAD obstruction that decreases resting blood flow, the LIMA patency rate is > 90% at 10 years post implantation^[12,43]. Failure of LIMA graft mostly occurs in patients with competitive flow between the LIMA and the native vessel as the result of low grade LAD lesion. The long-term patency of a LIMA graft to a diseased LAD guarantees normal perfusion to $\geq 50\%$ of the total myocardial mass for many years^[17,44,45]. In contrast to DES that only remedy a single focal LAD obstruction, a LIMA graft protects proximal and mid segments of the LAD against the development of new atherosclerotic lesions^[46-48]. Further, by restoring normal LAD blood flow at rest and during exercise a LIMA graft enhances downstream vascular endothelial function and thereby delays the progression of atherosclerosis^[47]. The LIMA endothelium is abundant in inducible nitric oxide (NO) synthase and thereby has a high NO concentration. The LIMA endothelium prevents graft thrombosis, slows the progression of target vessel atherosclerosis and maintains distal vessel patency^[46,48].

Observational rather than evidence-based data led to the overwhelming use of LIMA in CABG surgery^[49-51]. In the absence of evidence based data, one can only speculate on the role that the LIMA plays in the superiority of CABG surgery over PCI with 2nd generation DES in patients with complex mCAD^[47,52,53]. The absence of LIMA was found to be associated with redo operation and high mortality in patients after CABG surgery^[14,54].

In summary, despite a lack of evidence-based data, the LIMA to LAD bypass graft is largely thought to underlie the long-lasting and favorable outcome of contemporary CABG surgery.

DES

CABG surgery is rarely performed without implantation of a LIMA graft. Thus, the outcome of patients who undergo surgical revascularization with only SVG cannot be compared to that of patients who undergo PCI revascularization with current 2nd generation DES. In the absence of evidence-based data comparing the effects of SVG vs. DES on outcome measures, data must be compared from findings of individual therapeutic trials of these different CAR modalities^[55]. The incidence of in-stent thrombosis (ST) and SVG occlusions was similar at 5 years with 1st generation DES like the paclitaxel eluting stent (PES) in the SYNTAX trial^[56]. Advancements of stent technology, with the development of everolimus eluting stent (EES), have provided improvements in immediate 1-year ST rate (0.60% vs. 1.59%)^[57] and 5-year ST rate (1.30% vs. 1.86%)^[16] when compared to first-generation DES. However, as ST exerts a greater effect on mortality than SVG occlusion^[56], ischemia-driven target lesion revascularization (ID-TLR) may be a more apt comparison with SVG graft failure. In a meta-analysis from 2016, 5-year ID-TLR incidence was 7.53% with EES and 11.50% with PES^[58], which is favorable compared to a 75%-86% patency for SVG in 5-7 years^[42] [Table 1].

In summary we do not have randomized trials of surgical CAR with exclusive implantation of SVG vs. PCI endovascular revascularization with implantation of 2nd generation DES. Therapeutic trials of these 2 CAR modalities point to similar patency rate and outcome with SVG and 2nd generation DES at 5 years.

MULTI-VESSEL REVASCULARIZATION IN ACUTE CORONARY SYNDROME

Acute coronary syndrome, with its spectrum in disease from unstable angina to ST-elevation myocardial infarction (STEMI), portends future repeat cardiovascular events^[59]. CABG in STEMI is very rare, comprising about 5%-8% of STEMI presentations in the ACTION registry per year^[60]; 39% of those CABG cases were after primary PCI and median angiogram-to-CABG time was 23.3 h. Numerous studies have shown a correlation between total ischemia time and overall cardiovascular mortality in STEMI^[61-63].

Table 1. Rates of drug eluting stent target lesion revascularization¹, saphenous vein graft failure², and LIMA-LAD arterial graft failure²

	1-year	5-year	10-year
1st generation DES- Paclitaxel (PES)/Sirolimus (SES)	3.97% ³	11.50% ⁴	16.4% ⁶
2nd generation DES- Everolimus (EES)	6.03% ³	7.53% ⁴	14.8% ⁶
Saphenous vein graft	2.1%-19% ⁵	14%-25% ⁵	39% ⁸
LIMA-LAD graft	3.4% ⁷	11.9% ⁷	11.9% ⁷

¹Drug eluting stent occlusion was measured by rates of target lesion revascularization; ²saphenous vein graft patency was measured by angiographic evidence of vessel occlusion; ³1-year data from a meta-analysis comparing 1st generation DES (PES and SES) to 2nd generation DES (EES)^[57]; ⁴5-year data from a meta-analysis comparing 1st generation DES (PES) to 2nd generation DES (EES)^[16]; ⁵1-year and 5-year data from a meta-analysis comparing saphenous vein vs. arterial conduits^[42]. It should be noted that definitions of graft failure varied between the different studies; ⁶10-year data from a multicenter European randomized trial, which used pre-planned repeat angiography to reassess in-stent restenosis^[86]; ^{7,8}rates of SVG and LIMA-LAD were determined via angiography in their respective studies^[15,87]

As an attempt to reduce ischemic time and practical considerations of immediate revascularization after a diagnostic angiogram, PCI has become the most frequent revascularization strategy^[64]. There is a scarcity of data directly comparing DES with CABG in the setting of ACS, which mostly consists of subgroup analysis of larger PCI vs. CABG studies. A recent meta-analysis of these subgroups found reduced myocardial infarction (MI) incidence with CABG (3.8%) when compared to DES (7.5%) after non-ST elevation MI (NSTEMI), with similar rates of mortality (8.7% vs. 10.8%, $P = 0.248$) and stroke (2.6% vs. 2.8%, $P = 0.788$)^[65].

Recent PCI mortality data have favored immediate complete revascularization (the coronary intervention of both culprit and non-culprit obstructive stenoses) rather than culprit-lesion only PCI followed by staged-PCI of non-critical stenosis^[66,67]. When compared to culprit-only PCI, complete revascularization has shown similar rates of contrast-induced nephropathy^[68].

In summary, revascularization methods in the setting of acute coronary syndrome skew toward PCI in part due mortality benefit from decreased ischemic time. However, in head-to-head analysis there is a modest reduced subsequent MI benefit for CABG.

CONVENTIONAL HCR

HCR consists of 2 separate procedures: surgical CABG surgery with a LIMA graft to the LAD and PCI with implantation of 2nd generation DES to diseased non-LAD coronary arteries. The 2 procedures can be performed back to back in a 1-stage approach or on different days in a 2-stage approach [Table 2 and Figure 1].

The 1-stage approach is generally performed in a hybrid operating room (OR) with the LIMA graft being first anastomosed to the LAD followed by PCI with endovascular implantation of 2nd generation DES in non-LAD arteries^[19,69-71]. The surgical LIMA to LAD surgical anastomosis is commonly performed through an anterolateral thoracotomy at the level of the 4-5th intercostal space. The thoracotomy approach has the advantage of shorter ventilation time and post-operative length of stay over a conventional CABG sternotomy^[72-75]. However, thoracotomy may be associated with increased pain levels in the immediate post-operative period^[70,76]. The use of cardiopulmonary bypass is commonly left to operator preference. Percutaneous endovascular revascularization of diseased non-LAD arteries is performed after administration of loading dose of anti-platelet agents^[20]. The advantage of the 1-stage approach is the verification and possible repair of a defective LIMA-LAD anastomosis before initiation of anti-platelet therapy. Its disadvantage is the need for a hybrid OR.

The 2-stage approach encompasses an interval of 1-2 days between surgical and endovascular CAR^[71]. It allows for control of bleeding in the post-operative period before initiation of anti-platelet therapy and does

Table 2. 1-Stage vs. 2-stage hybrid coronary revascularization

	1-stage	2-stage
Advantages	Gives option for off-pump LIMA-LAD bypass, can avoid a median sternotomy by using thoracotomy with minimally invasive/endoscopic/robotic techniques, shorter ventilation and hospital time, verification and correction of LIMA-LAD grafts prior to antiplatelet therapy induction	Does not require hybrid operating room, increased time for prevention of post-operative bleeding, decreased inflammatory response to bypass surgery that may cause in-stent thrombosis, provide option for PCI-first of non-LAD lesions to avoid ischemia or infarction in those territories during LIMA-LAD surgery
Disadvantages	High cost, long operating time, requirement of hybrid operating room, increased coordination requirement between interventional cardiologist and cardiothoracic surgeon, thoracotomy option has increased pain	Increased inpatient admission time, no option for immediate repair of defective LIMA-LAD anastomosis, increased bleeding risk for PCI-first approach

LIMA: left internal mammary artery; LAD: left anterior descending; PCI: percutaneous coronary intervention

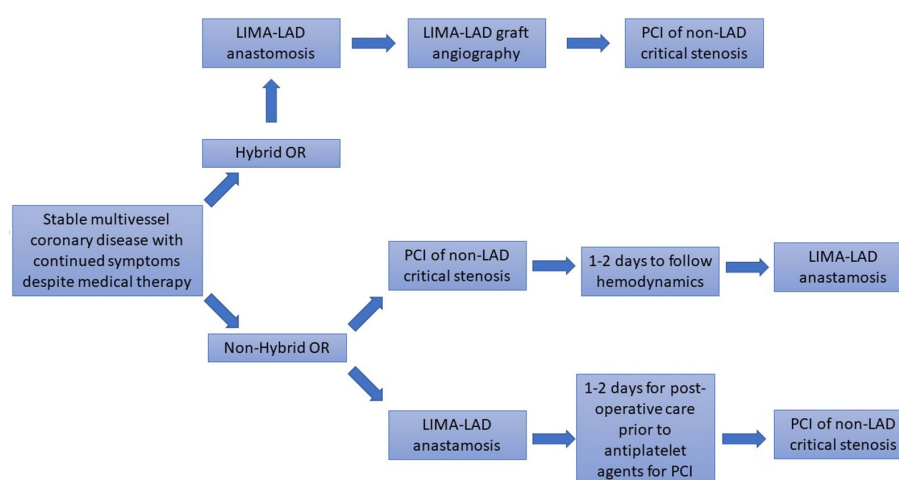


Figure 1. Hybrid Coronary Revascularization process flow-chart. This flow-chart details the steps of Hybrid Coronary Revascularization for both hybrid OR and non-hybrid OR settings. In LIMA-LAD anastomosis can be through mini-thoracotomy or sternotomy. In the hybrid-OR 1-step approach, LIMA-LAD graft can be immediately visualized with angiography. OR: operating room; LIMA: left internal mammary artery; LAD: left anterior descending; PCI: percutaneous coronary intervention

not require a hybrid OR. When patients have critical lesions of non-LAD arteries, PCI with endovascular implantation of 2nd generation DES in diseased non-LAD arteries can be first performed to avoid complications (hypotension and hemodynamic compromise) at the times of thoracotomy and the LIMA graft to the LAD^[77]. The PCI endovascular revascularization first approach increases the risk of bleeding as CABG surgery is then performed in patients who are receiving dual anti-platelet therapy^[77].

HCR IN CLINICAL PRACTICE

Given the complex multi-disciplinary nature of HCR and its pre-procedural planning, its clinical utility would be in the treatment of stable ischemic heart disease with refractory angina [Figure 2]. Coronary physiologic assessments such as fractional flow reserve^[78] have shown benefit in selecting a subset of patients who may benefit from PCI when compared to medical management. At this time there is no current data for potential use in acute coronary syndrome, but that may change over time with the increasing number of hybrid ORs and multi-disciplinary heart teams. HCR allows for a less invasive approach than traditional CABG, while providing added mortality/graft patency benefit of the LIMA-LAD over the multi-vessel PCI with DES^[79-81].

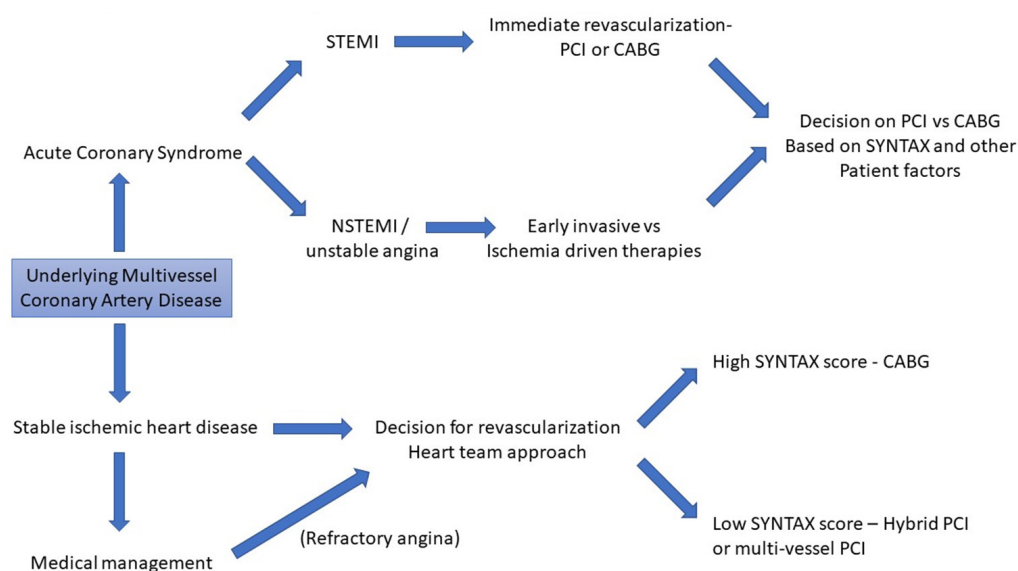


Figure 2. A proposed clinical-decision making flowchart with Hybrid Coronary Revascularization implementation. PCI: percutaneous coronary intervention; CABG: coronary artery bypass graft; STEMI: ST-elevation myocardial infarction; NSTEMI: non-ST elevation myocardial infarction

PRAGMATIC HCR

The 1-stage approach to conventional HCR requires a hybrid OR that is only available in one third of hospitals in the United States^[20]. The 1-stage approach also requires a close collaboration between interventional cardiologists and cardio-thoracic surgeons. Such collaboration is common for a transcatheter aortic valve implantation procedure but is not common practice for the management of CAD patients^[82]. The major aim of HCR is to provide CAD patients with the long-lasting beneficial outcome that LIMA affords and the complete revascularization including attention to chronic total occlusion that endovascular stenting allows^[83-85].

HCR is a most suited form of revascularization for patients with mCAD and low to intermediate SYNTAX score. Patients with mCAD and high SYNTAX score are better served by CABG surgery with implantation of multi arterial conduits and when needed SVG^[12]. A more pragmatic approach to HCR than that underlined in above-mentioned protocols is likely to facilitate its adoption by interventional cardiologists and cardio-thoracic surgeons. The 2-stage approach to HCR appears to be eminently more practical than the 1-stage approach in most American medical centers. CABG of LIMA to LAD may be conveniently performed through sternotomy as CT surgeons are more familiar with the sternotomy than the thoracotomy approach for CABG. An interval of 2-3 days between surgical and endovascular revascularization allows times for the inflammatory response to surgery to subside and for control of peri-operative bleeding before initiation of dual anti-platelet therapy. The 1-stage approach to HCR and the practice of HCR in patients with critical obstructions of non-LAD arteries will await encouraging results of the 2-stage approach to HCR.

CONCLUSION

In the age of PCI, HCR combines the known benefit of LIMA to LAD grafting with the minimally invasive approach of stenting non-LAD territories. The wide acceptance of HCR by the cardiovascular community will require demonstration of safety and long-lasting benefit on cardiovascular outcomes. A more pragmatic approach than currently outlined may help interventional cardiologists and cardiothoracic surgeons gain experience with dual surgical/endovascular coronary revascularization.

DECLARATIONS

Authors' contributions

Concept and design: Gacad V, Singh T, Motwani A, Samson R, Le Jemtel TH

Data acquisition: Gacad V, Singh T, Motwani A

Data analysis: Gacad V, Singh T, Motwani A

Manuscript preparation: Gacad V, Singh T, Motwani A

Critical revision and finalizing of the manuscript: Gacad V, Singh T, Motwani A, Samson R, Le Jemtel TH

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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Review

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Chronic total occlusion percutaneous coronary intervention complications: prevention and management

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Abstract

Percutaneous coronary intervention (PCI) of coronary chronic total occlusions (CTO) still represents a challenge in the field of interventional cardiology. Despite the rate of peri-procedural complications has decreased over the years, it remains higher than in non-CTO PCI. Coronary perforations are among the most common and serious complications. Furthermore CTO recanalization carries a risk of unique and specific complications such as donor vessel injury and equipment loss or entrapment. Other infrequent complications of non-CTO PCI such as contrast induced renal dysfunction and radiation skin injury, assume more relevance in this subset given the length and complexity of these procedures. Operators facing CTO percutaneous treatment should be aware of the potential complications and the available strategies for prevention and management, to achieve procedural success.

Keywords: Chronic total occlusion; complications; coronary perforation; donor vessel injury; equipment entrapment

INTRODUCTION

Percutaneous coronary intervention (PCI) of coronary chronic total occlusions (CTO) still represents a distinct challenge in the field of interventional cardiology. The rates of successful CTO recanalization is growing, due to numerous advances in terms of newer dedicated devices, improved techniques and extensive operators' experience. Nevertheless coronary CTO treatment continues to be a challenging issue



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Table 1. Complication incidence during CTO PCI^[1-5,16,17]

Complication	Incidence (%)
Perforation	2.6%-4.8%
Donor vessel Injury	1.1%-1.8%
Equipment loss or entrapment	-
Contrast-induced nephropathy	3.4%
Vascular access complications	0.5%-1.5%
Radiation skin injury	< 0.01%
Arrhythmia	0.8%
Stroke	< 0.01%
Urgent CABG	0.1%
Myocardial infarction	2.5%
Death	0.2%

CTO: chronic total occlusions; PCI: percutaneous coronary intervention

owing to multiple reasons including the fear for peri-procedural complications^[1]. Despite the rate of peri-procedural complications has decreased over the years, it still remains higher than in non-CTO PCI.

The most common and feared complications occurring during CTO PCI are perforations, donor vessel dissection and/or occlusion, equipment loss or entrapment, vascular access complications, contrast-induced nephropathy, radiation skin injury, periprocedural myocardial infarction, arrhythmias, transient ischemic attack or stroke [see Table 1]. There remains considerable variability in reported complications rates in literature due to data coming mainly from single centers experience. Nevertheless, more recently, data have been made available from multicenter registries enrolling 1000 (OPEN-CTO Registry^[2]) and even more (ERCTO Registry^[3]) patients in experienced CTO PCI centers.

Aim of the following chapter is to analyze the common periprocedural complications during CTO PCI, how to prevent them and the main solution techniques.

CORONARY PERFORATION

Coronary perforations (CPs) are one of the most common and serious complications occurring during CTO PCI. They have an estimated incidence of 2.6%-4.8%^[2-6] and are associated with a 5-fold increase in 30-day mortality. Cardiac tamponade occurs in approximately 10% of patients with a coronary perforation and could represent a life-threatening situation.

General approach: when perforation is confirmed, the following measures should be played out.

- Prompt specific perforation treatment (see section below for details).
- Fast echocardiography looking for pericardial effusion and cardiac tamponade indicators (right chambers collapse, respiratory variations of antegrade mitral flow). In this setting pericardial effusion can also be detected by fluoroscopy images, especially in postero-anterior views, as contrast staining in the pericardial space.
- Fluids infusion (saline solution, blood transfusion, colloids) or vasopressors in relation to the hemodynamic conditions.
- Pericardiocentesis when necessary. Autotransfusion should be taken into account in patients with persistent bleeding despite concerns have been raised due to the potential to induce hemolysis, coagulation abnormalities or possible infections, especially when large quantities are re-infused. However this procedure was shown to be safe and effective in a recent review including 30 consecutive patients^[7].
- Pain management and eventually sedation.
- In rare cases evaluate mechanical support of circulation (ECMO/Impella/IABP).

- Reversal of anticoagulation with protamine is not recommendable in perforations occurring during CTO interventions since it does not solve the mechanical problem underlying this complication and, on the other hand, it can dramatically increase the risk of thrombosis until all equipment are in coronary arteries.

Specific approach: different devices and techniques can be used in relation to the perforation site.

Coronary perforation have been classified by severity according to Ellis criteria^[8]. Ellis classification recognizes 3 grades of perforation severity (I, II and III): while grade I can occasionally resolve without intervention or just with reversal of anticoagulation, the grade III is usually referred to abrupt and large perforations with acute cardiac tamponade and hemodynamic instability and may cause myocardial infarction and death.

Nevertheless anatomic classification based on vessel location of perforation can be more helpful, orienting in the management approach. It distinguishes three types of CPs: main vessel (MV) perforation, distal target vessel perforation and collateral channel perforation^[9].

MV perforation occurs in either antegrade or retrograde approaches. Guidewire perforation during wire escalation or dissection and re-entry techniques are typically self-limited and rarely lead to cardiac tamponade. The risk of pericardial effusion dramatically increase when a device (i.e., microcatheter or CrossbossTM) or a balloon is advanced along a guidewire located in the pericardial space, outside the architecture of the vessel. The first highly important step is represented by prompt balloon inflation proximally to the area of contrast extravasation. If extravasation persists despite prolonged balloon inflation, then a covered stent should be implanted. Covered stent implantation generally requires a dual catheter technique (“ping-pong”) in order to minimize bleeding. While a balloon is maintained inflated through the first guiding catheter, a second catheter is advanced near the coronary ostium and covered stent is positioned proximal to the occluding balloon. Then the occluding balloon is deflated and withdrawn and the covered stent is advanced and deployed. Covered stents advancement can be difficult in tortuous and calcified vessels due to the lack of flexibility of these devices. In the same way operators must take into account that their delivery across not well prepared CTO lesions should be demanding.

Distal target vessel perforation typically represents a complication of antegrade approach, occurring after CTO crossing. It is usually due to advancement of stiff and/or highly hydrophilic wires into small branches. Dual arterial injection could help to prevent this type of complication, showing vessel anatomy beyond the distal cap of the CTO. Also in this case, prompt balloon dilatation proximally to the perforation site is the first treatment step. If bleeding persists, embolization with coils, fat, microsphere/beads or thrombine is required. In our experience coils release through common microcatheter (i.e., Finecross[®], Terumo) is the safer and more feasible option. We prefer detachable coils that allow a more accurate placement.

“Balloon-Microcatheter Technique” (BMT) using microcatheters compatible with 6 Fr guiding catheters can be used for treatment of this type of perforation. The BMT consists of simultaneous advancement of a microcatheter over a parallel wire distal to the occluding balloon, in order to continue to operate through the microcatheter itself without deflating the occluding balloon^[10]. This technique is able to accurately assess sealing of the perforation before and after the release of microcoils by tip injections from the jailed microcatheter, with no need of repeated deflations of the the proximal occluding balloon.

Collateral vessel perforation is a unique complication that may occur during retrograde CTO PCI. It is usually due to guidewires and/or devices advancement through the collateral or to balloon collateral dilation performed in order to facilitate retrograde devices passage. Progression to cardiac tamponade will depend on the location of the collateral vessel (i.e., septal vs. epicardial). Septal collateral perforation

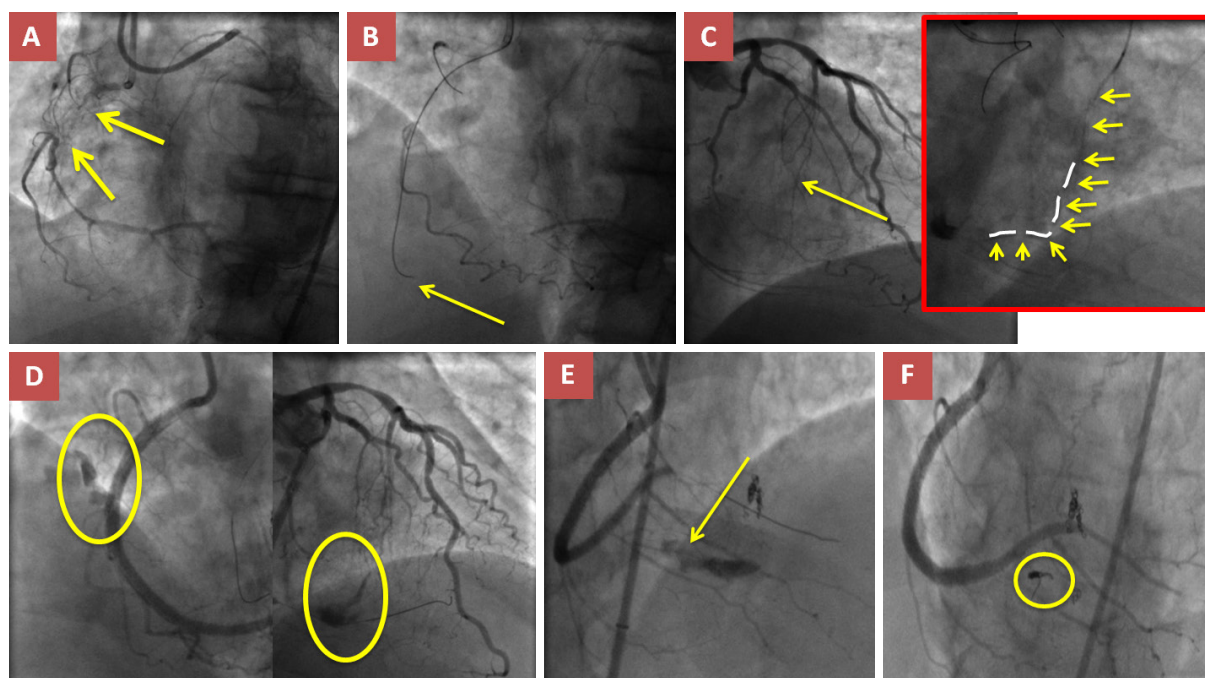


Figure 1. A: proximal RCA CTO; B: first antegrade approach was attempted but failed (guidewire outside of the vessel, yellow arrow); C: septal collateral was then chosen for retrograde attempt (see magnification in the red square); D: after reverse CART, guidewire externalization and stent implantation on RCA, perforation at level of mid RCA and septal branch were noticed (yellow circles); E: persistent extravasation and pericardial effusion increasing at echo despite covered stent implantation and coil release proximally to septal branch perforation; F: after coil release from retrograde source, no more contrast extravasation and stable pericardial effusion. CTO: chronic total occlusions; CART: controlled antegrade and retrograde tracking; RCA: right coronary artery

is typically considered a benign event because it commonly results in bleeding into interventricular septum. Septal wall hematomas that derive are usually asymptomatic, but can result in chest discomfort and rarely in heart block depending on its size and location^[11,12]. Even more rarely, a septal wall hematoma can progress to a septal wall rupture^[13] or to obstruction of the ventricular cavity with subsequent cardiogenic shock. Unusual cases of right ventricular intramural hematoma following septal perforation are also reported^[14]; they may be self-limiting or may result in hemodynamic compromise mimicking cardiac tamponade and then requiring emergency surgical evacuation. Septal vessel perforation can also take place into any cardiac chamber, including the coronary sinus: however, in this case, rarely any adverse clinical consequence occurs. In some cases septal perforations can be managed conservatively (especially when they have been caused by a guidewire) but embolization proximal to the perforation site should be considered for persisting extravasation. Anyway operators should keep in mind that in infrequent cases septal perforation may lead to pericardial effusion because these vessels can end in an epicardial course [Figure 1].

Epicardial collaterals perforation carries a high risk of pericardial effusion and rapid cardiac tamponade, due to their natural course. Moreover operators should remember that epicardial vessels receive blood flow from both antegrade and retrograde sources. Also in this case proximal balloon occlusion is the first maneuver to put in place. Then, unlike the cases above, the perforation should be approached both antegrade and retrograde. Usually embolization (i.e., coils) is the preferred treatment, both proximally and distally to the perforation site. In some cases perforation could be solved, on the antegrade side, only through a covered stent deployment in the MV interrupting blood supply to collateral. Obviously this approach presupposes that the CTO has been recanalized and the perforated vessel can be approached from both sides. At the end dual injection from both sides should be performed to confirm that there is no residual bleeding. If bleeding continues despite these measures, cardiac surgery may be required.

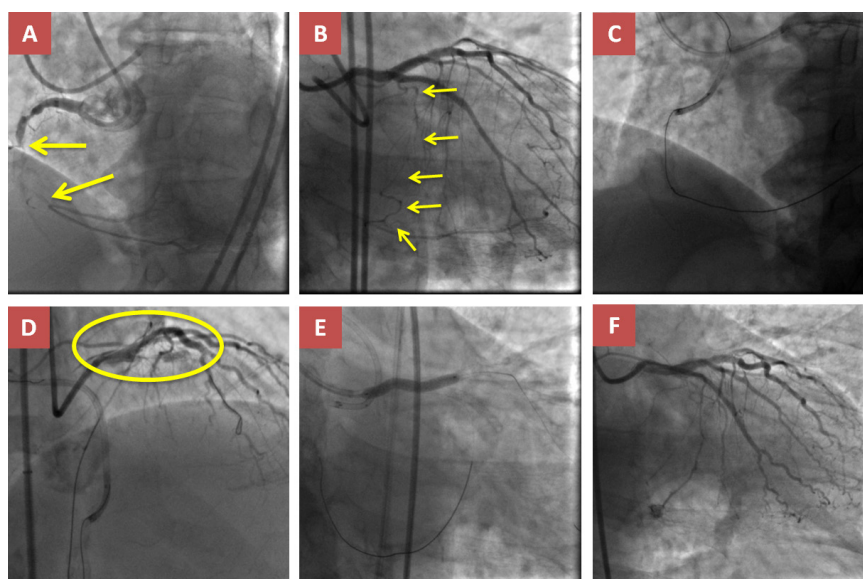


Figure 2. A:heavily calcified mid RCA CTO; B: epicardial collateral (yellow arrows) from LCx was navigated with Sion Black wire; C: then CTO was crossed retrograde with Gaia 3 wire that re-entered in right guiding catheter but the microcatheter wasn't able to cross retrograde despite double trapping; D: clinical destabilization suddenly occurred with chest pain and hypotension: angiography showed LM dissection with LAD occlusion (circle); E: LM-LAD stenting after LAD wiring; F: final angiographic result: patency of LAD and LCx, aborted CTO PCI. CTO: chronic total occlusions; RCA: right coronary artery; LAD: left anterior descending; LCx: left circumflex; LM: left main

One last consideration should be made on epicardial perforations occurring in patients with prior coronary artery bypass graft. Typically, bleeding may be focal and contained in spaces due to prior adhesions and surgical scarring, without tamponade. Nevertheless local chamber compression (i.e., atria) with focal tamponade not amenable to pericardiocentesis and requiring surgical intervention, can still occur.

Despite new materials and techniques availability in the prevention and management of CPs, a crucial role is played by the identification of the clinical/angiographic predisposing factors. A recent study of Kinnaired *et al.*^[15] investigates the principals features related to CPs during CTO PCI. Older age, female sex and previous PCI were found as main individual risk factors. Procedural factors associated with an increased risk of perforation were Crossboss/Stingray use, rotational or laser atherectomy, and microcatheter use.

DONOR VESSEL INJURY (DISSECTION AND/OR OCCLUSION)

Abrupt donor vessel injury represent a feared and unique complication occurring during retrograde CTO PCI. Its estimated incidence is 1.1%-1.8%^[16]. Leading mechanism of donor vessel injury are dissection (most common), thrombus formation/embolization, spasm or accidental air injection. Main effect of this complication is myocardial ischemia and its extension is related to the size of myocardium supplied. Thus, chest pain, electrocardiographic changes, hypotension and arrhythmias are common clinical features. Clinical and interventional management depends on the leading mechanism.

Dissection: usually retrograde CTO procedures require aggressive guiding catheters to gain the maximum support but at the expense of potential proximal donor vessel dissection. Furthermore specific attention must be paid in withdrawing equipment (wires, microcatheters), maneuver than can cause “deep intubation” and consequent dissection [Figure 2]. We recommend to place a protection guidewire along LAD at the beginning of the procedure, when retrograde approach from left coronary system is needed.

If not placed before, intra-luminal guide-wire should be promptly advanced and, if correct position is not certain, a microcatheter should be used (laminar blood back-flow and eventually tip-injection can help to confirm correct position). Also IVUS could be used to avoid intimal injection and confirm correct wire position. Once intra-luminal wire position is confirmed, balloon inflation and stent implantation usually resolve the dissection.

If sub-intimal, the wire should be left in its position and a second guide-wire should be placed distally ("parallel wire"). Whether not possible, a re-entry technique must be considered.

Thrombus formation/embolization: during CTO PCI the activated clotted time (ACT) has to be controlled every 30 min at least, to ensure correct anticoagulation. It must be maintained above 350 s. If ACT is low, correct with heparin infusion. In cases of heparin-resistance bivalirudin should be considered. When thrombosis occurs thrombus aspiration systems could be employed, followed by multiple balloon inflations but this strategy could lead to distal embolization. Gp2b/3a infusion should be also considered.

Spasm: guiding catheters and guidewires could induce an incremented vasoreactivity. In these cases intracoronary injection of vasodilators (es. Nitroglycerine, Adenosine, Nitroprusside) is recommended and usually resolve the spasm.

Accidental air injection: this is a rare eventuality that ideally should never happen. Meticulous bleed back from catheters and flushing after each device exchange should be practiced. Balloon rupture can also introduce air in the coronary arteries, though in small quantities. When occurring, air aspiration is crucial and could be combined to inotropic agents infusion. It is also possible to attempt bubbles breakdown with wires/balloons.

EQUIPMENT LOSS OR ENTRAPMENT

Equipment loss or entrapment during CTO PCI (guidewires, stents and other devices) usually represent a infrequent circumstance. Indeed it is a rarely reported complication so that its real incidence is unknown. The risk for this complication is related to lesion complexity, extent of calcifications, vessel tortuosity and techniques required for recanalization.

Knuckled wires have the potential of getting knotted. For this reason extreme caution should be paid when retrieving them in order to avoid entrapment. Guidewire fracture may also occur but rarely is associated with adverse clinical effects. Similarly microcatheters overtorsion could lead to entrapment. Balloon entrapment in highly calcified lesions and tortuous vessels a balloon could occur. In some cases balloons cannot be deflated for hypotube kinking. In both cases it is crucial to remove the device avoiding distal ischemic damage. A controlled traction on the device avoiding the system fracture should be applied as first measure. If traction is not sufficient it would be necessary to advance a "guide extension catheter".

When stent loss occurs, retrieval should be attempted in all cases by distal balloon inflation and withdrawn together with the lost stent into the guide catheter. Not retrievable stent should be crushed against the vessel wall by multiple balloon inflations and stent deployment. Finally, adequate lesion preparation, is crucial to avoid stent loss during delivery attempt.

CONTRAST-INDUCED NEPHROPATHY

CTO PCI can be long-lasting procedures with administration of high volumes of contrast medium. Accurate patient selection should be performed in order to identify those at higher risk of CIN (incidence 3.4%), carefully balancing the benefit-risk ratio of the procedure. Prevention of CIN is the first measure

to be considered in all patients undergoing CTO interventions. Many prophylactic measures have been proposed to avoid kidney damage, however hydration with saline solution administration (1 mL/kg for 6 h before and 12 h after the procedure) has been proven to have the best outcomes.

During CTO PCI, repeated contrast injection should be avoided. Retrograde approach usually requires less contrast, likely due to use of “tip-injection”. Similarly to non-CTO PCI, IVUS employment can contribute to reduce contrast amount in several ways (IVUS-guided antegrade puncture, vessel diameter and disease extension evaluation).

VASCULAR ACCESS COMPLICATIONS

CTO PCI usually requires dual arterial access and larger sheath diameter, compared to non-CTO PCI. Both conditions increase the probability of vascular access complications with reported rate of 0.5%-1.5%^[17]. Compared with femoral access, radial approach is associated with lower adverse cardiac events and major vascular complications rates across the entire spectrum of patients with stable or unstable CAD^[18]. Moreover, the development of thin-walled sheaths (e.g., 7 in 6 French Glidesheath SlenderTM, Terumo) and sheathless techniques made feasible complex PCIs requiring 7 French catheters also from the radial access. Indeed, even in CTO PCI, a fully transradial approach has been proven to be safe with a high rate of success and low complications incidence^[19]. On the other hand, due to technical aspects (e.g., multiple devices housing in the same catheter), 8 French catheters may be needed and in these cases femoral access is the most practical way. Furthermore, depending on anatomical characteristics, when strong back up support is desirable, 45 cm long femoral sheaths could provide it. For all these reasons vascular access choice is left to the operator's discretion.

RADIATION SKIN INJURY

Radiation skin injury is of particular concern in patients undergoing CTO PCI, as long fluoroscopy and cine-angiography exposure may be required to cross and treat the lesion. However radiation injury incidence is low (< 0.01%)^[5], but the data are under-reported in literature. Tricks to minimize radiation exposure are reducing frame rate of fluoroscopy and cine; another ploy is the use of stored fluoroscopy instead of angiography. Newer angiographic devices with low-dose settings could also reduce patient radiation exposure.

CONCLUSION

CTOs represent the most technically challenging lesions that interventional cardiologists face in everyday practice. However, due to newer dedicated devices and improved techniques, the rate of successful CTOs recanalization is increasing whereas the rate of complications is reducing. Nevertheless peri-procedural complications incidence, particularly in retrograde approach, is still higher than in non-CTO PCI. Operators approaching to CTO PCI should also be aware of the unique set of complications associated with CTOs recanalization. Consciousness of the potential specific CTO PCI complications is the first step to prevent and solve them. Then the knowledge of techniques and equipment available for complications management, combined with operators' experience, will contribute to safe percutaneous treatment of CTOs.

DECLARATIONS

Authors' contributions

Searched for literature data and wrote the paper: Colombo F, Bernardi A

Conceived the structure of the chapter and reviewed the manuscript: Garbo R

Availability of data and materials

All the literature data (articles, case reports and reviews) supporting this book chapter were collected from Pubmed and are open source available.

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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.

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Review

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The hybrid algorithm for chronic total occlusions

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Abstract

In the last years procedural success rate of chronic total occlusions (CTOs) percutaneous coronary intervention has improved primarily for two reasons: the evolution in materials and the new techniques and skills acquired by dedicated CTOs operators. In the last decade a lot of complex and advanced CTO techniques have been introduced. The hybrid algorithm allows to standardize the experience shared by CTO operators. The aim of the algorithm is to help the operators to choose the best strategy for the single case, in order to improve procedural success rate, to fasten the procedure, shortening failure modes, and to reduce X ray exposure and contrast load. The aim of our review is to highlight the most recent scientific evidence about the use of the hybrid algorithm for the treatment of CTO.

Keywords: Chronic total occlusion, percutaneous coronary intervention, interventional cardiology, coronary artery disease, hybrid algorithm

INTRODUCTION

In the last years the success rate of chronic total occlusions (CTOs) percutaneous coronary interventions (PCI) has rapidly improved, not only because of the evolution in materials (e.g., microcatheters, dedicated guidewires) but also thanks to the development of dedicated programs and shared strategies for operators approaching these very complex procedures. In addition, a major role has been played by the introduction of new complex techniques, including retrograde approaches and sub-intimal strategies. In details, dissection/re-entry techniques consist in gaining the sub-intimal space before/inside the CTO and re-entering in the true-lumen of the native vessel once the occlusion segment is overtaken. This can



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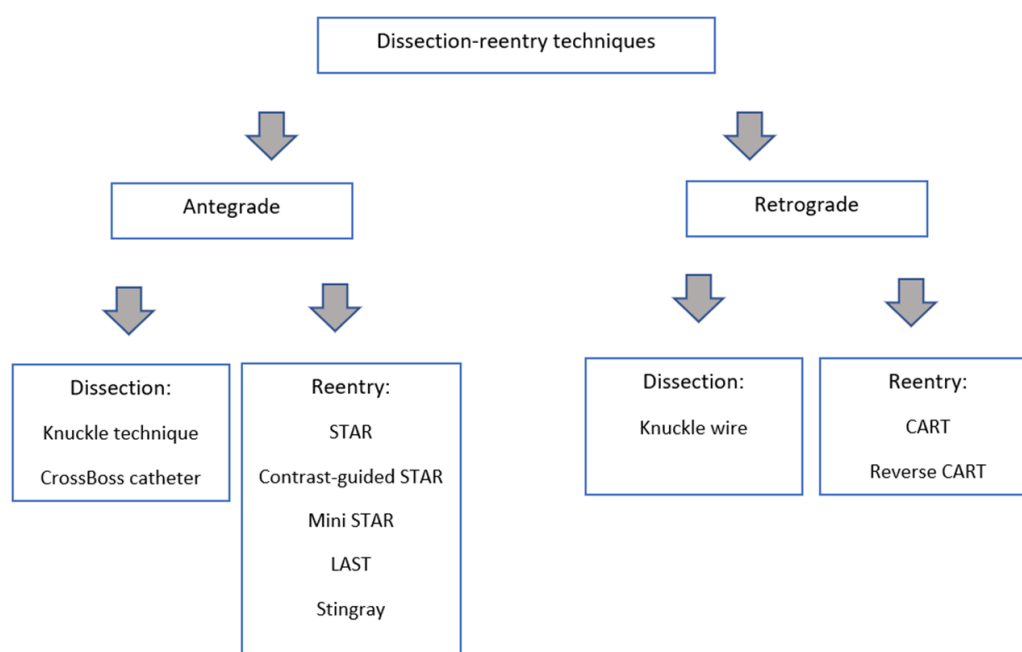


Figure 1. Flowchart showing the possible dissection/re-entry techniques. CART: controlled antegrade and retrograde subintimal tracking; LAST: limited antegrade subintimal tracking; STAR: subintimal track and reentry

be performed both via retrograde or antegrade approaches [Figure 1]. One of the first techniques was introduced by Colombo *et al.*^[1] namely the subintimal track and reentry (STAR) technique (this technique was first developed for peripheral interventions). To obtain the “dissection and re-entry” Colombo used a polymer jacket looped guidewire (“knuckle wire”) such as the Fielder-XT or the Pilot 200, that entered easily in the sub-intimal space before the occlusion (because of its hydrophilic properties) and could manage to spontaneously re-enter in the true lumen when approaching a bifurcation in a more distal segment of the vessel. Since its first description in 2005, this technique has experienced many evolutions according to different operators. In 2008, Carlino *et al.*^[2] reported for the first time its sub-intimal technique, performed by placing a microcatheter in the subintimal space, and injecting contrast to visualize the subintimal course and thus facilitating driving of the guidewire. This technique is known as contrast-guided STAR technique. In addition, the limited antegrade subintimal tracking (LAST) technique has been introduced by Lombardi^[3] in 2009: this consists in the progression of a guidewire in the subintimal space, performing a re-entry as proximal as possible after the occlusion. The guidewire must be previously knuckled to create a loop with a very acute angle, to facilitate the re-entry. With the introduction of the CrossBoss and the Stingray Catheters (Boston Scientific) since 2012 the success rate of the CTO dissection re-entry procedure has been improved. In the mini-STAR technique introduced by Galassi *et al.*^[4] since 2012 a microcatheter (generally the Finecross, Terumo, Tokyo Japan) is advanced up to the proximal cap of the CTO using a spring soft guidewire that must be exchanged with a Fielder wire that addressed the true lumen.

Focusing now on retrograde approach, the retrograde dissection can be obtained with a knuckle guidewire, the re-entry can be achieved with two different techniques. The first one is the controlled antegrade and retrograde subintimal tracking (CART) technique, introduced by Surmely *et al.*^[5] in 2006. As a first step, the operator should place the retrograde guidewire into the subintimal space. A balloon is advanced through the retrograde guidewire and inflated in the subintimal space in order to track the advancing of the antegrade guidewire into the distal vessel. The second (and currently most common) is the reverse CART technique, which is conceptually similar to the previous technique but inverted. The balloon is

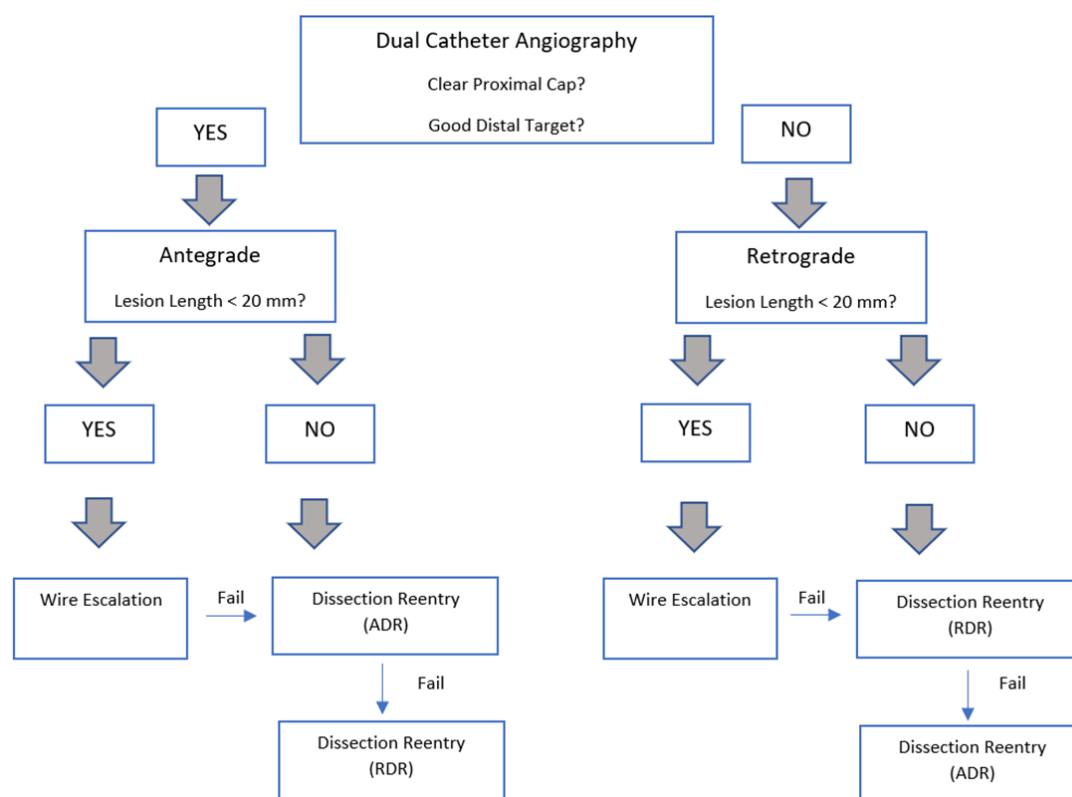


Figure 2. Illustrative representation of the hybrid algorithm. It is important to highlight the possibility to switch from one technique to the other if the previous technique is in failure mode. ADR: antegrade dissection and re-entry; RDR: retrograde dissection and re-entry

positioned in the subintimal space thorough the antegrade guidewire. Then the balloon is inflated to expand the subintimal space and to facilitate the transition of a retrograde guidewire from the subintimal space connected with the proximal true lumen.

The hybrid algorithm [Figure 2] was first described by Brilakis^[6] and colleagues in 2012, in contrast to the Japanese School that traditionally favors true lumen-to-true lumen techniques. Given this variety of new techniques and increasingly dedicated materials, the aim of the hybrid algorithm was to optimize not only success but also time and procedural steps by suggesting an easy switch among techniques to select the “right technique for the right CTO”. Theoretical additional advantage of such approach is also that of allowing reduction in exposure times for the operator and the patient. The hybrid approach indeed includes all available CTO techniques. It starts mandatorily with dual-injection angiography. In order to select the best strategies, the operator focuses on 4 main anatomic characteristics of the lesion: (1) proximal cap ambiguity; (2) distal target vessel; (3) interventional collateral; and (4) lesion length^[7]. After careful angiographic evaluation, bailout and consecutive strategies are determined upfront. It is recommended to change strategy after not more than 5-10 min without any progression.

Despite the excellent results of this algorithm in the USA and Europe, in the Asian regions this is rarely applied. The Asian Pacific CTO club has therefore introduced a new type of algorithm since 2017. There are three characteristics that guide the operator in choosing the primary strategy and if it has to be antegrade or retrograde. The three features are: proximal cap ambiguity, the quality of the vessel distally and if there are good caliber collateral branches. Unlike the Hybrid approach, the length of the lesion alone is not enough to determine whether a wire escalation or re-entry dissection technique should be more advisable^[8].

The aim of our review is to highlight the most recent scientific evidence about the use of hybrid algorithm for the treatment of CTO.

OUTCOMES OF THE HYBRID ALGORITHM

Multicenter US registry

An important Multicenter registry from the USA examined the procedural technique and outcomes of 1036 consecutive CTO PCI using the hybrid algorithm between 2012 and 2015 in 11 US centers. Technical success was achieved in 91% and a major procedural complication occurred in 1.7% of the cases. The initial crossing strategy was successful in 58% of the lesions, whereas 39% required an additional approach. In this registry, the application of the hybrid approach resulted in high procedural success and low complication rate, supporting its expanding use in CTO PCI^[9]. It is important to notice that the physicians participating in this registry were extremely experienced CTO operators with already years of expertise in CTO techniques, thus the external validity of the results of this registry in a group of physicians with less CTO expertise is debatable.

The RECHARGE registry

A solid source of data was also provided by the Registry of Crossboss and Hybrid procedures in France, the Netherlands, Belgium and United Kingdom (RECHARGE)^[10], which described the procedural and clinical outcome of the adoption of the hybrid algorithm in Europe in a group of physicians with definitely less expertise (during the registry) than the American doctors involved in the Multicenter US registry. In this prospective, multicenter registry, patients treated electively for CTO PCI were prospectively included from 17 centers between January 2014 and October 2015. A total of 1253 CTO PCI were performed. The average J-CTO score was 2 ± 1 and was higher in the failure group (2.6 ± 0.6 vs. 1.9 ± 1 , $P < 0.001$). Overall procedural success was 86% and major in-hospital complications rate was 2.6%. The primary strategy was successful in 60% of cases; a switch to a second-line strategy (as suggested by the principle of the algorithm) occurred in 34% of cases, resulting highly successful (74%). Median procedural time was 90 min (IQR 60-120 min) and median fluoroscopy time was 35 min (IQR 21-55 min), median contrast volume was 250 mL (IQR 180-340 mL). High technical success rates combined with a low event rate supported further use of this algorithm. Four techniques were mainly applied: antegrade wire escalation (AWE) was the primary strategy in 77% of patients, followed by a retrograde technique in 17% and antegrade dissection reentry in 7%. The primary strategy was successful in 91% of easy procedures (J-CTO score 0), 80% of intermediate (J-CTO score 1), 62% of difficult (J-CTO score 2) and 43% of very difficult lesions (J-CTO > 2). Second, third or more bailout crossing strategies were used in 34% of cases and were successful in 74% of the procedures, leading to an overall technical success rate of 86%.

Of note, a sub-analysis of the RECHARGE study was focused on investigating the feasibility and efficacy of the radial approach in CTO PCI. The authors analyzed the 1253 patients undergoing CTO PCI according to the hybrid algorithm and divided the population into two groups: a fully trans-radial approach (fTRA, single or bi-radial accesses) vs. transfemoral approach (TFA, defined as single femoral, bi-femoral or combined femoral/radial). A fTRA was applied in 306 (24%) cases, while 947 patients (76%) were treated in the TFA group. Technical success was achieved in 259 of 306 patients (85%) in the fTRA group and 816 of 947 patients (86%) in the TFA group, and these similar success rates were also confirmed after propensity score matching and stratification for J-CTO score. This was one of the first studies supporting the efficacy of the radial approach as a valid alternative to the conventional trans-femoral one in CTO PCI^[11].

The four angiographic characteristics of the hybrid algorithm

1. Proximal cap ambiguity: this refers to the possibility to clearly identify and define the entry point of the CTO lesion and to engage it.

2. Lesion length: lesions are divided in less versus more than 20 mm. Lesions ≥ 20 mm have a longer procedure time and lower success rate using standard wire escalation techniques^[12]. In this case the best primary approach may be dissection reentry techniques.
3. Target coronary vessel at the distal cap: this refers to the size of the lumen after the distal CTO cap, distally to the lesion itself, and the eventual presence of significantly visible side branches at the distal cap level; this characteristic has also an impact on the possibility to perform dissection/re-entry techniques.
4. Size and suitability of collateral circulation for retrograde techniques (so called “interventional collaterals”): optimal collateral vessels can be easily accessed with wires and microcatheters, are large enough to allow the passage of these devices, have minimal tortuosity and are not the only source of flow^[6].

DEVICES TO SUPPORT PCI

Wires

1. A hydrophilic and/or polymer-jacket 0.014” guidewire, low-gram force with tapered tip for outweigh microchannel or soft tissue, for collateral channel passage and for knuckle techniques (examples: Fielder XT, Fielder XT-A, Fielder XT-R, all Asahi Intecc, Nagoya Japan).
2. A non-tapered, polymer-jacket hydrophilic 0.014” guidewire for collateral channel crossing in retrograde approach. (examples: Fielder FC, Suoh 03, Sion black, all Asahi Intecc, and Pilot 50, Abbott Vascular Santa Clara, California).
3. A moderately high gram force (from 4 to 6 g) polymer-jacket non-tapered guidewire for complex lesion crossing, knuckle technique and dissection re-entry, such as Pilot 200 (Abbott vascular) guidewire.
4. A high-gram-force 0.014” guidewire with a tapered non-jacketed tip for penetration techniques, cap puncture, complex lesions crossing and lumen re-entry techniques, for example Miracle 12, Confianza Pro 12 wire (Asahi Intecc) or Hornet 14 (Boston Scientific).

Microcatheters

1. Corsair (Asahi Intecc): it's a 2.7 F microcatheter. It is originally designed for septal collateral crossing. The length for this use is 150 cm. Thanks to the screw like structure of the distal part, which uses 2 thick stainless-steel wires and 8 thin stainless-steel wires, it ensures very high crossability into tortuous vessels during retrograde approach. It reinforces the torque transmission to the guide wire and creates better backup for penetration of harder lesions. An antegrade version to support antegrade techniques (wire support and exchange) has been developed with a length of 135 cm. Both the 5 mm tapered soft tip with Tungsten powder mix and the reinforced tapered shaft after the screw like structure, are coated with a Hydrophilic Polymer, which provides lubricity and enhances maneuverability. It also allows super selective injection of contrast.
2. Caravel (Ashai): the lengths are 135 cm (for antegrade approach) and 150 cm (for retrograde approach), the tip entry profile is 0.48 mm and the microcatheter is coated with hydrophilic coating. It is compatible with 0.014” wire. It has good crossing profile and trackability and the low-profile shaft facilitates the crossing of microchannels.
3. Turnpike (Teleflex): it's a “family” of microcatheters containing a robust multi-layer shaft that provides enhanced flexibility, torque and tracking over a 0.014” guidewire. There are: the Turnpike (antegrade version 135 cm, retrograde 150 cm), the Turnpike Low Profile (LP, antegrade version 135 cm, retrograde 150 cm), the Turnpike Spiral (only antegrade 135 cm) and the Turnpike Gold (only antegrade 135 cm).

4. Small outer diameter over the wire (OTW) microcatheters like Finecross (Terumo-corporation), Quick Cross (Spectranetics corporation, Colorado) or Supercross (Teleflex) for wire support and exchange. Also of these catheters an antegrade and a retrograde version exists.

5. Small OTW balloons for support and exchange are in general not recommended in contemporary CTO PCI.

6. Tornus microcatheter (Asahi, Intecc): Tornus has a solid metal body, while the outer surface is hydrophobic coated. It is a penetration microcatheter that allows to expand the lumen of calcified vessels, providing easy access to other medical devices and improving the treatment of tight lesions. It allows to cross CTO through helical movements.

7. CrossBoss microcatheter (Boston Scientific): this is a metal OTW microcatheter with a rounded “olive-shaped” tip. Once inserted in the body of the CTO lesion, it is rotated rapidly in either direction to facilitate advancement through a CTO without the wire in the lead. It is a key device for controlled antegrade sub-intimal dissection/re-entry techniques^[13]. It creates a controlled blunt dissection of the segment tracked. Due to its shape, the chance to perforate is limited. The device tends to maintain a subintimal position during advancement. It is used only during antegrade procedures.

8. Stingray balloon and Stingray guidewire systems (Boston Scientific): it is a 1-mm flat dual-parallel balloon with 3 exits ports connected to the same guidewire lumen^[13]. It is a device for reentry purposes distal to the CTO segment (thus to be used only in antegrade procedures). The distal exit port is used to place the balloon in position. When the balloon is inflated it generates a flat surface (due to the 2 parallel balloons) that accommodates itself in the subintimal space around the intima. Once inflated, the other two exit ports, which are both just before the distal one, are 180° opposed, so one is oriented to the adventitia and the other to the lumen. It is used for true lumen re-entry^[6].

To manage complications like coronary perforation and cardiac tamponade the Cath lab must be equipped with covered stents, embolization coils, pericardiocentesis kit and the operators should be trained for the treatment of these dangerous complications.

TECHNIQUES

Access

The operator should use access routes which he/she is more familiar with^[14]. On one hand the femoral access allows the use of larger guiding catheters improving the support, on the other hand the radial approach improves patient comfort and reduces complications^[15]. Both are acceptable. In the sub-analysis from the RECHARGE trial, the use of a fTRA resulted feasible in the whole spectrum of difficulties for CTO revascularization^[11]. In these cases, more supportive guiding catheters are preferred to allow enough penetrative power and overcome the most resilient proximal cap. Guiding catheter size is usually limited to 6-7 French (occasionally 7F) from the radial approach, compared with standard 8 French used in transfemoral CTO PCI^[16]. All the operators but especially radial operators, should be familiar with all the techniques that can improve the support like balloon anchoring, mother and child techniques and guiding extension devices^[17]. The balloon anchoring is achieved by inflating a balloon into a small branch of the target vessel proximal to the CTO segment to obtain support to overcome the lesion. The mother and child technique provides to insert a smaller French catheter into a larger catheter to improve support to the guidewire. Nowadays guiding catheter extension devices such as Guideliner (Teleflex) and Guidezilla (Boston Scientific) are available and better choice than mother and child guiding catheters. It is recommended to start the procedure with ipsilateral and contralateral injection for a proper diagnostic

angiography, to be able to know adequately the anatomy and to choose properly the best primary approach and the eventual following approaches. We suggest unfractionated heparin as the best form of anticoagulation during CTO PCI, with an activated clotting time more than 350 s. Heparine is considered the safest anticoagulation method because in case of complications it can be rapidly reversed with protamine, if needed.

Antegrade wire escalation

It is the sequential use of guidewires with different characteristics from the proximal to the distal part of the lesion in order to try and remain intraluminal during the whole trajectory and to achieve directly the distal true lumen after the CTO. It is the most commonly used primary strategy in CTO PCI (around three quarter of the cases)^[10]. Following the hybrid algorithm, you can choose AWE as primary strategy in case of: (1) an unambiguous proximal cap; (2) < 20 mm occlusion length; and (3) good distal target vessel. Soft tapered polymeric wires are the initial choice. These wires can cross the occlusion through small invisible channels or by crossing the softest part of the lesion. After trying for a few minutes without progression, it is better to rapidly step up to stiff spring coil tapered wires to overcome hard and fibrotic/calcified segments, and then change again with a soft wire to complete the crossing of the CTO^[7]. Penetration force and maneuverability of the wire should always be supported by a microcatheter^[18]. The CTO crossing wires should be exchanged once they access the distal true lumen, with safer wires, to prevent distal small vessel perforation. A possible AWE technique can be applied when the first wire is constantly directing into the false lumen: the parallel wire technique. The first wire is left in the false lumen and a second wire is passed parallel: by doing so, the first wire keeps the dissection channel closed and acts as marker for advancing the second wire^[19].

Anterograde dissection re-entry techniques

Antegrade dissection occurs when a guidewire or a microcatheter is advanced within the subintimal space. This can be obtained with the CrossBoss catheter or a knuckle wire technique^[4,6]. In the latter approach, which is also the first step of the STAR technique, a 360 degrees looped polymer jacketed wire (the knuckle wire) is advanced in the direction of the distal cap of the CTO. It is important not to rotate the knuckle wire to avoid entanglement. This approach is known to be much safer than advancing stiffer guidewires without knuckle, as the knuckle tracks the vessel anatomy in the subintimal space without perforating the vessel. The CrossBoss catheter has the advantage of creating a smaller sub adventitial space than the knuckle wire, further it can facilitate the use of the Stingray system for final re-entry in the true lumen, as it created a larger subintimal channel. After subintimal crossing there are two primary method to re-entry: (1) LAST Method; (2) device-based (Stingray system).

The LAST Method

After gaining the sub-intimal space, the wire is advanced after the distal cap of the CTO, and the microcatheter is positioned near the desired re-entry point. A dedicated guidewire (stiff polymer jacket or stiff tapered) is selected and directed to the distal true-lumen in order to obtain a successful re-entry^[6].

Stingray-based reentry

The Stingray balloon has a flat shape with two side-exit ports. Upon low-pressure (2-4 atm) inflation, one exit port is automatically oriented toward the true lumen and the other one toward the vessel adventitia^[20]. The delivery catheter shaft is 0.014" guidewire compatible. The Stingray guidewire is a stiff guidewire with a 20 cm distal radiopaque segment, a 1.5 mm, 28° angle, distal bend, and a tapered tip with a 0.0035" distal prong. The Stingray guidewire is advanced through one of the two side ports of the Stingray balloon under fluoroscopic guidance to re-enter into the distal true lumen.

These techniques, specifically the Stingray-based approach, are less frequently adopted, and were often considered as the last chance in the beginning. Indeed in the RECHARGE registry and also in the data

from the US registry the adoption rate was respectively 18% and 26%^[9,10]. This can be attributed to the higher difficulty and lower predictability of success of this technique and the need for more expensive materials to obtain successful reentry. However, with expertise, this technique is gaining more and more space in the current strategical approach to CTO.

THE RETROGRADE APPROACH

Retrograde wire crossing

Retrograde wire crossing means CTO crossing from distal to proximal, with successful access to the true lumen in the section of the vessel upstream the lesion^[21]. After successful passage, the operator brings the retrograde wire and microcatheter into the antegrade guiding catheter. The last step of this technique is to exchange the retrograde wire for a long wire to be externalized from the retrograde guiding through the microcatheter in the antegrade guiding. This externalized wire is then used as an antegrade wire from the antegrade guiding catheter^[22].

Kissing wire technique

This procedure mixes antegrade and retrograde techniques. Lesion penetration is performed antegradely. If the operator finds a microchannel or if the CTO lesion is relatively soft, the antegrade wire can be advanced; the operator can stop the wire halfway through the occlusion. The retrograde wire is then advanced aiming the tip towards the antegrade wire. Eventually, the antegrade and retrograde guide wires will meet (or “kiss”). The retrograde wire is substantially used as landmark to be followed by the antegrade wire. This technique is generally used to reduce contrast exposure for the patient and to reduce the possibility of potential ambiguity regarding the course of the vessel, thus making advancement of the antegrade wire safer. After crossing the whole lesion, the antegrade wire has to be in the true lumen of the vessel downstream the CTO lesion, the balloon catheter is advanced into the occlusion and the operator can start to dilate the lesion. The kissing wire technique is rarely performed by experienced retrograde operators, as the reverse CART technique provides a more consistent method for connecting the channels in refractory cases^[17].

Knuckle technique

As previously discussed, in the knuckle technique a dissection is created by forming a loop in the retrograde wire. Ideal wires for this purpose are soft hydrophilic wires. The use of a microcatheter is crucial for allowing increased support in advancing the knuckle wire and to allow the finalization of the technique^[19]. Conventionally used wires for the knuckle technique are the Fielder XT (generating smaller knuckle and often minor sub-intimal areas) and the Pilot 200 (generating instead bigger knuckles and wider sub-intimal areas, because of its higher stiffness; this is especially useful in performing sub-intimal techniques in the right coronary artery). In more complex scenarios, where the knuckle of the wire alone is not strong enough to allow advancement through the occlusion, the use of “microcatheter knuckle” has also been described by experienced operators. Basically, this technique consists of forming a knuckle with the wire and the microcatheter together, which is then pushed forward as a unique system in order to allow for more force to overcome extreme calcifications and resistance when performing sub-intimal tracking. The “knuckle” is a key technique in the hybrid approach armamentarium. In fact, when CTO conditions appear adverse to a common true-lumen to true-lumen technique (such as AWE or retrograde wire escalation, especially when bendings > 90° and occlusion length > 20 mm coexist), creating an entry-dissection and advancing the knuckle grants a relatively easier, faster and safer way to cross the occlusion, opening the possibilities to the various re-entry techniques.

CART technique

In the CART technique, the principle is to create a dissection upstream the CTO lesion for antegrade crossing. A guidewire with the support of a microcatheter is overlapped to a balloon catheter advanced on

a guidewire placed in the subintimal space via the retrograde approach. The antegrade and the retrograde systems meet each other in the subintimal space. Multiple dilatations of the retrograde balloon create a connection between the antegrade and the retrograde spaces so that the antegrade wire may regain the true lumen distal to the occlusion^[19]. This technique is rarely used nowadays, mainly due to the complexity of tracking a balloon through the retrograde way. The reverse CART technique is currently preferred.

Reverse CART technique

Another method to connect the proximal and the distal lumen is the reverse CART technique. This method is the most commonly used. A microcatheter is placed across a collateral vessel into the subintimal space of a CTO segment, over a guidewire (via retrograde approach). An antegrade wire is advanced into the CTO segment alone or with a microcatheter or a balloon. The balloon is placed adjacent to retrograde microcatheter and inflated. The balloon angioplasty creates a connection between the two spaces. The retrograde wire can now be passed into the proximal vessel and wire externalization can be performed^[7].

DISCUSSION

According to the hybrid algorithm, a first and key point is the performance of a proper dual injection to optimally view the CTO lesion and estimate its length, the proximal cap characteristics and the quality of the distal target vessel to choose the optimal initial strategy^[23]. According to Christopoulos *et al.*^[9] the dual injection has been demonstrated crucial for achieving high success rates in CTO PCI. Indeed, the hybrid algorithm improves the success rate in CTO PCI when applied by experienced operators. In the RECHARGE^[10] the success rate was 86% with a low incidence of adverse events (2.6%). In the multicentre US registry^[9] the success with the initial crossing strategy resulted 58%, but after adoption of additional strategies a final technical success rate was 91% with a low incidence of adverse events (1.7%). The only problem could be the interpretability of the angiographic characteristics, for example the proximal cap ambiguity is subjective, and it depends on operator experience. In the RECHARGE, despite the high degree of several negative angiographic characteristics, AWE was the most frequently used first technique (77%). AWE was successful in 62% of the cases with low success rate in very difficult CTO PCI. This problem could be potentially bypassed using intravascular ultrasound imaging which would help better defining the proximal cap^[10,20]. In the same registry the algorithm's suggestion that lesion length ≥ 20 mm should drive the strategy to dissection and re-entry techniques, was less followed than expected. In 50% of cases the first strategy applied was still AWE. Two possible factors should be kept into consideration: (1) lesion length is often not clear, even if the dual injection should reduce this risk of misinterpretation; (2) operators still prefer to wire the softest tissue of the CTO with dedicated wires and new microcatheters (lower crossing profiles), while shifting to a dissection/re-entry technique is always a possible strategy in case of AWE failure. Importantly, the threshold to change strategy should be within 15 to 30 min of failure mode, to reduce contrast and radiation exposure. Overall the availability of subsequent strategies and the ability to shift between different ones is crucial to increase the success rate from 60% to 85%-90%^[10]. Indeed, as shown in the US registry, an easy switch in strategies allowed for an increase of technical success from 64% with the firstly adopted technique to a final 91%. An alternative algorithm, the minimalistic hybrid approach, was described by Zivelonghi *et al.*^[24]. The authors developed it in order to minimize the use of double access, large bore catheters and femoral approach, thus reducing patient's discomfort and procedural complications. In this sequence of steps all the techniques are included: antegrade, retrograde as well as sub-intimal and intraluminal techniques. The focus of this algorithm is to perform all the possible techniques of the hybrid algorithm minimizing the unnecessary "damage" to the patient (for example, no second access if not needed, 6F guiding catheters instead of 7-8F in case of "a priori" belief that the procedure can be performed with 6F guiding only, default radial access in case of 6F guiding).

CONCLUSION

The application of the hybrid strategies allows to achieve extremely highly procedural success rates with limited complication incidence. Although AWE is often used as an initial technique, this approach alone has a low success rate in the most complex CTO lesions. As suggested in the hybrid algorithm, when the characteristics of the CTO-lesion appear not favorable (especially when the lesion length is more than 20 mm) the direct use of a dissection re-entry technique can be considered -both antegrade or retrograde- depending on the proximal cap characteristics and the distal vessel morphology. Implementing this strategy could result in higher success rate, lower complication rate and a limitation of the radiation exposure for both operators and patients, as well as contrast exposure for the patients. Another important consideration is that the operators should be trained for the management of potentially life-threatening complications, like coronary perforation and cardiac tamponade.

FUTURE DIRECTIONS

The hybrid approach has been proven to be very effective and has also shown to reduce the rate of complications. The next step is to train potential CTO operators so that the correct diffusion of this algorithm will further improve success rates, especially in lower CTO PCI volume centers. In our view proctoring is a crucial step for a steep increase in expertise of new CTO operators. Moreover, we need efforts to further improve patient comfort and to reduce access related complications by minimizing the use of dual access, large bore catheters and femoral approach when it is possible, without impacting the efficiency of the hybrid algorithm.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Zivelonghi C, Budassi S, Agostoni P

Performed data acquisition, as well as provided administrative, technical, and material support: Budassi S, Zivelonghi C, Agostoni P

Availability of data and materials

Not applicable.

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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.

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Review

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Atrial septal defect repair in the age of transcatheter devices

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Abstract

The aim of this review is to discuss the management of atrial septal defects (ASD) in the adult patient paying special attention to the elderly population and the most recent transcatheter advancements. ASDs are characterized by the following categories: ostium secundum, ostium primum, sinus venosus, and coronary sinus defects; though multiple defects may exist concurrently. Intervention for closure of ASDs are indicated with the development of right ventricular volume overload, or in the clinical context of paradoxical embolic stroke. Previously, there was significant disagreement regarding the timing of ASD closure in adult patients, but there is now general consensus that adult patients with clinical evidence of right ventricular overload should undergo closure of ASDs at the time of presentation. The present review describes the typical presentation of patients with symptomatic ASD's, medical management, and whether surgical or percutaneous approach should be pursued. We will also discuss other important considerations for patient selection and potential early and late complications of transcatheter ASD closure such as congestive heart failure, device embolization, and tissue erosion. At the time of this writing, there are currently three FDA-approved devices for percutaneous VSD closure including the Amplatzer™ Septal Occluder (ASO, St. Jude Medical, St. Paul, MN), Gore HELEX™ Septal Occluder (W.L. Gore and Associates, Newark, NJ), and Gore CARDIOFORM™ Septal occluder (GCSO, W.L. Gore and Associates, Newark, NJ) devices. Many premarket approvals were granted for devices that never went to market due to poor investigational study performance. Likewise, the HELEX device has since been discontinued upon bringing the GCSO device to market. We will focus primarily on the ASO device with a brief review of current investigations into the GCSO device, both of which carry an indication for closure small to medium sized ASDs in the ostium secundum position. Additionally, this review covers the safety of transcatheter closure of ASDs with currently available devices, review studies associated with devices available outside the United States, and perioperative considerations for transcatheter



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intervention. Obstacles to device employment and countermeasures to overcome operational challenges will also be discussed. To this end, variations or similarities of currently approved devices will be emphasized throughout this discussion where possible. Lastly, we will offer insights into device evolution trends with the expectation of new device developments on the horizon. We will briefly discuss up and coming areas of active research, including the emerging fields of novel biomaterials and gene therapy.

Keywords: Atrial septal defect repair, transcatheter, endovascular, elderly, current methods

INTRODUCTION

Atrial septal defects (ASD) are one of the most common congenital cardiac abnormalities reported both in adolescent and adult populations. The incidence of newly diagnosed atrial septal defects are second only to bicuspid aortic valves as the most common congenital heart disease in children, with ASDs accounting for the majority of congenital malformations diagnosed in adults^[1]. ASDs may be detected in asymptomatic patients, though physical findings may be subtle at best making detection prior to associated symptoms difficult in most clinical settings^[2]. Though patients with ASDs may remain asymptomatic well into adulthood, undetected ASDs may lead to potentially irreversible complications such as arrhythmias, pulmonary hypertension, stroke or their associated sequelae^[3,4]. The true incidence of ASD may be significantly underestimated due to the nature of their relatively silent clinical course. One study estimates 941 per one million live births have an ASD based on the metaanalysis of 43 studies, which accounts for an estimated 30%-40% of adult congenital cardiac abnormalities^[5-7]. Ostium secundum defects are the most commonly reported ASD as compared to defects associated with the septum primum, sinus venosus, or unroofed coronary sinus which occur in descending frequency respectively^[8]. Although surgical closure of ASD is considered to be safe, efficacious, and time-tested, it requires open heart surgery, longer hospital stays, and may not be suitable for elderly patients with concomitant comorbidities^[9].

MORPHOLOGY AND CLINICAL FEATURES OF ASDS

Location, Morphology, and suitability for surgery vs. transcatheter intervention

It is important to note that morphological variations of different types of ASDs, which determines whether a particular defect is amenable for transcatheter closure. Briefly, ASDs fit into four major classes: ostium secundum, ostium primum, sinus venosus, and unroofed coronary sinus [Figure 1]. Ostium secundum defects are characterized by enlarged foramen ovale with insufficient septum secundum development, causing incomplete closure and fusion of the atrial septum. Secundum type defects are the most common atrial septal malformation accounting for up to 80% of ASDs^[10]. Secundum type defects are considered ideal for transcatheter ASD closure due to their size and surrounding tissue for device fixture. Ostium primum defects, also known as endocardial cushion defects, are defects at the level left or right atrioventricular valves. Sinus venosus defects are located in proximity to either the superior or inferior vena cava. Lastly, coronary sinus septal defects are characterized as an “unroofing” of the coronary sinus in which allows communication between the coronary sinus and the LA. Mixed defects, or those involving multiple defect types are also possible, though less commonly reported are also typically repaired with open surgery. Although, surgical repair is considered as the standard method of treatment for all but secundum type defects, case reports exist describing multiple ASDs and coronary sinus defects which transcatheter closure was successful without significant valvular impairment or conduction disturbance^[11,12].

ASD variation with age

Clinical characteristics of ASDs differ significantly in pediatric populations as compared to adults. ASDs are detected in asymptomatic children with increasing frequency due to non-invasive screening modalities such as echocardiography, routine ECG, and even prior to birth during routine obstetric

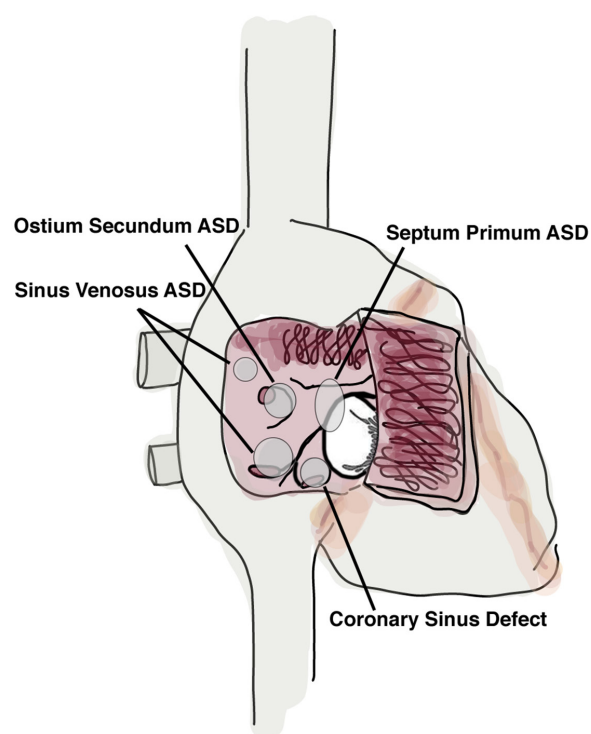


Figure 1. ASD locations. ASD: atrial septal defects

wellness sonograms^[13]. Furthermore, studies show referral to specialty services occurs at an earlier age if subtle findings are detected on physical examination despite lack of symptoms. One such single center retrospective study indicated the median age of diagnosis was 5 months of age^[14]. Areas of increasing interest in pediatric management include predictors of spontaneous ASD closure. Many predictors of spontaneous closure have been proposed; the longest held predictor appears to be size of the ASD at time of detection with small defects (3-5 mm) having up to 87% closure rates and large defects (> 8 mm) conferring much lower closure rates (0%-8%)^[14-16]. Others suggest the use of patient age at time of detection or normal weight gain after detection as clinical predictors for spontaneous ASD closure^[17].

Adult populations with ASDs are typically asymptomatic with great variability in the onset of symptoms. More common symptoms appear to be early onset atrial flutter or atrial fibrillation due to atrial stretch, and less commonly decompensated right heart failure in patients under 40 years of age^[18]. The natural history and subsequent prognosis were reported by Campbell^[19] with progressive worsening mortality approaching 90% by the 6th decade in patients with uncorrected defects.

Untreated atrial septal defect in the elderly

Elderly patients with hemodynamically significant defects more frequently encounter complications with long-term adverse consequences such as atrial arrhythmia, pulmonary hypertension, and atrioventricular valvular insufficiencies related to chronic ventricular volume overload^[20]. These patients have comparatively higher prevalence of co-morbid diseases including diabetes mellitus, stroke, systemic hypertension, chronic lung diseases atherosclerosis and coronary heart diseases^[21-23]. Longstanding left to right shunt at the atrial level further results in a progressive atrial stretch and right ventricular dilatation which in turn eventually leads to tricuspid insufficiency^[24]. The left heart may also be influenced by way of increased atrial pressure, chronic volume under load, and co-morbid diseases like systemic hypertension or coronary heart disease^[6]. Furthermore, chronic LV unloading due to the left to right shunt, and diastolic compression of left ventricle

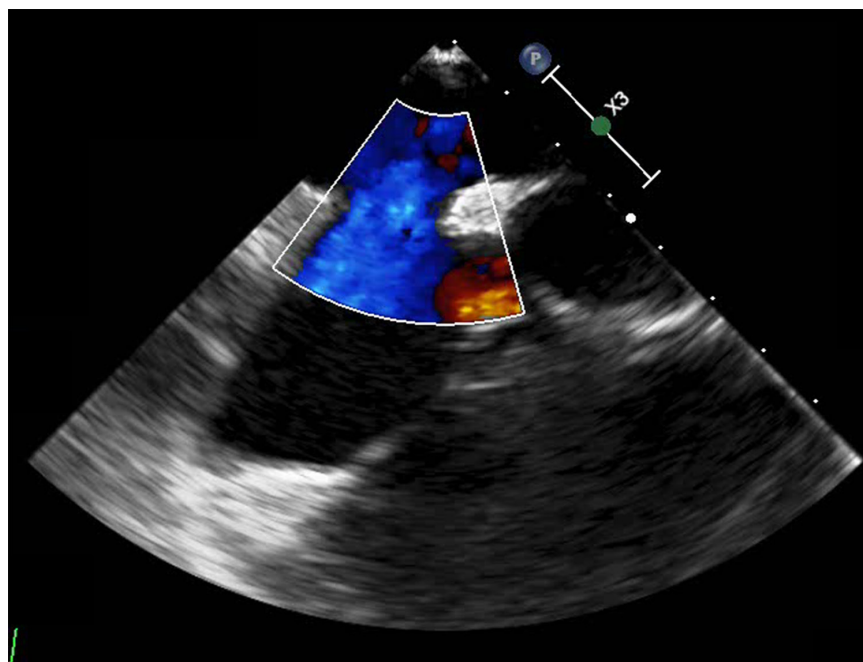


Figure 2. 2D Doppler Echo demonstrating atrial septal defects left-to-right shunt

by the dilated right ventricle may further reduce the LV end-diastolic volume in the chronic state. This so called “masked LV restriction” may lead to development of pulmonary edema secondary to LV dysfunction and left atrium (LA) pressure increase after ASD closure^[22,25]. Due to the chronic nature of the condition, patients usually adjust their activity level to adapt to their relative disabilities, and invasive interventions are placed under increasing scrutiny due to the paucity of evidence for survival benefit. Prospective studies evaluating quality of life improvements, or elucidating risk *vs.* objective benefit are called for to establish the role of ASD closure in the elderly.

IMAGING MODALITIES FOR ASD EVALUATION

Echocardiography

Conventional transthoracic echocardiography (TTE) is capable of identifying the presence of ASDs, characterizing chamber dilatation, estimated pulmonary artery pressure, shunt ratio, and other coexisting cardiac conditions. [Figure 2](#) demonstrates doppler imaging of an unrepaired ASD. Tissue doppler imaging may be of particular use in elderly patients who suffer pronounced LV diastolic dysfunction. One recent study suggests patients at risk for post ASD closure congestive heart failure by measuring early mitral annular velocity to help direct volume management during and after ASD closure^[26]. In regard to assessment of ASD morphology, including maximum defect dimensions and characterization of the surrounding tissue rim, 2D TTE is somewhat limited. These limitations are surmounted with the adjunct of transesophageal echocardiography (TEE) which offers a stepwise enhancement in characterizing the size, location, and tissue rim surrounding ASDs to determine suitability for transcatheter repair. TEE is considered a semi-invasive procedure so is undertaken only after initial evaluation with TTE^[27,28].

3D echocardiography

3D echocardiography provides better spatial visualization than conventional echocardiography. An example of a diagnostic 3D TEE visualizing an unrepaired defect can be seen in [Figure 3A](#). 3D TEE can also depict 3D structures in great detail with high-resolution images allowing for enhanced understanding of complex valvular and congenital heart defects^[27]. Initially, 3D echocardiography was reconstructed from serial 2D

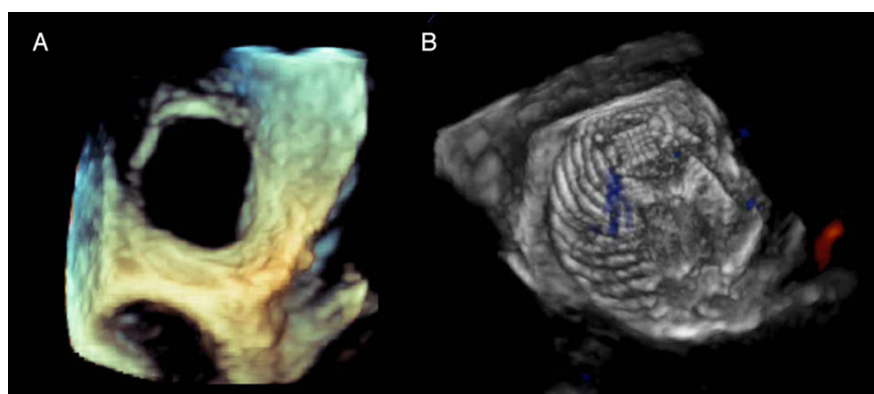


Figure 3. 3D transesophageal echocardiography visualization of atrial septal defects (A); same lesion after deployment of Amplatzer device (B)

images, which is time-consuming and resource intensive. Nowadays real-time 3D echocardiography with matrix array transducer is available in TTE as well as TEE. 3D TTE is a promising technology capable of providing comprehensive en face images of ASD with the added benefits of being noninvasive, lower cost than TEE, portability, and carries better accessibility than TEE. 3D TTE has a potential to provide accurate information on ASD morphology such as size, location, and surrounding rims for treatment both in children and adult populations. Furthermore, real-time 3D TEE has utility in providing accurate real-time information about complex ASDs, especially those in patients with multiple ASDs, and allowing for real-time feedback of device deployment positioning^[29,30].

Intrauterine Sonographic Detection of ASD

The International Society of Ultrasound in Obstetrics and Gynecology recently published guidelines on the detection of fetal cardiac anomalies in 2017 with the goal of improving early detection by obstetricians and family practitioners^[31]. The feasibility of ASD detection via intrauterine sonogram has been demonstrated by many isolated case reports, retrospective analysis, and prospective studies^[32-34]. Despite this, there are few reports on the sensitivity of intrauterine ASD detection (30%-74%), and should be relegated to pregnancies that carry high risk for cardiac abnormalities^[34-36].

Transcranial doppler ultrasonography

Transcranial doppler ultrasonography (TCD) is a viable alternative to TTE or TEE for screening and follow-up evaluation of ASD. It offers a relative degree of comfort over TEE, and offers sensitivities equivalent to TTE in terms of identifying right to left shunts, but cannot detect other associated defects that echocardiography can^[37].

Intracardiac Echocardiogram

Intracardiac echocardiography (ICE) offers superior visualization of the septal morphology during transcatheter device deployment^[38]. It is, as the name suggests, invasive and requires additional femoral vessel access for deployment. Real time 4D ICE also appears to be on the horizon with reports on its development and pilot study usage are now emerging^[39].

MANAGEMENT OF ASD

Surgical vs. Medical management of Secundum ASD

ASDs are considered for closure in symptomatic patients where a left to right shunt is present with evidence of right heart pressure overload (right atrial or ventricular enlargement), and pulmonary to systemic blood flow ratio (Qp:Qs) is greater than 1.5:1^[40]. In addition to right heart overload and Qp:Qs > 1.5:1, in

asymptomatic patients, those whose pulmonary artery pressure is less than 50 percent systemic arterial pressure, and pulmonary vascular resistance is greater than one third the peripheral vascular resistance, without exercise induced cyanosis, are recommended for ASD closure^[40-42].

Of great interest to clinicians in the age of readily available transcatheter repair of secundum type ASDs is the decision to pursue transcatheter or open surgical repair. One such study at Mayo Clinic sought to evaluate outcomes of surgically managed ASD cases as compared to medical management alone with a follow-up interval of 27 to 32 years after the index surgery. Study findings demonstrated that the survival rate was 74% as compared to 85% for age sex matched medically managed controls. In cases where surgical intervention occurred below the age of 24 years, survival rate reported was the same as age matched controls. Independent predictors of long-term survival were age at the time of operation and main pulmonary artery systolic Pressure (PASP)^[43]. The more recent study of the Danish population found that there was a significant reduction in the life expectancy and lower quality of life in patients with small ASDs that did not meet criteria for repair when compared to the general population^[44]. In another study, surgical vs. medical management were compared prospectively in a randomized clinical trial of 473 patients over the age of 40 years with a median follow up period of 7.3 years. Overall mortality rate was not statistically different. However, there was a higher rate of recurrent pneumonia noted in the medical arm. There was indeed a trend higher complication rates such as CHF, sudden cardiac death, and overall mortality in the medical arm, but was ultimately not statistically significance^[45].

More recently the 2018 ACC/AHA task force undertook a meta-analysis seeking to understand differences in outcomes of medical vs interventional management of secundum type ASDs. Their analysis found 11 studies that met criteria for inclusion, and in most instances found a protective effect with bearing on reduction of symptoms, functional capacity, and improvement of hemodynamic characteristics following either surgical or transcatheter intervention^[46]. Interestingly, in the same analysis, there was either insufficient data to determine relative risk of death, or a weakly positive protective effect after intervention, from the included studies. Furthermore, a nationwide study of patients with corrected and uncorrected ASDs were compared to the general population with interesting results; their findings demonstrated a relative reduction in mortality for patients who underwent repair of ASDs. But whether repaired or not, patients with ASDs patients experienced a shorter lifespan when compared to the general public^[47]. Taking the results of these, and similar studies, may help establish important expectations when discussing intervention; quality of life and reduction of symptoms, rather than preventing mortality, may lead discussions pertaining to goals of therapy.

Surgical vs. transcatheter intervention

At the time of this writing, only secundum type defects have transcatheter devices approved for intervention. Primum, Sinus Venosus, and Coronary sinus defects still carry the recommendation of open surgical intervention with only rare reports of transcatheter interventions published^[48-51]. In the pediatric population there are similar long term outcomes between surgical vs. transcatheter intervention, however cost analysis demonstrates a better value in terms of overall cost as well as shorter length of stay for patients undergoing transcatheter repair^[52]. In the adult population, mortality between transcatheter and surgical intervention are similar, but long-term reintervention rates appear to vary between the two. In a review of 718 procedures, Kotowycz *et al.*^[53] report that long term reintervention rates for transcatheter repair are more common than compared with conventional surgical approach. Other studies report similar findings - but prospective randomized studies have not been undertaken to determine true differences. Furthermore, patients more likely to undergo transcatheter repair are often higher risk than patients deemed appropriate for surgical intervention potentially skewing attempts to study differences in outcomes.

Of growing interest is the prospect of treating ASDs associated with sinus venosus defects, which are traditionally treated with open surgical repair. Presently, there are only case reports describing transcatheter

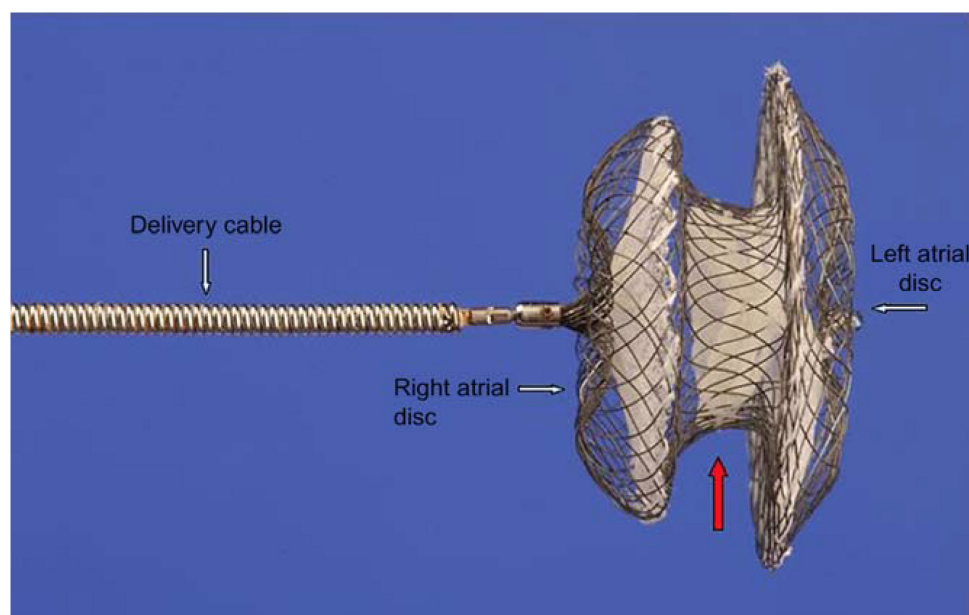


Figure 4. St Jude Amplatzer Device, reproduced under creative commons license, Thomson and Quereshi 2015

approaches. Two such case reports technical success with correcting partial anomalous pulmonary venous return of the right upper pulmonary vein by deploying a covered stent graft into the affected pulmonary vein^[54,55]. Abdullah *et al.*^[56] describe an approach that combines covered stent grafts and occlusion devices to correct sinus venosus defects successfully in four patients; of which two required re-intervention with an additional covered stent or PFO closure device, but all without significant complications at the 12-month follow-up point^[56]. Others have reported success with the immediate release patch, which has been under investigation in translational animal studies as a potential alternative to metallic devices^[57,58].

Transcatheter devices available today for closure of secundum ASDs

The origins of transcatheter ASD repair can be traced back to King's report of non-operative ASD closure during cardiac catheterization in 1976^[59]. Formal development of a device for ASD, however, is attributed to the Atrial Septal Defect Occluding System (ASDOS) submitted by Babic *et al* in 1990^[60]. Though successive iterations made the device more user friendly and showed early promise, the ASDOS was abandoned in 2001 with the development of newer generations of transcatheter devices. A history of transcatheter device evolution has been detailed by Nassif *et al.*^[61]. Today, transcatheter ASD closure is associated with low complications, short duration anesthesia, short hospital stay, and well documented long-term symptom follow up^[62-64]. Transcatheter ASD closure is now considered the first choice of treatment as opposed to surgical intervention. The most widely employed device worldwide, and one of two FDA approved devices for use in North America is the Amplatzer device, shown in Figure 4. Echocardiography, either ICE or TEE play a considerable role in the guidance of these procedures and in further assessment of the final results. Areas of active research focus on examining other imaging modalities like magnetic resonance imaging or computed tomography to construct 3D topographical visualizations of the heart and associated defects prior to transcatheter ASD closure^[65-69]. A review of recent publications describing the outcomes and population size of the respective studies are listed in Table 1, including devices otherwise available outside of the United States.

ASD characteristics amenable for percutaneous closure

Two crucial parameters should be evaluated in patients with secundum septal defect prior to intervention: maximal ASD and surrounding rim dimensions. Presently, the Amplatzer device is capable of closing

Table 1. Recent publications describing ASD device outcomes

Device name	Manufacturer	Approval	Recent/ongoing studies	n	Significant findings
Cocoon	Vascular Concepts Pakkret, Thailand	CE Mark	Lairakdomrong <i>et al.</i> ^[70] , 2013 - retrospective	63	100% closure at 12 mo, 3 early embolization requiring surgical
			Thanopoulos <i>et al.</i> ^[71] , 2014 - prospective observational	92	100% closure at 6 mo, no adverse events
Ultrasept II	Cardia Eagan, MN, USA	CE Mark	Mijangos-Vázquez <i>et al.</i> ^[72] , 2018 - retrospective	30	100% closure at 6 mo, no adverse events
			Bartel <i>et al.</i> ^[73] , 2010 - case series	2	2 reports of fabric erosion requiring surgical removal
			Aubry <i>et al.</i> ^[74] , 2014 - case series	9	2 out of 9 experienced fabric erosion requiring surgical removal
			Bozyel and Özcan ^[75] - 2017, retrospective	9	3 out of 9 patients with device required surgical removal
			Chamié <i>et al.</i> ^[76] , 2016 - case series	4	4 out of 70 developed early fabric erosion, treated with device in device
Nit Occlude ASD-R	PFM Medical Mepro Köln, Germany	CE Mark	Peirone <i>et al.</i> ^[77] , 2014 - prospective observational	73	98.6% closure at 11 mo, no adverse events
			Bulut <i>et al.</i> ^[78] , 2016 - prospective observational	30	98% closure at 10 mo, 1 erosion requiring surgical removal
Ceraflex ASD		CE Mark	Astarcioğlu <i>et al.</i> ^[79] , 2015 - prospective non-randomized	58	100% closure at 6 mo, no adverse events
			Apostolopoulou <i>et al.</i> ^[80] , 2018 - retrospective	183	100% closure at 22 mo, no adverse events
Figulla Flex II	Occlutech Jena, Germany	CE Mark	Kenny <i>et al.</i> ^[81] , 2018 - prospective randomized	107	94.4% closure at 6 mo, 1 device embolization
			Haas <i>et al.</i> ^[82] , 2016 - retrospective	1315	97.3% closure at 12 mo, 5 device embolization, 3 AV block
			Godart <i>et al.</i> ^[83] , 2014 - retrospective	31	90.3% closure at 36 mo, 1 device embolization, 1 AV block
			Roymanee <i>et al.</i> ^[84] , 2015 - retrospective	77	97.4% closure at 43 year, 2 device embolization, non-inferiority to ASO
			Aytemir <i>et al.</i> ^[62] , 2013 - retrospective	58	99.3% closure at 12 mo, 2 device embolization, 4 embolic events, 2 device thrombosis
Cardioform	WL Gore Flagstaff, AZ, USA	CE Mark	Kim <i>et al.</i> ^[85] , 2019 - retrospective	152	100% closure at 25 mo
			GORE Assured Study, ongoing ^[86]	522	Clinical Trial NCT02985684, enrollment complete, final results by 2022
			Hemptinne <i>et al.</i> ^[87] , 2017 - retrospective	26	100% closure at 6 mo, 5 wire frame fractures
			Kim <i>et al.</i> ^[85] , 2019 - retrospective	17	100% closure at 23 mo
Amplatzer	St. Jude Medical St. Paul, MN, USA	CE Mark	Grohmann ^[88] - 2016, retrospective	173	95.4% closure at 20 mo, 4 device embolization, 3 AV Block
			Turner <i>et al.</i> ^[89] , 2017 - prospective	1000	97.9% closure at 24 mo, 1 embolization, 3 cardiac erosion
			Spies <i>et al.</i> ^[90] , 2007 - retrospective	170	100% closure at 12 mo, 4 embolization, 1 TIA,
			Tomar <i>et al.</i> ^[91] , 2011 - retrospective	529	100% closure at 56 mo, 96.7% symptom free, 1 stroke
			Kim <i>et al.</i> ^[85] , 2019 - retrospective	98	100% closure at 29 mo, 1 embolization
			Post Market Surveillance (ASO 522) ^[92]	602	Clinical Trial NCT02353351, study terminated, results not published yet

defects with a maximum defect diameter less than 38 mm^[93]. An example of balloon sizing as assessed intraoperatively with balloon sizing as compared to real time TEE sizing can be seen in Figure 5. ASDs typically give the appearance of with ellipsoidal geometry that varies throughout the cardiac cycle^[94,95]. Selection of optimal device size, particularly in patients undergoing the procedure without balloon sizing or multiple defects, involves measuring the major axis diameter of the defect during of ventricular end systole. More recently, real time 3D TEE is challenging the need for balloon sizing with stop-flow technique as an adjunct to prevent underestimation of defect size or tissue rims^[96,97].

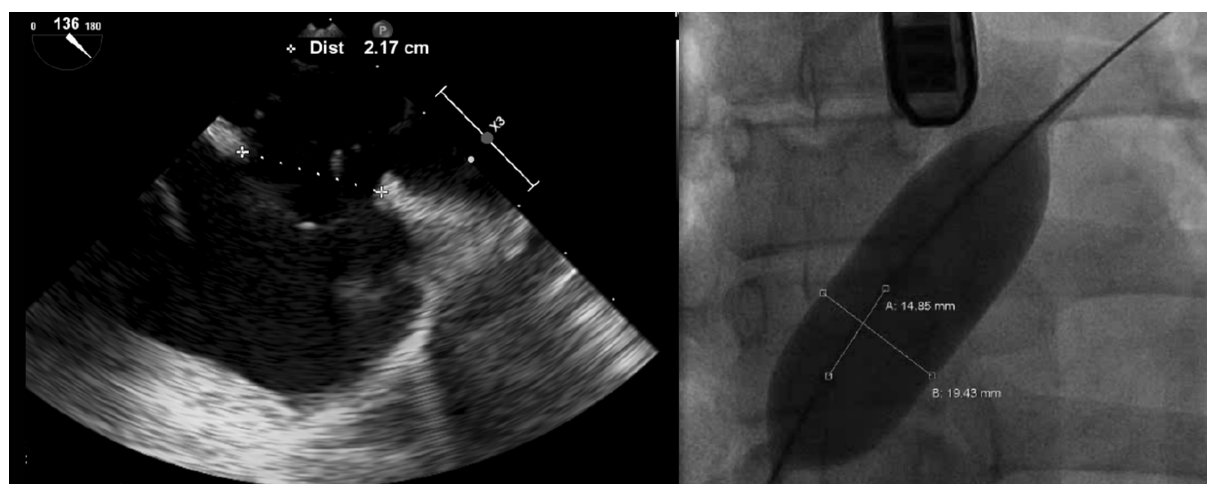


Figure 5. Echo Atrial septal defects sizing (left) vs. Balloon sizing of defect (right)

Transcatheter closure of ASDs with a maximal native diameter > 30 mm can be quite challenging, and alternative techniques for deployment may be required, which will be discussed later. In regard to classification of surrounding rims, although there are some differences noted among studies, distances from ASD to aorta, superior vena cava, right upper pulmonary vein, inferior vena cava, coronary sinus, and atrioventricular valve are evaluated. Adequate tissue rim is defined by at least 5mm from the defect edge to the surrounding structures so as not to impinge on the vena cava, pulmonary vein, coronary sinus, tricuspid or mitral valve^[28]. Figure 6 depicts areas of interest in measuring surrounding tissue rim dimensions.

Figure 7 illustrates the tissue rim measurements as seen via intraoperative TEE. Tissue measurements are best taken as follows: AV valve and right upper pulmonary vein tissue rim are best viewed in the 4 chamber view, SVC and IVC rims are best measured in the Bi-Caval view, and the Aortic and posterior rim measurements are best taken in the short axis view. These are recommendations, but individual body habitus and variations in heart orientation may necessitate obtaining alternate views to accurately measure tissue rims. Interestingly, Yan *et al.*^[98] describe generating a custom 3D model to visualize and assess device closure feasibility based on 3D TEE end systolic dimensions with 29 of 30 patients found to have deficient posterior-inferior rim size (< 3 mm), providing a proof of concept for simulated in-vivo device fitment prior to undergoing transcatheter intervention^[98]. Though, caution should be maintained regarding attempting transcatheter closure with inadequate rim size, as many studies demonstrate increased risk for device embolization with difficult retrieval or conversion to open surgery^[99-102].

Special issues in the management of elderly patients with ASD

Comparative benefits from ASD closure in the elderly population have historically been underreported as compared younger populations. The paradigm of non-operative management of previous generations had, in some ways, stymied broad acceptance and given cause to thwart intervention where there was no perceived benefit. However, percutaneous management of ASD in elderly patients has gained reluctant enthusiasm, as evidenced by analyzing trends in hospitalizations captured by the National Inpatient Sample Database^[103-106]. The promise of shorter hospitalization time and reduced complication rates is tempered with the many difficulties faced perioperatively due to the tendency toward combined comorbidities. Realistic benefits of ASD closure include symptomatic relief, improvements of functional status as well as the overall improvement in the quality of life^[25].

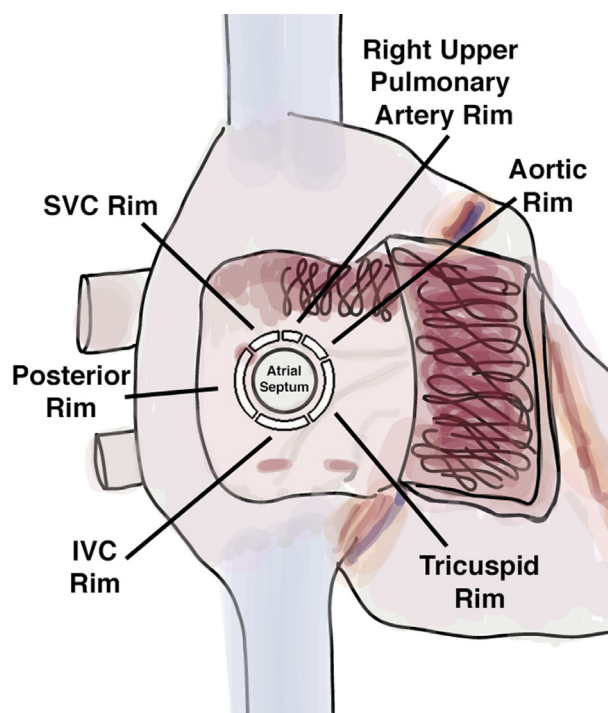


Figure 6. Tissue rim measurement areas

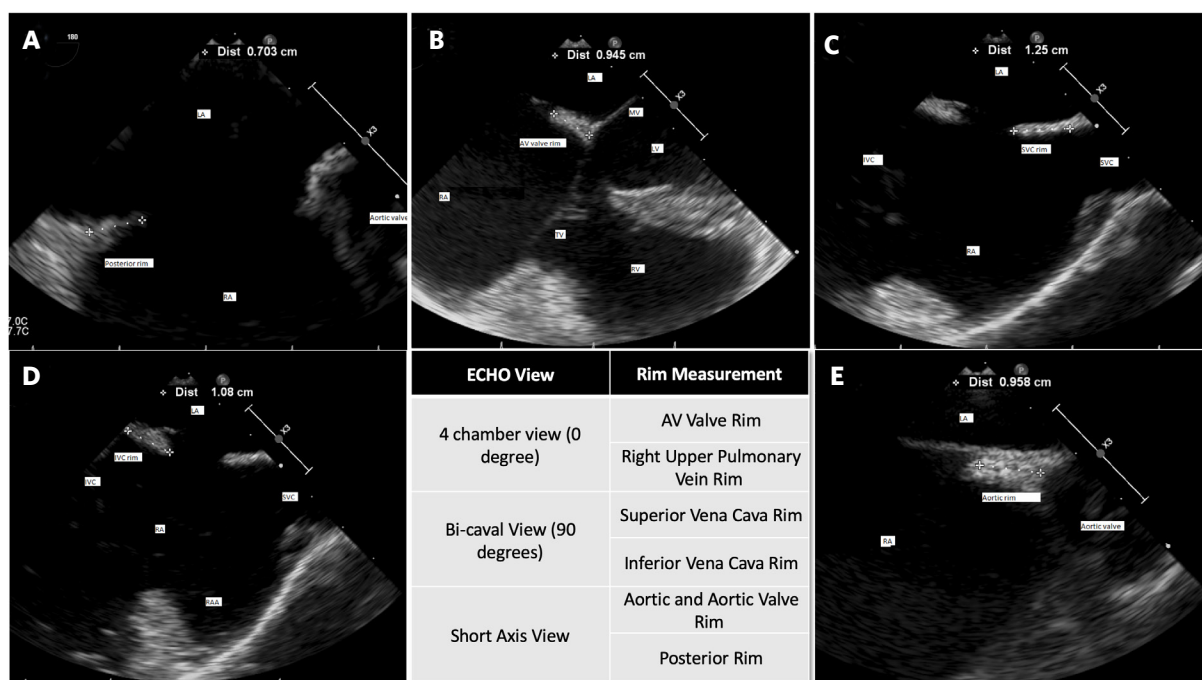


Figure 7. Posterior Rim in Short Axis View (A); AV Valve Rim in 4 Chamber View (B); SVC Rim in Bicaval View (C); IVC Rim in Bicaval View (D); and (E) Aortic Rim in 4 Short Axis

Outcomes of ASD closure in elderly Patients:

In two separate studies Swan and Khan both found that following ASD intervention, a small cohort of geriatric patients with a median age of 70 years old, saw improvement in their New York Heart Association (NYHA) class, 6 minute walk time and improvement in overall physical/mental health score in addition

to an extremely high procedural success rate (98%)^[23,107]. Furthermore, Nakagawa *et al.*^[103] reported that after intervention in a population composed of patients 70 years or older with hemodynamically significant ASD, percutaneous closure is efficacious and safe. The intervention led to a significant improvement of PA pressure and NYHA functional class, as well as reversal of RV enlargement^[103].

Similarly, in 2014 Komar *et al.*^[108] studied the mid-term outcome of patients over the age of 60. Interestingly, their primary outcome was focused more on quality of life indices and functional benefits rather than complications or long term survival. Metrics such as time of sustained exercise before feeling short of breath, VO₂max, and the SF-36 quality of life questionnaire to gauge the benefits of ASD closure. Symptomatic parameters like incidence of shortness of breath or time of exercise before shortness of breath both improved significantly; furthermore 88% of patients surveyed had a significant subjective improvement in quality of life 12 months following their index surgery^[108].

Obstacles in transcatheter atrial septal defect closure in elderly patients:

The most salient issue in elderly cases is not their primary pathology, but their co-morbid systemic and cardiac diseases. This necessitates careful preoperative evaluation of the associated risk factors as an essential aspect of successful treatment. Approximately one third of the patients showed systemic hypertension and systemic diseases like diabetes mellitus, and a considerable extent of pulmonary and neurological disease conditions were also present^[109]. Among the cardiac co-morbidities pulmonary hypertension is reported in nearly 50 % of the cases, chronic atrial arrhythmia in more than 20% and ischemic heart disease in about 15% of the patients^[110,111]. Post-closure pulmonary edema developed because of “masked LV restriction” may appear in 2% to 4% of the elderly cases may be evaluated with a balloon occlusion prior to ASD closure^[112].

Similarly, diastolic dysfunction and stiffening of the LV causes increased left to right shunting, which may explain in part why the late diagnosis is established in elderly patients who were previously asymptomatic. Careful assessment of left ventricular and left atrial pressures via left heart catheterization during defect balloon occlusion and weighing potential hemodynamic consequences *vs.* perceived benefits of intervention, are especially important in the elderly patient population. Miranda *et al* report that left ventricular end diastolic pressure may help predict left atrial pressures in those undergoing ASD repair. They found that the vast majority of patients who had a baseline left ventricular end diastolic pressure > 15 mmHg developed significantly elevated left atrial pressure during balloon occlusion of ASD^[113].

ASD and pulmonary arterial hypertension

Due to chronic right ventricular volume overload, elderly patients with hemodynamically significant ASDs have a tendency to present with pulmonary hypertension. Pulmonary hypertension develops as a result of increased pulmonary blood flow due to left-to-right shunting. However, the anomalous rise in pulmonary blood flow creates secondary physiologic changes such as pulmonary vascular intimal proliferation and medial hypertrophy that affect pulmonary vascular resistance^[114,115]. The consequence of such changes has been observed to be reversible in younger patients, but may not be fully reversible in the elderly^[116]. It is well understood that the natural course of ASD and the associated effect on pulmonary hypertension is notably worse than in patients without pulmonary hypertension^[117]. Thus, pulmonary hypertension is traditionally considered an absolute contraindication to ASD intervention, especially surgical closure^[118]. The expansion of therapeutic options for treating pulmonary hypertension may offer new avenues for ASD closure. An area of active research is the role of ASD closure in combination with new pulmonary hypertension treatments such as prostanoids, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors, even if initial hemodynamic parameters are unamenable to ASD closure^[119-122]. More recent studies such as the North American Atrial Septal Defect Pulmonary Hypertension (NAAPH) Study demonstrate feasibility of ASD closure in patients with PAH with an aggressive “treat to repair” strategy

which first addresses underlying pulmonary hypertension^[123]. Optimization of elderly patients with concomitant pulmonary hypertension prior to ASD closure remains an area of active research.

Cardiac erosion after percutaneous ASD intervention

In patients with superoanterior rim deficiency, the increased risk of serious complication, i.e., “cardiac erosion” may increase after implantation of the device. The exact mechanism of “cardiac erosion” is not been well understood; previous clinical experience proposed that an aortic rim deficiency and oversized occlusion device may be highly correlated with cardiac erosion^[124]. In response, updated instructions-to-user were published for the Amplatzer device with specific guidance for aorto-superior rim size specifications^[125]. One recent case series reported that absence of the aortic rim was common finding among patients who developed erosion^[126]. Subsequently, other putative risk factors were also reported as physicians modified their practices and over sizing became less common^[127]. Specifically, deficient aortic or SVC rim size, along with balloon sizing were associated with increased risk of erosion^[128]. It should be noted, however, that these studies are retrospective in nature, and prospective studies have not yet been undertaken to determine true causal relationships for erosion relating to rim size.

FUTURE DIRECTION OF TRANSCATHETER INTERVENTION FOR ASD

The transcatheter ASD repair has evolved from employment in select patients unable to undergo open surgical repair, to applications in pediatric populations, and is now gaining traction in the elderly. Where currently secundum type ASDs and limited case-reports of closure in other variants of ASD are now being reported, we may expect future devices to address these limitations. On the other hand, complications arising from this procedure, especially cardiac erosion, are still being reported. Progress over the last several decades in terms of safety and efficacy are impressive and point to a bright future in the treatment of congenital heart defects. We conclude this review by looking to the near and long-term future in the state of the field.

New devices for difficult ASD closure

Several technical modifications have been introduced over the years to address difficult transcatheter ASD closure, including delivery sheath modification, position deployment, or additional material to hold the left atrial disk inside the LA. Some advocate deployment with balloon assisted placement^[129]. This technique, however, may cause injury to the pulmonary vein. The development of steerable catheters may offer improved techniques in positioning ASD devices^[130]. Use of such a steerable catheter has been described in case reports, but has not yet been implemented in commercially available devices, offering an opportunity for future development^[131].

Endovascular retrieval of embolized devices

A well described early and mid-term complication of transcatheter ASD closure is device dislodgement and embolization. The rote response, if the device has been fully deployed, is to convert to open surgery for retrieval and repair. Improving techniques for endovascular retrieval are supported by case reports, case series, and retrospective reviews of experience^[132-134]. Common embolization sites are the left ventricle, abdominal aorta and femoral vessels^[135,136]. Lastly, Martins, Mendez, and Anjos provide an excellent pictorial stepwise description of various retrieval techniques and devices, and even include demonstrative videos^[137]. Protective devices to prevent embolization during surgery may be an area of future interest to prevent distal embolization periprocedurally^[138].

Salvage of residual shunt with device-in-device intervention

Intracardiac devices that are malfunctioning, whether dislodged, malpositioned, or sub-optimally effective, are typically treated with open heart surgery for removal and remedy. At the present, there are only case reports describing “device-in-device” salvage to return function to such malfunctioning devices^[75,139]. The

concept of device-in-device salvage involves deploying a second device through residual defects the first device did not completely close, in order to provide an adequate seal zone the first device did not provide. These early reports may eventually become the foundational principals for guidelines to prevent conversion to open surgery, but no definitive conclusions can be drawn with such limited data. Likewise, residual shunts following surgical repair of ASDs may be of great interest in the case of secundum ASDs.

GENE THERAPY, TISSUE ENGINEERING, AND STEM CELL THERAPY FOR ASD

The goals of treatment in congenital cardiac malformations are ever shifting. Seventy-five years ago, researchers and clinicians sought to find appropriate screening criteria where risk factors for ASDs were poorly understood. With the advent of better screening methods and guidelines, the difficult decision of who should undergo surgery or medical management then became the diagnostic dilemma. With newer, safer, conventional and endovascular procedures well established, the next logical progression in the field is primary prevention of the disease process. Several reports proposing genes associated with ASD that may inform progress toward potential targets for gene therapy in genetically linked variants of ASD^[140-142]. Furthermore, elucidating the temporal and spatial relationships among terminally differentiating cells during cardiogenesis may provide further insight into the precise moment in congenital defects begin^[143,144].

Until primary prevention with gene therapy is technically feasible, other interventions may be on the horizon. Tissue compatibility of ASD closure devices remains an area of interest for researchers and clinicians alike. Biocompatible and bioabsorbable based devices are currently under investigation^[145]. Those at the beginning of their surgical career may see 3D printed custom devices or bio inspired devices in the form of surgical glue or gels that can be deployed endovascularly emerge within their career^[54,146-148].

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Authors' contributions

Made substantial contributions with initial draft, subsequent revisions, and approved final draft: Zimmermann E, Hussain H, Avgerinos D

Made substantial contributions with subsequent revisions and approval of final draft:

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Review

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Characterizing the mechanical properties of the aortic wall

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Abstract

Characterizing the physical properties of the aortic wall is essential to understanding the causes of cardiovascular diseases, such as aneurysms. Modelling compliant, anisotropic, multilayered tubes such as the aorta has proven to be a challenge. *In vitro* studies of the mechanical properties of arteries incorporate a variety of testing methods; however, the majority of these tests fail to replicate the complex, transmural loading conditions arising from pulsatile flow. These methods include typical tensile tests, both in uniaxial and biaxial set-ups, bulge inflation tests and extension-inflation tests. Bulge-inflation tests grant material information in response to biaxial loading but still do not mimic proper cylindrical loading conditions. Extension-inflation tests capture the cylindrical loading but have only been performed with static pressurization and with rigid boundary conditions in effect. This review aims to present the current state of the biomechanical characterization of arterial walls, particularly the aorta, through discussion of testing methods and their findings. We emphasize literature that focuses on prediction of aneurysm rupture risk. Moreover, overarching concepts such as histological effects, age dependent effects, segmental effects, hemodynamic effects, viscoelastic modelling and torsion will be briefly explored. An understanding of the current limitations of testing will hopefully lead to the development of more robust *in vitro* test methods that will further elucidate the relationship between changing vessel wall mechanics and cardiovascular disease.

Keywords: Aortic aneurysm, biomechanical testing, aortic stiffness, aortic rupture

INTRODUCTION

Aortic aneurysm can be a life-threatening condition, representing a serious mortality risk of 80% if rupture occurs^[1]. There is a significant decline in mortality risk if aneurysms are electively treated with aortic



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replacements when compared to non-elective replacements^[2]. Therefore, early predictions of aneurysm rupture risk can be of great benefit to patients. Currently, an aortic aneurysm diameter greater than 5.5 cm is an indication for intervention. However, in patients with connective tissue disorders, where structural changes in the aortic wall are well defined, a much lower threshold of dilatation is warranted for intervention; in Marfan Syndrome, a diameter of greater than 5.0 cm is an indication for surgery with a lower threshold of 4.5 cm in the presence of further risk factors^[3].

Aneurysm diameter and growth may not accurately predict the risk of aneurysm rupture^[4] and might lead to undertreatment of those who have other contributing factors such as aortic stiffness or a connective tissue disorder. Alternatively, overtreatment is also a possibility: Trabelsi *et al.*^[5] found that even at a diameter of 6 cm only 31% of patients with aneurysms developed complications. Moreover, in the general population, there is a large number of individuals with aortic diameters between 5.0 and 5.5 cm whose risk of complications are not well understood^[6]. The Laplace Law is the theoretical basis of the diameter guidelines; however, this law is valid for simple spheres and uniform cylinders and might not adequately appreciate the complex geometrical nature of the native vessels^[1].

The biomechanical analysis of diseased aortas could therefore represent an intriguing opportunity to further elucidate the mechanisms leading to rupture even in the absence of connective tissue disorder. It has long been recognized that a blood vessel cannot be considered as a passive conduit for blood flow. Rather, a blood vessel is a continuously adapting, dynamic element with the purpose of maintaining optimal function in response to changing hemodynamic conditions^[7]. Therefore, a more thorough understanding of the biological composition and biomechanics of the vascular system is warranted. There is a need for experimental data with an effort to determine the response of the aorta to a pulsatile waveform. Biological tissues, though subject to conservation of mass, momentum and energy, have unique constitutive equations that differentiate them from inorganic materials, and thus make them harder to characterize. This review aims to present, in brief, the current state of the biomechanical characterization of arterial walls, particularly the aorta, through discussion of testing methods and their findings. Moreover, overarching concepts such as histological effects, age dependent effects, segmental effects, hemodynamic effects, viscoelastic modelling and torsion will be briefly explored.

CURRENT TESTING METHODS

Uniaxial tensile test

Uniaxial tensile tests have been widely used to test biological tissues *in vitro* due to their simplicity and ability to give precise information regarding local properties of soft tissues. While extending a piece of the sample (either rectangular in shape, or dog-bone shaped), the displacement and resulting force are recorded until fracture [Figure 1A]. This data can be used to obtain a variety of stress-strain relations, and depending on the constitutive model chosen, authors may make use of different stress and strain parameters outlined throughout Continuum Mechanics such as Cauchy stress, engineering stress, Second Piola-Kirchoff stress, Green strain, true strain, and engineering strain.

With uniaxial tensile testing, one can obtain the ultimate tensile strength (strength most often recorded at failure), the yield strength, as well as the strain at failure. A single value of Young's modulus, which is useful for defining non-biological materials, cannot be adequately used for blood vessels since they are characterized by a non-linear elastic behaviour. A common method to define this parameter for biological tissues is to calculate the modulus incrementally and report it for a specified range of stress. Additionally, most authors make use of the maximum tangential modulus to describe vessel stiffness and make succinct comparisons between intra-study specimens. Currently there is no standard for testing protocol, and variations in experimental parameters such as the force range and number of cycles for preconditioning,

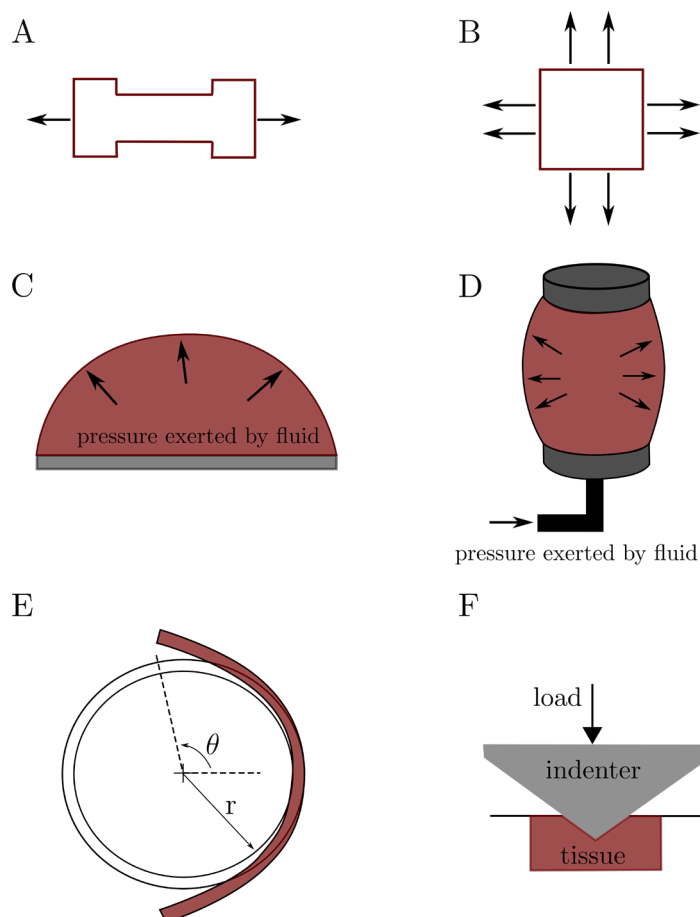


Figure 1. Schematics of the mechanical tests described for aortic mechanics where A: shows a uniaxial test; B: biaxial testing; C: the bulge-inflation test; D: depicts inflation-extension testing; E: opening angle testing: Upon cutting an intact circumferential segment of an artery in an unloaded state (described by radius, r), an expansion of the segment is observed over time, leading to an equilibrium zero-stress state (described by radius, R , and opening angle, θ). The residual strains can be obtained from comparison between the two states; F: nano-indentation test: an indenter of known material properties is pressed into the tissue with a known loading pattern, after which the area of the indentation is observed, and the hardness of the material can be calculated

loading or strain rate, or the strain measure used to calculate elasticity parameters, make lateral comparisons between papers difficult at best^[4,8].

A focus of uniaxial testing on the aneurysmal aorta has been considering the region- and layer-dependent variations of the wall behaviour. Iliopoulos *et al.*^[9] first showed that the ascending thoracic aortic aneurysm (ATAA) exhibits heterogeneity between the anterior, posterior, right and left lateral regions. Note that the following studies presented in this section divided specimens into these same four regions. The data from the circumferential direction suggested this direction to be stiffer than the longitudinal direction at physiological and high stresses. While no differences in the peak elastic modulus were observed between the four regions in the circumferential direction, the longitudinal direction results indicated that the anterior region was significantly less stiff than the other regions. The results showed that no correlation existed between failure stress and the diameter of the whole ATAA^[9]. The layer-specific differences of the tunica intima, media, and adventitia from uniaxial data of the ATAA were documented by Sokolis *et al.*^[10]. In general, the circumferential stiffness was recorded as higher than the longitudinal direction in the adventitia and media, but not the intima, though all layers had a higher failure stress in the circumferential direction. This is true for all four regions (previously introduced) of the media, but only for the anterior and posterior regions of the adventitia. This study also brought up the important concern that the adhesive connections between the three layers needs to be considered when drawing conclusions regarding the overall mechanical

properties of the wall from those of the individual layer behaviour. Khanafer *et al.*^[11] performed uniaxial tests on the ATAA and highlighted the importance of the elastic modulus in assessing risk of aneurysm rupture. Sassani *et al.*^[12] examined the regional uniaxial response (collecting bi-dimensional strain data) of the intimal, medial, and adventitial layers of the aneurysmal ascending aorta in the circumferential and longitudinal directions, again separating the specimens into four regions. A decoupled microstructure-based formulation, resulting in a reduced two-fiber model, was used to describe the uniaxial behaviour in either direction, concluding that material parameters are highly dependent on differences within the underlying wall composition. Further studies on these histological effects are presented under the section titled Factors Affecting Arterial Mechanics.

Biaxial tensile test

While uniaxial tests describe failure properties of vessel walls adequately, as shown by the studies cited in the previous section, characterising loading response along a singular axis does not reflect *in vivo* conditions, where the blood vessel wall is subject to multi-axial stresses. Guinea *et al.*^[13] performed uniaxial tests on healthy thoracic aortas, with samples obtained from persons deceased from non-cardiovascular causes. They acknowledged the limitations of the uniaxial test, stating that it poorly reproduces the complex loading conditions seen at the physiological level, which are better described by biaxial testing. In addition, uniaxial testing does not provide a true understanding of the anisotropic properties of the vessel wall, as the circumferential and axial test strip come from different locations. Biaxial testing is done with a square sample, which is placed in a loading rig with four arms, generally by means of hooks, at 90 degrees to each other [Figure 1B]. A significant volume of literature has focused on biaxial testing of both healthy and diseased tissue^[14-18]. Alreshidan *et al.*^[19] used biaxial testing on resected ascending aortic tissue to compare to and validate the use of *in vivo* speckle tracking transesophageal echocardiography to estimate aortic stiffness to better stratify the risk of aortic aneurysm rupture. The advancement of *in vivo* imaging techniques gives rise to more reliable data; however, the behaviour of the aorta with these tests is limited to patient-specific physiological conditions. In addition, *in vivo* testing presents multivariate data, as it is not possible to disregard other non-loading effects, such as the effect of surrounding perivascular adipose tissue. Drawbacks of biaxial testing arise from the attachment method, i.e., the failure of the tissue usually occurs at the attachment location and rupture is also influenced by the components used to hold the tissue^[20]. In addition, testing samples of various shapes does not allow for accurate comparison since the geometry, in addition to the clamping method, can alter the mechanical properties observed, as was described by Waldman *et al.*^[21].

Bulge inflation test

The bulge inflation test, also referred to as the membrane bulge test, gives information on biaxial behaviour, and was used most recognizably in the work by Mohan and Melvin^[22] on healthy human thoracic aorta specimens. This test involves using a square specimen, which is secured in the inflation device through an airtight seal [Figure 1C]. A fluid, generally water, is released at a specified rate, and the expansion of the specimen is tracked optically with complex digital imaging techniques to obtain the strain field. This technique has also been applied to analyze the mechanical behaviour of aneurysms^[5,23,24]. Romo *et al.*^[23] utilized the bulge inflation test on the ascending aorta to observe the most likely location of aneurysmal rupture many stages of deformation before the rupture took place by noting the region that had the most amount of localized thinning. They created local thickness over pressure maps to predict the site of aneurysm rupture and they posited that the site of rupture mostly occurs not at the location of maximum stress but at the location of greatest wall weakening.

Inflation-extension test

An inflation-extension test differs from the aforementioned techniques in that it replicates the *in vivo* cylindrical loading scenario of a blood vessel. In general, an intact portion of a blood vessel is extended

by some means to replicate axial loading, and fluid is run through the conduit to enact circumferential loading [Figure 1D]. Optical methods may be used to track displacement and force of pre-set markers on the blood vessel^[25,26]. Courtial *et al.*^[27] present, in detail, an inflation device that was coupled with non-invasive imaging techniques such as ultrasound, in order to identify the parameters of a silicone tube based hyperviscoelastic model that represented the native aorta. They found that the inflation-extension test was adequate in validating this model. The inflation-extension property of the aorta was further tested by Horny *et al.*^[28] in an analytical simulation, using parameters from autopsy measurements. They established that while axial pre-stretch, which allows the aorta to minimize deformation during systole and diastole, decreases with age, the prespecified value of axial pre-stretch can still have a significant effect on the mechanical properties of the vessel wall. Inflation-extensions tests are valuable for understanding the operating mode of arteries. However, inverse analysis and simplification is typically required to find the stress-strain relationship from the pressure-diameter measurements^[13].

Opening angle test

Residual stresses are present in the circumferential direction of the arterial wall, and this can be visually shown by cutting open a ring segment of an artery along the axial direction^[25,26]. Subsequently the opening angle formed by the specimen may be optically measured until the cut section stabilizes [Figure 1E]. Accounting for these residual stresses, which have been shown to be present in the axial direction as well^[29], leads to a significantly lower circumferential stress gradient along the thickness of the vessel^[30]. This observation is better explained when considering the circumferential residual strains, wherein accounting for these strains works to decrease the stretch ratio at the inner surface, while increasing that of the outer surface^[31,32]. In essence, to account for residual stresses, “state 0” (stress-free state) is taken as the reference configuration, rather than “state 1” (unloaded state - outlined in Ref.^[30]). Moreover, the presence of residual stresses in arteries results in a more uniform stress distribution throughout the vessel wall^[33,34]. Zheng and Ren^[35] further explored the effects of three-dimensional residual stresses in each of the three arterial layers. They showed that the residual circumferential stress is compressive within the intima, while tensile in the remaining two layers, and that the bending of the media in the longitudinal direction noticeably affects the mechanical behavior of the arterial wall. Cardamone *et al.*^[36] attribute the origin of this residual stress to non-uniform growth and remodelling as well as temporo-spatial variances in wall components.

Sokolis *et al.*^[37] examined the regional distribution of circumferential residual strains in the human aorta according to age and gender to gain a more detailed understanding of the zero-stress and no-load states of the human aorta. The opening angle measurement, which characterizes the circumferential residual strain, was highest in the aortic arch and declined in the descending aorta. Opening angle measurements for aneurysmal tissue are difficult to obtain due to the irregular shape of the diseased vessel wall. Sokolis^[38] discussed the residual stresses within the ATAA in great depth, presenting several important conclusions regarding the vessel wall behaviour: (1) change in the wall composition, mainly the decreased presence of elastin within the aneurysmal vessel wall, results in greater viscoelastic behaviour; (2) residual strains vary along the circumferential direction within each of the three layers of the wall; and (3) analysing the residual stresses of the layers individually reveals that the intima is held in compression, while the media is in tension. Contrary to previous studies performed on the abdominal aorta, the adventitia was shown to be under compression in the circumferential direction, though still under tension longitudinally. Higher opening angles were measured in the aneurysmal tissue and it was postulated that this might be a compensatory mechanism to increase the vessel's resistance to dissection^[39]. This is perhaps similar to a compensatory increase in opening angle measurements with increased stiffness of the aorta with age. Most recently, Sokolis *et al.*^[40] employed opening angle testing to study the axial residual strain variations in the human aorta according to age and gender. Interestingly, they found that the axial opening angle and residual stress decreased with age as compared to the circumferential residual strain which had increased with age.

Nanoindentation test

Nanoindentation tests may be used to characterize local deformation and properties in multilayer materials [Figure 1F]. Though it is known that the artery is made up of three distinct layers, a large portion of the aorta's biomechanical modelling describe it as a single, homogenous layered vessel. That being said, experimental results regarding the behaviour of the individual layers has been performed on ATAA tissue in detail, and strain-energy-functions were fit to the data to describe the behaviour. Reiterating the discussion from Uniaxial Testing, it was shown that the intima was the weakest of the three layers, with the adventitia exhibiting the highest failure stresses and resistance to excessive deformation, preventing rupture. Sokolis *et al.*^[10] revealed that the behaviour of the intact aneurysmal wall was most similar to the intima and media layers, however, drawing inferences regarding the overall layered vessel wall behaviour remains a challenge due to the complex attachment and interaction between layers.

Hemmasizadeh *et al.*^[41] introduced a custom nanoindentation technique showing that the inner layer is more compliant than the outer layer and sustains higher strains. Therefore, rupture of the aorta travels outwards from the inner layer. This is verified by Manopoulos *et al.*^[42] by uniaxial tests performed on ascending thoracic aortic dissected tissue to understand the failure properties for the vessel wall as divided into an inner (intima-media) and outer (media-adventitia) layer. They found that the inner layer displayed a significantly lower strength than the outer layer, in addition to a sharp decline of stress following rupture, unlike the outer layer which exhibited a slow decrease to zero stress. Interestingly, the relative strengths of the two layers differed significantly in magnitude from what has previously been reported on the individual layers^[10,42]. The outer layer was shown to be stronger than the adventitia alone, and in comparing the properties of the origin of inner layer failure to distal locations, it was evident that dissection occurred where the layer was thinner and exhibited increased stiffness. These results offer valuable insight as to why the specimens studied^[42] dissected, rather than undergoing full rupture.

FACTORS AFFECTING ARTERIAL MECHANICS

Histological effects

Soft tissues, including blood vessels, contain collagen, elastin, and ground substance. The relative amount, density, and arrangement of these three constituents greatly affect the mechanical response of the tissue^[43,44]. Therefore, it is important to consider histological composition when characterizing the physical properties of the aortic wall. Distribution of elastic fibres in soft tissues and its relation to mechanical properties have been studied extensively^[43,45]. Bellini *et al.*^[18] better defined the mechanical environments of the media and adventitia layers within the arterial wall by highlighting the roles that smooth muscle cells and fibroblasts play in arterial homeostasis. Taghizadeh *et al.*^[46] evaluated the mechanical properties of the aortic wall while accounting for the lamellar structure within the media layer. Cardamone *et al.*^[36] confirmed that elastin contributes significantly to the shortening of arteries observed upon cutting along the circumferential direction (residual stresses), and to the opening angle phenomenon. Holzapfel *et al.*^[47] combined histological data with computational modelling to create a general constitutive model of the anisotropic collagen fibre dispersion in arterial walls. In terms of aneurysmal tissue, studies focusing on the ascending aorta report reduced levels of elastin, but normal levels of collagen, throughout the vessel wall when compared to a healthy aorta^[48,49]. Aneurysm development in the ascending aorta has been shown to be associated with higher stiffness of the wall, resulting in increased wall stresses, but not leading to a weakening of the wall for age-matched subjects^[48].

Segmental effects

There are segmental differences in the structure and mechanical properties of the aorta, and these are dependent on the magnitude of stress that the wall is subjected to regularly under normal conditions. Schriebl *et al.*^[50] incorporated the themes of segmental and histological analysis to study the layer specific distribution and orientation of collagen fibres in the abdominal and thoracic aorta. They concluded that

there were distinct fibre families, directions and dispersion present in the three arterial layers: these variations between the layers underlie their different mechanical and functional properties. Sassani *et al.*^[12] utilized a four fiber microstructure based model to characterize region and layer-specific material properties in ascending aortic aneurysmal tissue samples; their novel hypothesis that the fibers are able to support compressive forces provides further insight into the role of elastin and collagen in withstanding mechanical loads. The aortic wall shows an increase in viscoelastic creep further from the aortic root, which can be attributed to a decrease in elastin content. Irregular variability in the opening angle was observed over the length of the vessel, the pattern of which was determined to be similar across both young (less than 40 years of age) and old subjects^[37]. Haskett *et al.*^[14] delved into the critical importance of gaining a better structural model of the aorta through quantification of microstructural and mechanical changes. Specifically, they looked at anisotropy and extra-cellular matrix microstructure changes as a function of location and age of the aorta.

Age-dependent effects

Another concept of interest is the age-dependent elastic behaviour of the arterial wall which is closely related to the histological compositional changes that occur with age. These alterations with age are explored by Maceri *et al.*^[51]; they introduce a multiscale mechanical model that accounts for nanoscale effects of cross-link stretching, as well as micro- and macroscale mechanisms. This model further supports experimental evidence regarding the histological alterations in the aortic wall that occur with age, showing evidence between the influence of cross-link density and stiffness on the elastic modulus. With age, there is an increase in aortic diameter, thickness, elastin stiffness and collagen cross links and there is a decrease in collagen amount, collagen fiber radius and waviness. Carallo *et al.*^[52] concur and describe the effects of ageing as a change in the arterial wall with an increased collagen presence and reduced elastin function, leading to a larger and stiffer vessel. Moreover, this increase in aortic stiffness and thickness is greatest circumferentially^[14].

Cavalcante *et al.*^[53] present a comprehensive review on arterial stiffness associated with age and, in particular, the acceleration of aortic stiffness due to hypertension. They identify arterial stiffness as an important prognostic factor and emphasize its deleterious effect on the Windkessel function of the aorta. Moreover, they identify pharmacological and non pharmacological therapies targeted against increased aortic stiffness. This further highlights the importance of identifying contributing factors that affect vessel function so that more targeted therapies can be employed. A monotonic decrease in circumferential and axial tensile strength of the arterial wall with age is evident as well^[13,54,55]. Guinea *et al.*^[13] utilized uniaxial tensile tests on donor thoracic aortas and found that the tensile strength of the thoracic aorta falls rapidly after age 30 and Morrison *et al.*^[55] found that circumferential and longitudinal strain decreased by 50% with increasing age (patients with a mean age of 68 compared to patients with a mean age of 41). Dejeva *et al.*^[56] found a negative correlation between failure stress and stretch with increasing age in all three layers of the ascending aorta and a similar correlation was found by Iliopoulos *et al.*^[57] in tissue samples of patients with Sinus of Valsalva aneurysms. Tracy and Eigenbrodt^[58] found that a disproportionate increase in vessel wall thickness with age causes a deviation from the Laplace law: they introduced age specific constants into the Laplace equation to make their data conform to Laplace expectations. This further highlights the need for a more comprehensive guideline to assess aneurysm rupture risk, especially in an ageing population, than the current diameter-based guidelines which were formed on the basis of the Laplace Law.

Hemodynamic effects

Hemodynamics is of particular interest as a potential factor in arterial disease formation and progression. Dynamic *in vitro* tests could show the effect of flow on the healthy arterial structure and subsequent disease formation while still allowing for control of the loading conditions and isolating mechanical effects. Pulsatile arterial hemodynamics has been explored in numerous studies^[52,59,60]. The scope of this

review is not intended to cover studies on hemodynamics and associated arterial mechanics; however, to fully understand the complexity of characterizing the vasculature wall and the mechanisms that may lead to cardiovascular complications, the fluid-structure interactions - for example, wave propagation in arterial walls, local hemodynamics, and temporal wall shear stress - should be considered. Therefore, in brief and without discussion of methods employed, several key concepts are mentioned. While the flow of blood is generally laminar, in some cases this flow can be disrupted and result in transitional conditions [Supplementary Figure 1], which may contribute to certain cardiovascular pathologies. Transitional flow in the presence of hypercholesterolemia has been proven to prime the vessel wall for the pathogenesis of atherosclerosis^[61]. Transitional blood flow has also been suggested as a cause of post-stenotic dilatation, however this association may be due to the common occurrence of turbulence alongside stenosis of the blood vessel wall^[62,63].

Transition has been proven to significantly increase pressure and shear stress within aneurysmal regions. Khanafer *et al.*^[11,64] suggest that this may result in a self-perpetuating mechanism of further dilatation and subsequent increase of turbulence in the region. It has been suggested that the associated hemodynamics through an aneurysm, such as recirculating flow, may result in the formation of thrombi^[64,65]. The majority of the literature; however, supports the hypothesis that the formation of an aneurysm is a multi-factorial, degenerative process^[66], not solely affected by hemodynamics and mechanical wall stress, but including inflammation and immune response, molecular genetics, and degradation of surrounding connective tissue^[64].

Viscoelastic modelling

Large arteries are viscoelastic, which entails distinct mechanical behaviour compared to typical elastic models and calls for analysis of time-dependent behaviour. Due to this viscous component, there is energy retained within the arterial wall upon unloading, which is seen through hysteresis present in the stress-strain, and pressure-diameter curves of arteries^[67,68]. Hysteresis loops may be used to estimate damping capacity, which is associated with the ratio of the dissipated energy to the stored energy^[69]. An interesting study on strain-rate effect of mechanical properties by Delgadillo *et al.*^[70] revealed that at a stretch ratio of 1.5, the experienced load within the arterial wall is reduced by 20% when the strain rate is increased from 10 to 200 %/S. They suggested that: "this behavior might be a consequence of the faster fluidization and small re-solidification that occurs in the cell at higher deformation rates".

Torsion

While the response of arteries to axial stretching and circumferential stretching has long been studied and used to quantify failure of the arterial wall, the effect of torsion has been explored to a lesser extent, despite the fact that *in vivo*, arteries are often subject to twisting along the longitudinal direction with body movement^[71,72]. Klein *et al.*^[72] found that there was a significant change in arterial length, curvature and twist in the femoropopliteal arteries when subjects were cross legged compared to straight legged. Furthermore, the abdominal aorta and common iliac arteries exhibit significant morphological deformations from musculoskeletal motion. Hence, torsion is of particular concern since it has been identified as a possible contribution to failure of stents in the more mobile arteries^[71-74]. Further study on the shortening, twisting and bending patterns of these arteries with stenting is required.

CONCLUSION

The inter-individual heterogeneity of the aorta's geometry and composition, and the distinct differences in regional mechanical properties^[26], fuel the difficulty behind understanding the underlying mechanics of the aorta. Uniaxial tests provide data regarding local mechanical properties and provide base comparisons between diseased and healthy arteries^[4,8-10,12,75]. However, biaxial tests provide a better estimate of the multiaxial and anisotropic properties of arteries^[14-18]. Both tests allow for data collection of incremental

elastic modulus until specimen failure, thus providing rudimentary data about rupture conditions. Bulge inflation tests further provide information on biaxial behaviour^[5,22-24]; however, inflation-extensions tests replicate *in vivo* loading scenarios and are more suited to explore the in-depth loading mechanisms under which healthy tissue may become diseased^[26-29]. The opening angle test illustrates the residual stresses that are present in the circumferential direction in arterial walls^[25,26,29,37,39]. Lastly, the nanoindentation test may be used to characterize local deformation and inter-layer properties of arteries^[10,41]. There has been great effort put into quantification of the mechanical properties of the aorta; however, acquiring whole tissue is difficult and the importance of standard testing cannot be overlooked. The development of more robust *in vitro* test methods and further research with respect to spatial and temporal variations in aortic composition will lead to a clearer understanding of the biomechanical contributions to vascular pathologies such as aneurysms. This will potentially lead to a more comprehensive stratification of aneurysm rupture risk and will hopefully lead to more targeted treatment strategies.

DECLARATIONS

Authors' contributions

Performed literature review and composed manuscript: Pejcic S, Hassan SMA

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Consent for publication

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Review

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Mechanical ventilation and cardiopulmonary bypass: a narrative review of the mechanistic lung protective measures

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Abstract

Postoperative pulmonary dysfunction is a multifactorial complication in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB). Numerous risk factors including individual, surgery- and anesthesia-related have been identified. Exacerbated systemic and pulmonary inflammatory response to CPB is one of the most studied mechanisms of lung injury in this patient setting. However, current literature lacks specific intraoperative mechanical ventilation (MV) strategies associated with a significant improvement in patients' outcomes. We reviewed the randomized clinical trials and other reports published within the last 5 years involving patients undergoing cardiac surgery with CPB in order to summarize the existing MV strategies used in these patients and their associated outcomes. Moreover, we described the pathophysiological mechanisms involved in post-CPB lung injury and the mechanistic effects of protective ventilation.

Keywords: Cardiopulmonary bypass, mechanical ventilation, postoperative pulmonary complications, protective mechanical ventilation

INTRODUCTION

Impaired postoperative pulmonary function is a common and multifactorial complication after cardiac surgery^[1,2]. Exacerbated cellular and humoral activation is a widely-known response ensuing from cardiopulmonary bypass (CPB), being the major cause of postoperative lung injury^[3,4]. Protective



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mechanical ventilation (PMV) strategies such as the use of continuous positive airway pressure (CPAP) have shown benefits in non-cardiac surgeries^[2]. Likewise, CPAP, low tidal volume (V_T) and recruitment maneuvers have been used in patients undergoing cardiac surgeries under CPB aiming to ameliorate lung mechanics and to decrease postoperative pulmonary complications (PPCs)^[5,6].

Mild respiratory dysfunction is commonly reported after cardiac surgery under CPB with a small percentage of patients developing severe lung dysfunction^[7]. Even though protective ventilation strategies have been associated with decreased levels of pro-inflammatory cytokines and improved lung mechanics, its impact on other postoperative long-term outcomes such as PPCs and hospital length of stay (LOS) remains unclear.

A comprehensive review of current literature was carried out aiming to describe the pulmonary physiopathological changes experienced by patients undergoing cardiac surgery with and without CPB and treated under different ventilation strategies. Likewise, the incidence of PPCs in patients with and without continuous MV during CPB was analyzed.

METHODS

A literature search on PubMed, Embase, and Cochrane Library databases was carried out in order to identify manuscripts published between 01 Jan 2014 and 31 Jan 2019 describing MV and pulmonary complications in patients undergoing CPB surgery. We used Medical Subject Headings involving the terms “MV” (combined with “CPB”, “CPB and lung injury”, “CPB and morbidity”, “CPB and mortality”, “CPB and pulmonary perfusion”, “cardiac surgery and oxygen diffusion”), “CPB” [combined with “pulmonary complications”, “CPAP”, “positive end-expiratory pressure (PEEP)”, “lung injury”, “lung mechanics”], and “lung protective ventilation in CPB surgery”. Our search was limited to manuscripts in English language, involving adult patients only, clinical trials (including phase I-IV studies), narrative reviews, and systematic reviews (with or without meta-analysis). Case reports were only considered if they were needed to support specific clinical findings not previously discussed. Moreover, we excluded manuscripts referring to CPB surgery outside the scope of this review, conference abstracts, thesis, and trials involving children or patients undergoing other cardiac surgeries different from CPB.

RESULTS

Initially, we identified 207 manuscripts out of which 46 were duplicates. After title/abstract screening, 113 manuscripts were out of the scope of this review and thereby excluded. Therefore, 48 articles qualified for full-text revision. Thirty-five ($n = 35$) articles were excluded due to no CPB surgery or intraoperative ventilation was discussed ($n = 27$), case reports ($n = 2$), protocol design ($n = 2$), trials involving cardiac surgeries in children ($n = 1$), thesis ($n = 1$), and no full-text available ($n = 2$). Therefore, 13 articles were included for further description in our qualitative analysis: systematic review and meta-analysis ($n = 1$)^[2], meta-analysis ($n = 1$)^[8], randomized clinical trials or RCTs ($n = 3$)^[9-11], prospective observational ($n = 1$)^[12], and reviews ($n = 7$)^[4-6,13-16]. Figure 1 describes the flow diagram corresponding to our search.

MV during CPB and serum inflammatory markers

A total of 3 RCTs and 1 prospective observational trial ($n = 157$ patients) studied the impact of intraoperative MV on inflammatory markers such as cytokines in patients undergoing cardiac surgery with CPB [Table 1]^[9-12]. Two RCTs involved one group with low V_T (3-4 mL/kg) MV and PEEP whereas no ventilation was administered in a second group^[9,10]. Another RCT assigned patients to either one of the following groups: patients without MV (MV group), patients receiving protective ventilation with continuous low V_T ventilation (LTV), and patients with CPAP of 10 cmH₂O (CPAP group)^[11]. Moreover, one prospective observational study allocated patients into 2 groups based on: MV or apnea with PEEP

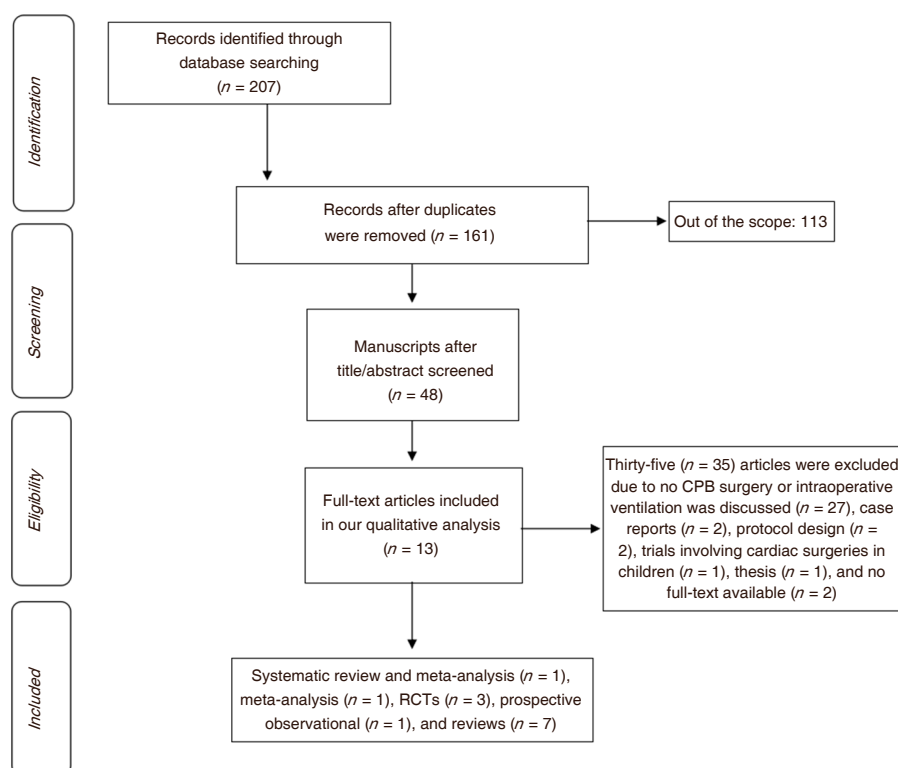


Figure 1. Flow diagram

received during CPB^[12]. Analyzed inflammatory markers varied among studies: chemokines (CCL2, CCL4, CCL20)^[9]; matrix metalloproteinase (MMP)-8, MMP-9 and lipocalin-2^[10]; tumor necrosis factor alpha (TNF- α) and interleukin (IL)-10^[12]; and IL-6, IL-8, and IL-10^[11]. Table 1 summarizes the main reported findings for each study.

MV during CPB and perioperative outcomes

Perioperative clinical outcomes (e.g., atrial fibrillation, perioperative myocardial infarction, and pericardial tamponade) and 28-day mortality after cardiac surgery were assessed in 2 of the RCTs included in this review^[9,10]. Moreover, the ratio between the arterial oxygen partial pressure (PaO₂) and the inspired fraction of oxygen (FiO₂) or PaO₂/FiO₂ ratio was reported in the only prospective observational study^[12]. Likewise, one meta-analysis by Chi *et al.*^[8] included 17 trials and 1,162 patients undergoing cardiac surgery evaluating the oxygenation index (PaO₂/FiO₂ ratio) and the alveolar to arterial oxygen difference (AaDO₂) after CPB. Rate of PPCs, shunt fraction, hospital LOS, and postoperative AaDO₂ (4 h after CPB) were also estimated. Authors used the GRADE system to assess the level of evidence for each outcome [Table 1].

A recent systematic review and meta-analysis described the impact of different MV strategies during CPB on postoperative outcomes in adult patients undergoing cardiac surgery. A total of 15 RCTs were included in this analysis, 13 trials in patients undergoing coronary artery bypass grafting (CABG) and 2 trials in patients undergoing valve surgery. Subsequently, only 5 studies (134 patients in total) reported the use of CPAP during CPB and its impact on oxygenation. Other primary end-points were PaO₂/FiO₂ ratio (5 studies), the alveolar-arterial O₂ gradient or P(A-a)O₂ (9 studies), hospital LOS (6 studies), and the duration of postoperative MV (6 studies)^[2].

Seven review manuscripts have summarized some of the current findings in terms of MV strategies and perioperative lung mechanics in patients undergoing cardiac surgery. Table 1 describes the main reported conclusions for each one of them^[4-6,14-16].

Table 1. Summary of the manuscripts included in our review

Authors	Year	Research Method	Sample Size	Main outcome	Comments
Bechtel <i>et al.</i> ^[13]	2014	Review	NA	Describe current literature about anesthetic management in patients undergoing CPB	Protecting ventilation techniques during CPB may be associated with decreased inflammatory response. However, no significant overall improvement in respiratory and oxygenation parameters has been reported
Beer <i>et al.</i> ^[9]	2014	RCT	30	Chemokines serum levels	CCL4 serum levels from POD1 to POD5 were significantly reduced in the ventilated patients when compared to the non-ventilated group ($P < 0.05$). Perioperative clinical outcomes and 28-day mortality were comparable among groups
Young ^[14]	2014	Review	NA	To describe current strategies to reduce the postoperative inflammatory lung injury in patients undergoing CPB	Increased resistance in the pulmonary circuit may result from no ventilation during CPB. Further RCTs are required to elucidate the impact of mechanical ventilation during CPB on postoperative pulmonary outcomes
Beer <i>et al.</i> ^[10]	2015	RCT	30	Matrix metalloproteinases levels	Matrix metalloproteinases levels were significantly reduced at different time-points in patients who underwent mechanical ventilation during CPB. However, clinical implications should be addressed in future trials
Ferrando <i>et al.</i> ^[5]	2015	Review	NA	Review pulmonary protective strategies during CPB	CPAP, recruitment maneuvers, and low V_T during CPB have been associated with better postoperative lung mechanics. In addition, maintaining certain level of pulmonary perfusion during CPB may positively impact these outcomes
Gaudriot <i>et al.</i> ^[12]	2015	Prospective Observational	50	Impact of Mechanical ventilation during CPB on postoperative immune response	Pro-inflammatory TNF- α and immunosuppressive IL-10 were significantly reduced in patients who received mechanical ventilation during CPB ($P < 0.05$). Moreover, non-ventilated patients had a lower postoperative lymphocyte count when compared with the ventilated group ($P = 0.04$).
Huffmyer <i>et al.</i> ^[6]	2015	Review	NA	Pulmonary complications after CPB: etiology, risk factors, and prophylaxis	Intermittent ventilation, low V_T and recruitment maneuvers have been associated with reduced atelectasis and improved lung mechanics. Mixed results have been reported in terms of inflammatory markers and clinical outcomes.
Lellouche <i>et al.</i> ^[15]	2015	Review	NA	Mechanical ventilation strategies in patients undergoing cardiac surgery	Protective ventilation strategies are associated with improved lung mechanics, decreased pro-inflammatory cytokines, and reduced postoperative intubation time and ICU LOS
Bignami <i>et al.</i> ^[16]	2016	Review	NA	Postoperative lung dysfunction and mechanical ventilation strategies to prevent it in patients undergoing CPB	No ventilation during CPB has been linked to increased lysosomal enzymes in lungs circulation and increased incidence of ARDS. Low V_T 6-8 mL/Kg of IBW, PEEP, recruitment maneuvers, and $FiO_2 < 80\%$ have been associated with decreased morbidity, hospital LOS, and PPCs. Ventilation before and after the CPB may significantly affect lung mechanics as well. Mixed results have been reported in terms of CPAP use during CPB and its association with improved postoperative pulmonary outcomes. Only one trial has reported high-frequency ventilation during CPB with no significant respiratory improvements reported
Chi <i>et al.</i> ^[8]	2017	Meta-analysis	NA	Impact of mechanical ventilation during CPB on PPCs when compared to non-ventilated patients	Mechanical ventilation during CPB results in an improved oxygenation and gas exchanged. However, comparable incidences of PPCs and hospital LOS have been reported among groups
Toikkanen <i>et al.</i> ^[11]	2017	RCT	47	Mechanical ventilation and its effect on cytokines levels after CABG	CABG with CPB is associated with an increased pro-inflammatory cytokines pulmonary passage when compared to patients where CPB was not used. Moreover, lung ventilation did not change cytokines concentration in patients undergoing CABG with CPB. Main limitation: sample size, patient selection (e.g., lung disease was excluded), and no subgroups (ventilation vs. non-ventilation) in patients undergoing CABG without CPB
Bignami <i>et al.</i> ^[4]	2018	Review	NA	Describe current status of protective ventilation strategies and their impact on postoperative outcomes	In patients undergoing cardiothoracic surgery, protective ventilation strategies are associated with a decreased inflammatory response and should be considered in patients at high risk of PPCs. CPAP, low V_T , and non-ventilated lungs are among the options for mechanical ventilation during CPB

Wang <i>et al.</i> ^[2]	2018	Systematic Review and Meta-analysis	NA	Different strategies used for mechanical ventilation during CPB and postoperative outcomes	CPAP between 5-15 cm H ₂ O during CPB may be associated with short-term benefits such as improved gas exchange and oxygenation. However, no significant differences in these variables were found when comparing patients undergoing mechanical ventilation during CPB and those non-ventilated
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CPB: cardiopulmonary bypass; RCT: randomized clinical trial; CCL: chemokine ligand; POD: postoperative day; CPAP: continuous positive airway pressure; VT: tidal volume; TNF- α : tumor necrosis factor alpha; IL: interleukin; ICU: intensive care unit; LOS: length of stay; IBW: ideal body weight; PEEP: positive end-expiratory pressure; FiO₂: inspired fraction of oxygen; PPCs: postoperative pulmonary complications; CABG: coronary artery bypass graft; ARDS: acute respiratory distress syndrome

DISCUSSION

Multifactorial mechanisms affecting pulmonary function during and after CPB

Postoperative respiratory dysfunction is the most common postoperative complication in patients undergoing cardiac surgery under CPB affecting 10% to 25% of these patients and also associated with high mortality rates^[17-19]. Patient-specific, anesthesia- and surgery-related factors contribute to the onset of a complex mosaic of pathophysiological events that result in severe respiratory mechanics and gas exchange impairment ensuing postoperative pulmonary dysfunction^[18,20-23].

Patient-specific factors

Chronic obstructive pulmonary disease, low ventricular ejection fraction (EF) (i.e., EF < 30%), hypertension, blood transfusions, emergency surgery, previous cardiac surgery, combined procedures (i.e., cardiac and aortic procedures), active endocarditis, age > 70 are some of the patient-related risk factors associated with respiratory insufficiency after cardiac surgery^[24-26].

Anesthesia-related factors

Several reports identified a strong association between general anesthesia and impaired postoperative pulmonary function. Prolonged time in supine position and muscle relaxation have been linked to a significant reduction in both, functional residual capacity (FRC) and lung volume, resulting from a cephalic displacement of the diaphragm and the loss of balance between the elastic recoil of the lung and the outward forces of the chest wall. This reduction in FRC promotes alveolar collapse (i.e., atelectasis) and increases airway resistance with subsequent increased resistance to thoracic blood flow circulation. Furthermore, the volatile agents inhibit pulmonary hypoxic vasoconstriction whereas intravenous agents may decrease the hypoxic and hypercapnic ventilatory response. Intubation along with the aforementioned mechanisms may result in ventilation-perfusion mismatch, abnormal shunt fraction, and wider AaDO₂^[27,28].

Surgery-related factors

Sternotomy incision, sternosynthesis, and left internal mammary artery dissection

Numerous reports describe the association between the surgical technique and changes in respiratory mechanics and lung function^[22,29-33]. Median sternotomy disrupts sternum integrity, provokes chest wall instability (i.e., uncoordinated rib cage expansion, decrease compliance), and reduces lung volumes with subsequent impaired pulmonary mechanics^[29,31]. The combination of sternotomy and dissection of the left internal mammary artery (LIMA) has a significant impact on respiratory mechanics^[29,31]. LIMA harvesting maneuvers not only interfere with sternum stability but also may affect blood supply to the sternum, intercostal muscles, and left phrenic nerve functionality. Moreover, instillation of saline slush in the pericardial cavity has been also associated with phrenic nerve injury or dysfunction during cardiovascular surgery^[22]. Therefore, chest wall mechanics and diaphragm mobility impairment results in significant changes from pre-sternotomy breathing patterns (abdominal) to an upper thoracic pattern with reduced lung volumes thereby, promoting atelectasis^[34]. Retraction of the chest wall during LIMA harvesting produces additional trauma to the costovertebral joints ensuing an unstable rib cage with impaired diaphragm contraction^[34,35]. Likewise, altered thoracic wall mechanics and diaphragmatic dysfunction have been associated to a reduced postoperative abdominal motion^[22,29,31,34,36]. Nevertheless, disruption of the anterior insertions of the diaphragm seems to recover shortly after surgery^[37].

In addition to direct nerve injury (i.e., neuropraxia), the LIMA retractor has been also associated with lesion of the left internal oblique abdominal, external oblique abdominal and rectus abdominis muscles^[35,38,39]. In contrast, sternotomy with intact pleura maintains respiratory system and chest wall elastance unchanged. However, the opening of the parietal pleura leads to lung collapse with decreased lung elastance and resistance^[32]. In addition, the use of LIMA for grafting requires the insertion of a pleural subxyphoid or left intercostal tube for drainage, being subxyphoid placement associated with lesser impairment and postoperative pain when compared to intercostal insertion^[33,40,41].

Blood transfusion

Blood transfusion is used in 30%-60% of patients undergoing cardiac surgery and the reported incidence of transfusion-related acute lung injury (TRALI) is 2.5%^[42-44]. Blood transfusion has been linked to an increased risk of postoperative morbid events^[45], being transfusion of > 3 red blood cells units an independent risk factor for increased hospital LOS after cardiac surgery^[46]. Presence of bioactive lipids and antibodies in the stored blood, and the activation of transfused neutrophils in the setting of an exacerbated host's systemic and pulmonary inflammatory response are some of the mechanisms involved in the TRALI^[47].

Cardiotomy for suction

Tissue plasminogen activating factor, pro-inflammatory mediators (i.e., cytokines, activated leukocytes, lipids), pro-coagulants, and platelet factors are present in the cardiotomy suction blood. Numerous reports have shown the detrimental effects associated with these mediators during re-transfusion of unwashed blood collected in the pericardium including an increased inflammatory response with impaired lung function and hemostasis^[48,49]. Cell salvage devices helps to remove these activated mediators from the blood obtained from cardiotomy suction^[50,51].

Extracorporeal circulation

In spite of innovations in biocompatibility of CPB circuit's surfaces, the inflammatory response associated to extracorporeal circulation with subsequent anti-inflammatory response as well as the ischemia-reperfusion injury, continue to have a significant impact on postoperative morbidity and mortality after cardiac surgery^[52]. The exposure of plasma proteases to CPB circuit's non-endothelial surface ("contact activation") immediately activates the complement pathways and factor XII (XIIa). Likewise, classic complement pathway is activated by heparin-protamine complexes, coagulation and fibrinolysis byproducts (from the activation of the extrinsic coagulation pathway after vascular injury). Activation of classical and alternative pathway promotes the release of pro-inflammatory cytokines (TNF α , IL-1, IL-2, and IL-8), production of activated-polymorphonuclear leukocytes, endothelial cell damage, and capillary permeability^[21,52-54]. The combination of the aforementioned factors along with ischemia-reperfusion injury results in endothelial, alveolar and interstitial edema, increased airway resistance and atelectasis^[21]. Moreover, hemodilution (required to prevent embolism and hemolysis during CPB) may exacerbate pulmonary edema^[55].

Cessation of ventilation and altered surfactant production and function

Type II alveolar cell dysfunction, inactivation of large aggregate by alveolar edema fluid, and/or large aggregate leakage across the damaged alveolar capillary membrane are some of the effects of apnea and lung collapse to FRC during CPB, being the inflammatory response triggered by the use of a foreign bypass circuit during extracorporeal circulation^[56]. These biochemical and functional disturbances significantly affect surfactant concentration and functionality, contributing to the onset of atelectasis. Cyclic alveoli stretch is necessary to produce a signal transduction responsible of stimulating surfactant secretion by Type II alveolar cell^[57-59]. Therefore, apnea during CPB may significantly reduce surfactant secretion. Govender *et al.*^[60] reported that patients who underwent off-pump coronary bypass with MV using PEEP experienced higher postoperative large aggregate concentrations when compared to patients who

underwent CABG under CPB. Moreover, absence of ventilation has been associated with hydrostatic pulmonary edema, poor pulmonary compliance, and higher incidence of lung infections^[61,62].

Ischemia-reperfusion injury

Under physiologic conditions, bronchial circulation represents 3%-4% of the pulmonary blood flow and may decrease during CPB^[63]. The ischemic phase depletes the energy stores (i.e., ATP), increasing lactate levels in the pulmonary blood flow^[53,64]. The reperfusion and re-oxygenation phase after aortic cross-clamp release stimulates the production of reactive oxygen species (ROS) resulting in dysregulation of intracellular and mitochondrial calcium transport, increased inflammatory response (i.e., cytokines, complement and activation of neutrophils), endothelial cell damage, and increased vascular permeability^[60,65-67]. The systemic and pulmonary inflammatory states originated during and after CPB generate a compensatory anti-inflammatory response characterized by the production of anti-inflammatory cytokines (IL-10) and leukocytes^[68,69]. Monocytes downregulation follows this chain of events resulting in an increased susceptibility to postoperative infections^[52,70].

Hyperoxia

Increased oxygen concentrations are commonly administered during CPB in order to avoid cellular hypoxia, reduce gaseous micro-embolism, and improve neutrophils' functionality^[71]. Nonetheless, enhanced production of ROS, cardiovascular dysregulation, and increased injury due to ischemia-reperfusion are some of the systemic effects linked to hyperoxia^[72,73].

Could MV be a mechanistic strategy to protect the lungs during CPB?

Different strategies such as CPAP with and without PEEP have been implemented during MV under CPB. Current evidence about the use of MV as a mechanistic strategy for lung protection during CPB remains controversial. Early studies examined the effects of CPAP during CPB without showing any significant beneficial effects on oxygenation^[74,75]. Nevertheless, recent studies have reported that CPAP pressures of 10 cmH₂O were more effective in achieving and maintaining better postoperative PaO₂/FIO₂ ratio than lower CPAP pressures in patients undergoing cardiac surgery with CPB^[61].

Even though only a small amount of patients undergoing cardiac surgery may develop acute respiratory distress syndrome (ARDS), the reported mortality rates may reach up to 50%^[17,24]. An increased body of evidence supports the benefits of PMV (low V_T, FiO₂, and PEEP) in patients with ARDS^[76-80]. The rationale for using PMV during CPB lies in the fact that postoperative pulmonary dysfunction in cardiac surgery is characterized by alterations in lung mechanics and gas exchange abnormalities, which may resemble some of the ARDS physiologic and clinical features. Although many surgeons prefer the lung collapsed during CPB in order to improve the surgical field, recent published reports suggest that PMV may be associated with a significant reduction of pathophysiological events and pulmonary dysfunction after cardiac surgery^[81-89]. However, MV also entails some risk of pulmonary damage such as alveolar over distension (resulting from high V_T), alveolar rupture (due to cyclic opening), inactivation of surfactant, and excessive lung stress inducing elevated transpulmonary pressure^[90-93].

John and Ervine^[84] randomized patients undergoing CABG under CPB to either MV with low V_T/no-PEEP (ZEEP) or non-ventilation. Patients who were ventilated during CPB presented lower extravascular lung water content and shorter extubation times when compared to the non-ventilation group (530 ± 50 mL vs. 672 ± 32 mL, *P* = 0.028 and 3.60 ± 0.3 h vs. 4.8 ± 0.4 h, *P* = 0.038 respectively). Paradoxically, the cyclic expansion of the lungs may further reduce the bronchial blood flow during the pulmonary exclusion phase of extracorporeal circulation^[94].

Gagnon *et al.*^[86] studied 40 patients undergoing CABG with CPB. Patients were randomized into two groups, no ventilation (group I) and ventilation with low V_T (3 mL/kg) and ZEEP during CPB. Endothelial function was assessed through the changes in pulmonary vascular resistance index (PVRI) after the injection of acetylcholine (ACh) into the pulmonary artery. Although patients in the ventilated group had a better vasodilatory response to ACh, the difference in PVRI between the two groups was not statistically significant neither after declamping of the aorta ($P = 0.32$) nor at 1 h after CPB ($P = 0.28$). In addition, LTV with or without PEEP has been associated with attenuation of the systemic and pulmonary immune-inflammatory response and thereby, its effect in the lungs^[9,10,12,87].

MV and pulmonary perfusion during CPB

Discontinuation of the pulmonary artery circulation during CPB significantly affects the bronchial blood flow and metabolic demand which results in ischemia-reperfusion injury. Nevertheless, maintaining pulmonary circulation and ventilation during CPB have been associated with reduced ischemia-reperfusion damage in preclinical models^[95].

In humans, the impact of continuous pulmonary perfusion during extracorporeal circulation on reducing postoperative lung injury remains controversial^[96-98]. Santini *et al.*^[96] compared pulsatile pulmonary perfusion during CPB with conventional CPB in patients undergoing cardiac surgery. The pulsatile pulmonary perfusion group showed increased $\text{PaO}_2/\text{FiO}_2$ and lung compliance with reduced neutrophils in the bronchoalveolar lavage when compared to the conventional group. Moreover, pulmonary perfusion has been also associated with an increased postoperative oxygenation when compared to the use of histidine-tryptophan-ketoglutarate solution during CPB in patients undergoing cardiac surgery^[97]. Even though pulmonary perfusion during CPB reduces the postoperative inflammatory response and improves oxygenation, long-term benefits are yet to be determined. However, its implementation may considerably increase surgeons' workload.

CONCLUSION

A variety of MV strategies may have potential benefits in patients undergoing cardiac surgery with CPB. PMV is a useful mechanistic strategy during CPB associated with reduced systemic and inflammatory responses and thereby, lung injury. Nevertheless, the impact of these findings on postoperative morbidity and mortality has not been clearly established. Future prospective RCTs should address the need for clinical data describing both, short- and long-term outcomes in patients undergoing cardiac surgeries with CPB under MV.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and outline of this manuscript: Echeverria-Villalobos M
Made substantial contributions to data search, interpretation, and writing: Echeverria-Villalobos M, Munlemvo DM, Fiorda-Diaz J, Essandoh MK

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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.

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Review

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Surgical treatment for heart failure: cell-based therapy with engineered tissue

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Abstract

This review will outline cell-based therapy for heart failure focusing on tissue engineering to deliver cells to the damaged heart. We will present an overview of the central approaches focusing on pluripotent stem cell-derived cells, mechanisms of action, autologous vs. allogeneic cell approaches, immunologic modulation, and safety considerations. We will outline the progress that has been made to-date and define the areas that still need to be investigated in order to advance the field.

Keywords: Heart failure, induced pluripotent stem cells, tissue engineering

INTRODUCTION

The ability to differentiate specialized functional cells from pluripotent stem cells (PSCs) has opened up the possibility of new therapeutic approaches that provide the functional units to solve the underlying causes of disease. Work using cells differentiated from embryonic PSCs first appeared 1998 when Jamie Thomson and colleagues published a report of deriving embryonic stem cell lines from human blastocysts^[1]. This same laboratory described creating induced PSC lines from human somatic cells in 2007^[2]. In 2012, the Nobel Prize in Physiology or Medicine was awarded to John B. Gurdon and Shinya Yamanaka for



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the discovery that mature adult differentiated somatic cells can be reprogrammed to a pluripotent state to create an unlimited source of any cell in the body. The ability to generate human induced PSC-derived cardiomyocytes (PSC-CMs) from adult cells has further broadened interest and study in the use of these cells in the heart. A number of investigators are now culturing these cells, developing methods to maintain differentiated cell phenotype, and studying healthy and diseased human cardiomyocytes^[3-6]. Much of this work looks at reprogramming somatic cells from healthy and diseased donors to study known mutations and deficiencies of cardiomyocytes, as well as identify potential treatments. Hurdles regarding target identification and drug development have been attributed to variability in phenotype, thus the growing body of work in this area^[7,8].

Prior to the development of PSCs, the early work with cell-based therapy for acute myocardial infarction and chronic heart failure (CHF) involved administering varying adult somatic cell populations. With the exception of the skeletal myoblasts^[9], these approaches proved safe but had limited therapeutic efficacy with respect to improved cardiac function and structural remodeling^[10,11]. With the advent of PSCs it became possible for investigators to create the functional building blocks of the heart allowing for the development of tissue engineering (TE) approaches, cellular reprogramming, and gene editing to optimize the delivery of tissue function specific cells to the damaged heart^[12-23].

AUTOLOGOUS VS. ALLOGENEIC PREPARATIONS

Early in the evolution of cell-based therapies there was enthusiasm to use autologous approaches with the patient's own cells so as to avoid immune rejection. However, it has become clear that limitations with autologous approaches exist: sample collection and preparation from individual patients is time-consuming, costly and may ultimately lack efficacy because of problems with cellular genome stability and regenerative potential. To overcome these limitations, allogeneic approaches have been adopted using screened, optimal donors. This donor vetting improves both the quality and potency of the cell product, increases cell availability, and decreases cost through scaled manufacturing. The paradigm switch from autologous to allogeneic was observed with the first clinical trial using PSC derived cells for age-related macular degeneration, which started with autologous preparations but subsequently changed to using allogeneic cells^[24].

IMMUNE CONSIDERATIONS

Immunologic rejection is something relevant for all proposed cell-based therapies^[25]. The concept of accounting for the immune system is based on the belief that PSC derived cells are not immune-privileged because of Human Leukocyte Antigen (HLA)/ABO antigens. Investigators have suggested that the expression of these antigens in PSC-derived cells equates them with any other organ transplants, all of which require immune suppression^[26]. Immunosuppressive agents such as cyclosporine and tacrolimus have been used for solid organ transplant and described extensively in pre-clinical studies of TE preparations. However, they are costly, may be cytotoxic, and cause adverse effects. There is a clinical trial in Japan using PSC-CMs in patients with CHF patients where the patients receive non-HLA mapped allogeneic cardiomyocytes and are given immune suppression^[27]. The use of HLA mapping is one option by which to circumvent immunosuppressive agents; allogeneic cell lines of HLA-typed donors could be generated to maximally match each recipient, though an identical match is statistically improbable. Such a strategy would require creating and maintaining large cell banks. Generating personalized products for individual patients from the bank would be expensive and require HLA typing of every patient. The ability to edit candidate therapeutic cell lines is also being explored, to make a hypo-immune cell, one that would be considered "universal". Each cell line could be edited to remove HLAs while still maintaining markers of identity so as to go undetected as foreign by the immune system, theoretically eliminating the need for

immunosuppression^[28]. Gene editing may prove valuable in the future to generate cell therapy candidates that integrate or persist in the host without losing potency.

ENGINEERED TISSUE TO TREAT HEART FAILURE

The development of cell-based TE approaches is exciting and in essence has the potential to generate a therapeutic tissue with properties and function of the myocardium^[13]. Investigators have constructed tissue patches that could be manipulated with respect to anisotropic components, degree of electrical conductance, and mechanical qualities such as cardiomyocyte alignment and electromechanical coupling or using decellularized starting material as a substrate^[29,30]. Cardiac patches have been engineered with nanotopographically-defined hydrogels meant to enhance cardiac regeneration by providing functional cell-material interfaces^[31]. Three-dimensional (3-D) printing has also been utilized to generate TE scaffolds by organizing special patterning of stem cells. The 3-D generated patches proved sufficient to promote rapid vascularization with the potential to improve cardiac function and reverse maladaptive left ventricular (LV) remodeling^[32]. Cardiac scaffolds have been made from electro-conductive acid-modified silk fibroin-poly (pyrrole) substrates. With PSC derived cardiomyocytes these grafts showed enhanced gap junction distribution and cardiomyocyte maturation^[33,34].

Lancaster *et al.*^[35] have developed a TE cardiac patch composed of a bioabsorbable scaffold embedded with human neonatal fibroblasts and PSC-CMs that is implanted on the epicardial surface of the heart to treat CHF. The fibroblasts proliferate and synthesize glycosaminoglycans, collagen, elastin, fibronectin and laminin while providing a secretome of growth factors and cytokines. The fibroblasts support the incorporation of the cardiomyocytes into the scaffold and the cardiomyocytes provide additional complementary secretomes. This cardiac patch increases myocardial blood flow, reverses maladaptive LV remodeling, activates endogenous growth factor secretion, recovers hibernating myocardium and improves LV function and improves electrical propagation through the previously scarred myocardium and enhances electrical stability of the healthy tissue-scar interface^[14-16,35]. The specific growth factors are angiopoietin-1, β -myosin heavy chain, connexin 43, insulin like growth factor and vascular endothelial growth. This patch is robust; it is easy to handle for open surgical or minimally invasive implantation. [Figure 1](#) and the [Supplementary Video 1](#) demonstrate implantation on the epicardium in an infarcted Yucatan mini swine via a mini median sternotomy where the patch is positioned to broadly cover all damaged tissue. We envision that multiple patches and/or multiple patch applications could be applied if needed. [Table 1](#) summarizes previous work done with TE approaches using iPSC-CMs to treat heart failure. The epicardium may play an important role in regulating regeneration and may be the catalyst of the beneficial effects we and other investigators see when we implant cell-seeded scaffolds on the epicardium^[36-38,48-50].

Implanting TE scaffolds in preclinical animal models of heart failure has proven safe. The data have shown no teratoma formation, accompanied by hemodynamic benefits, and attenuated LV remodeling, however data show limited long-term engraftment with the implanted cells despite providing immune suppression^[20]. Menasché *et al.*^[19] have transplanted PSC-derived cardiac progenitor cell fibrin patches in 6 patients with decreased LV function and showed that the treatment was safe. No arrhythmias were noted by ICD interrogations and no tumors were reported^[19]. Based on this work, these investigators performed a second trial of 10 patients, but these results are still forthcoming. To date, there is only one clinical trial has commenced using PSC-CMs to treat patients in CHF; this trial is currently ongoing in Japan^[27]. Cardiothoracic surgeons are implanting cell sheets on the epicardial surface of the heart in immune-suppressed patients. [Table 1](#) summarizes some of the TE scaffolds developed to treat heart failure progressing from early work with rat neonatal cardiomyocytes to the clinical trial in Japan with PSC-CMs on cell sheets.

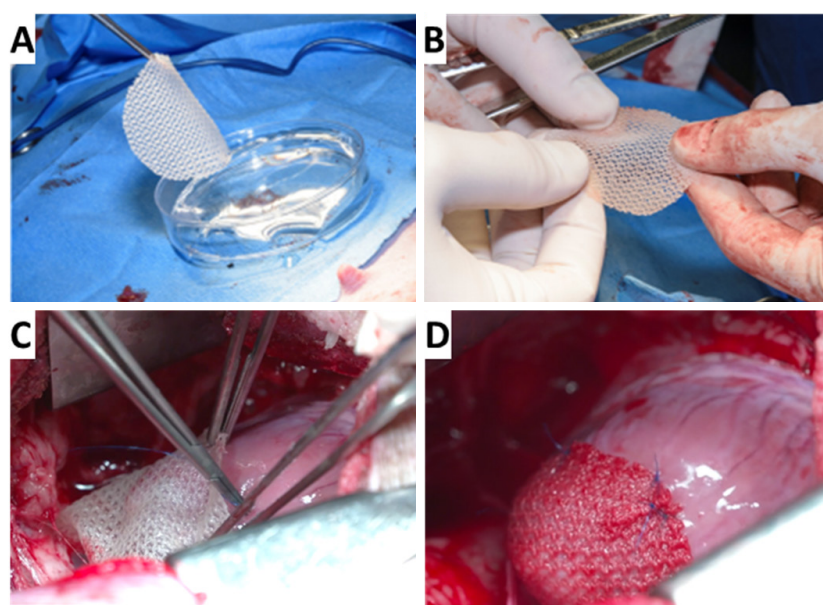


Figure 1. Intra-operative implant of cardiac patch in mini-swine one month after myocardial infarction. A: the patch is picked up by surgeon (KF) in the operating room; B: easily handled by surgeons prior to implantation; C: implanted in mini-swine through a mini median sternotomy; D: close up view of the patch successfully implanted on the heart

Table 1. Summary of TE approaches using pluripotent derived cardiac cells for heart failure

Cell type	Auto/allo	Scaffold	Stage	Investigator ^(Ref)
Neonatal rat cardiomyocytes	Auto	EHT	Pre-clinical	Zimmerman <i>et al.</i> ^[39]
Neonatal rat cardiomyocytes	Auto	Bioabsorbable polymer	Preclinical	Lancaster <i>et al.</i> ^[15,16]
Vascular smooth muscle	Auto	PCLA	Pre-clinical	Matsubayashi <i>et al.</i> ^[40]
Skeletal myoblasts	Auto	Cell sheet	Clinical	Sawa <i>et al.</i> ^[41]
Skeletal myoblasts	Auto	Cell sheets	Clinical	Yoshikawa <i>et al.</i> ^[42]
ES derived cardiac progenitor	Allo	Fibrin	Clinical	Menasché <i>et al.</i> ^[19]
PSC-CMs	Allo	Cell sheets	Pre-clinical	Kawamura <i>et al.</i> ^[20]
PSC-CMs	Allo	EHT	Pre-clinical	Yorgan <i>et al.</i> ^[43]
Bone marrow stem cells	Auto	Fibrin	Pre-clinical	Liu <i>et al.</i> ^[44]
Mesenchymal progenitor cells	Allo	Fibrin	Pre-clinical	Godier-Furemont <i>et al.</i> ^[45]
PSC-CMs	Allo	3-D scaffold	Pre-clinical	Gao <i>et al.</i> ^[17]
ES cardiomyocytes/ progenitors	Auto	Fibrin	Pre-clinical	Liau <i>et al.</i> ^[29]
Human cardiac progenitor	Allo	hdECM	Pre-clinical	Jang <i>et al.</i> ^[32]
PSC-CMs	Allo	Silk fibrion-poly (pyrrole)	Pre-clinical	Tsui <i>et al.</i> ^[33]
PSC-CMs	Allo	Cell sheets	Pre-clinical	Matsuura <i>et al.</i> ^[46]
PSC-CMs	Allo	Cell Sheets	Pre-clinical	Sasagawa <i>et al.</i> ^[47]
PSC-CMs	Allo	Bioabsorbable polymer	Pre-clinical	Lancaster <i>et al.</i> ^[35]
PSC-CMs	Allo	Cell sheets	Clinical	Cyranoski/Sawa ^[27]

TE: tissue engineering; Allo: allogeneic; Auto: autologus; hdECM: decellularized extracellular matrix; ES: embryonic stem; EHT: engineered heart tissue; PSC-CMs: human induced pluripotent stem cell-derived cardiomyocytes; PCLA:sponge polymer of epsilon-caprolactone-co-L-lactide reinforced with knitted poly-L-lactide fabric

MECHANISMS OF ACTION OF CELL-BASED THERAPY

It is likely that the mechanism of action that improves LV function with cell-based therapy is multi-modal and is shaped by the fate of the cells, as the cells can be intended as either integrating or non-integrating. Integrating cells directly replace lost myocardium and theoretically contribute to mechanical function. Non-integrating approaches are transient in that the cells persist for days, weeks or months, imparting a beneficial effect, before ultimately being cleared from the tissue. In both integrating and non-integrating

approaches, a secretome of soluble factors such as growth factors, exosomes, and miRNAs are thought to be responsible for the beneficial effects^[48-50]. The specific paracrine stimulation may vary by implanted cell type and may be modulated by the cell culture environment, delivery platform, and other relevant variables.

In pre-clinical studies Lancaster *et al.*^[14-16,35] did not immune suppress their animals and long-term physiologic benefits were seen when implanting human xenografts in immune-competent animals. The transplanted PSC-CMs did not persist beyond four weeks post-implantation, but the initial functional benefit continued to persist at ten weeks post-implantation^[35].

SAFETY CONSIDERATIONS

The use of PSC-derived cells offer potential as a source for therapeutic advancements in all tissues, not just the heart. The safety of these cell preparations when implanted in patients is obviously important. The potential for tumorigenicity resulting from undifferentiated PSCs contaminants is a concern, particularly with integrating cell preparations. The Food and Drug Administration requires extensive preclinical testing for teratoma formation before approving PSC-derived preparations for clinical use. During somatic skeletal myoblasts injections into the myocardium, ectopic foci were established that generated VT from spontaneously depolarizing cells^[9]. The finding of increased incidence of ventricular tachyarrhythmia has also been reported with human embryonic stem cell-derived cardiomyocyte injections into non-human primates and swine hearts^[51,52]. Interestingly in preclinical studies with PSC-CMs TE scaffolds implanted on the epicardium, the animals remain in normal sinus rhythm without any arrhythmias^[20,35].

PROSPECTS FOR THE FUTURE

Looking into the future to predict the next advances of TE approaches to treat CHF is difficult but while creating a cardiac patch has received a lot of interest, there are a number of other potential approaches. Investigators have proposed decellularizing entire hearts and then repopulating with new PSC-derived cells^[53]. There are also efforts to use 3-D printing to print entire organs^[54]. Synthesizing microvesicles such as exosomes or secretomes that are secreted from the PSC-derived cells are being explored as a way to bypass cell-based therapy and still retain the paracrine effect^[48]. Some of the issues outlined previously need to be addressed such as the best strategies for immune suppression in patients, required maturity of PSC-derived cells, use of integrating or non-integrating approaches and duration of transplanted cell survival in order to result in the most beneficial effects. A recent review summarizes the “bottlenecks” for the future of TE cardiac scaffolds^[55]. Despite these issues, we see a bright future for using TE approaches for regenerative cardiology and all of medicine. The FDA now has a rapid approval process for cell therapy and therapeutic TE products. The Regenerative Medicine Advanced Therapy designation of the 21st Century Cures Act is designed to help accelerate medical product development and bring new innovations and advancements to the patients who need them urgently^[56].

CONCLUSIONS

The availability of PSC-derived cells and ability to generate TE approaches have introduced a unique opportunity to develop novel strategies to treat patients. While there remain points of concern that need to be addressed with respect to PSC-derived therapy, such as defining the mechanisms of action and the potential need for immune suppression the field as a whole is moving forward and TE surgical therapies for regenerative cardiology are closer to reality today than ever before.

DECLARATIONS

Authors' contributions

Conception, design, analysis and interpretation of the data: Lancaster JJ, Koevary JW, Chinyere IR, Daugherty SL, Fox KA, Goldman S

All the authors made substantial contributions to this article.

Availability of data and materials

Not applicable.

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Conflicts of interest

Drs. Lancaster, Koevary, Goldman and Ms. Daugherty have a financial interest in Avery Therapeutics, a biotechnology company that is commercializing platform technologies to deliver cell-based therapies for clinical use. The other authors (Chinyere IR, Fox KA) report no conflicts.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Original Article

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Finite element analysis of polyether ether ketone 450G biomaterial used as cardiovascular stent implant

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Abstract

Aim: This research paper aims to modeling and finite element analysis of PEEK 450G biomaterial used as cardiovascular stent implant.

Methods: Commercially available CATIA V5 and ABAQUS 6.0 software were used for modeling and finite element analysis of cardiovascular stent implant to evaluate the radial displacement, stress distribution, and plastic strain in the proximal area of PEEK 450G biomaterial under pressure load conditions of 0.8, 1.0, and 1.2 MPa.

Results: The results show that, both in non-linear bending analysis and non-linear pressure analysis, that PEEK 450G stent exhibits very good radial expansion and lowest stress concentration in plaque and also which is well below the yield level (100 MPa), however plastic strain is quite high.

Conclusion: The blood circulation will be appropriate and also chances of vessel damage may be reduced more. Hence the study reveals that PEEK 450G can be best alternate biomaterial appropriate for cardiovascular stent implant.

Keywords: Biomaterial, polyether ether ketone 450G, biocompatibility, finite element analysis, stent implant



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INTRODUCTION

The innovation of technology in the field of medical and cardiovascular implantation has increased the demand for metal-free restoration and has led to the growth of innovative alternative materials. Biomaterials are frequently used for clinical applications in cardiovascular implantation, dental implants, heart valves, coated hip implants, surgery and for drug delivery “A biomaterial is a non-viable material used in a medical device proposed to interact with biological systems”^[1].

Cardiovascular artery disease is the most universal cause of death in the globe^[2]. Around 2300 Americans die of coronary disease daily, the average of one death for every 38 s. Every year there are more than 17.9 million of deaths, is projected to rise to 23.6 million deaths by 2030. The total direct and indirect clinical costs of cardiovascular disease are likely to increase to 749 billion \$ by 2035^[3].

Cardiovascular disease occurs when excess of cholesterol attach to the blood vessels wall, causing coronary artery disease (CAD)^[4]. There are different measures are available to revascularise a jammed vessel, including bypass surgery, atherectomy, angioplasty and coronary stenting^[5].

Forty years ago, coronary artery bypass surgery was the trendy revascularization action used to cure blocked CAD. Regular coronary closures occur and thus urgent surgery was essential^[5,6]. Compared to other possible treatments stenting shows some advantages, has less complications, less pain and also has faster recovery. Therefore, the use of stents in cardiovascular activities has quickly improved from 10% to over 80% in current practice^[7].

The present problem in cardiovascular field is that, Co-Cr L605 alloy stent implant has higher modulus of elasticity; owing to higher level of stresses developing in stent expansion which affects the coronary artery, hence chances of vessel damage is very high. In addition, the alloy has very poor plasticity and machinability. Further the presence of Nickel in Co-Cr L605 alloy is a risky aspect from the point of view of allergic problems^[8].

Polyether ether ketone (PEEK) boasts of outstanding fatigue resistance and toughness. The Young's modulus is extremely close to that of the bone. It is available for implantable devices viz cardiovascular, dental, orthopedics and spine applications. In addition, PEEK offers several advantages over metals, were excellent biocompatibility, chemical resistance, and superior in machinability, low co-efficient of friction, lessening of stress shielding.

Ortega-Martínez *et al.*^[9] have studied and recommended PEEK is an alternative material for medical implants because of several advantages over metals. The drawbacks of metals include high elastic modulus which causes high stress, allergies, and radiopacity. Guo *et al.*^[10] have investigated and suggested application of PEEK used as medical implants because of high modulus of elasticity (approximately equal to human bone), and high strength. The excellent biocompatibility and chemical resistance makes PEEK an attractive alternative compared to other biomaterials. Pargaonkar *et al.*^[11] have made comparison between different biomaterials and suggested that PEEK is the excellent biomaterial for medical devices. It has superior biocompatibility, chemically and physically stable, radiolucent and Young's modulus is similar to bone. Sagomonyants *et al.*^[12] have conducted cytocompatibility and mineralization *in vitro* studies on pure Titanium alloys and PEEK polymer, and recommended unfilled grade PEEK can propose a versatile base material for medical implants. Since the conventional material such Titanium alloy can release metal and ion debris resulting in blocking the implant area and stress shielding.

By comparison with costly experiments performed in laboratories and hospitals, numerical simulation performed by computers offer benefits of both cost and flexibility. This research article aims to modeling

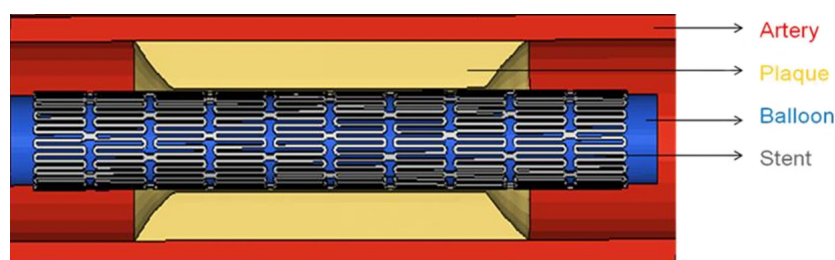


Figure 1. Assemble model of stent, balloon, vessel with plaque

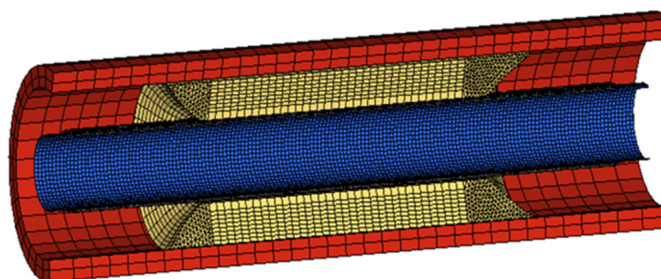


Figure 2. Finite element meshed model of stent, vessel, balloon and plaque

and finite element analysis of cardiovascular stent implant to evaluate the radial displacement, stress distribution, and plastic strain in the proximal area of PEEK 450G biomaterial under pressure load conditions of 0.8, 1.0, and 1.2 MPa.

METHODS

Finite element analysis

At present there are different coronary stent designs available in the market. In modern days the use of stents in vascular procedures has speedily increased. In order to improve outcome of the coronary stent implantation, it is essential to study the biomechanical performance of the stent before manufacturing and utilized. One of the effective methods used is finite element analysis to study the performance of the cardiovascular stent to alter the design of the coronary stent and its presentation.

Finite element models

This section covers modeling of different parts used in the study of biomechanical performance of cardiovascular stents. The modeling of coronary stent, vessel, plaque and balloon are illustrated in [Figure 1](#). Commercially accessible CATIA V5 software was used for modeling and the IGES file was imported in HYPERMESH V11.0 for meshing the model. [Figure 2](#) represents the finite element meshed model of stent, vessel, plaque and balloon.

Cardiovascular stent

In this research article a balloon expandable coronary stent was modeled. To create prime model of cardiovascular stent, commercially accessible software was used. The modeling of cardiovascular stent produced on source of imagery^[13]. The stent length = 15 mm, stent outer diameter = 1.915 mm, and stent thickness = 0.05 mm. The element type used for modeling of stent in ABAQUS as C3D8R (linear 8-noded solid element). The FE model of the coronary stent contains of 40,852 elements 108,080 nodes.

Table 1. Mechanical properties of PEEK 450G biomaterial^[15]

Material	Material Properties			
	Modulus of Elasticity E in (MPa)	Density ρ in (kg/mm ³)	Poisson's Ratio ν	Yield Stress σ_y in (MPa)
PEEK 450G	3700	1.30×10^{-6}	0.36	100

PEEK: polyether ether ketone

Balloon

Geometrical illustration of balloon model was formed based on standard reference^[14]. The length of the balloon was 25 mm and as a medium to inflate the coronary stent. The balloon thickness and outer diameter were 0.05 mm and 1.79 mm. To signify the balloon a polyurethane rubber form material was used. The balloon was modeled by using Mooney-Rivlin hyper elastic strain energy potential “W” and polyurethane is incompressible. Like coronary stent, the element type used for modeling of balloon in ABAQUS as M3D8R (linear 8-noded membrane element). The FE model of the balloon consisted of 13,750 elements 13,805 nodes.

Vessel

The model of vessel was created by assuming of isotropic and homogeneous material. The vessel was modeled by using hyper elastic Ogden material behavior and as a one- layer blood vessel. The vessel length was 23 mm, vessel outer diameter was 5 mm, and vessel thickness was 0.5 mm. The element type used for modeling of vessel in ABAQUS was C3D8R (linear 8-noded solid element). The FE model of the vessel consisted of 2352 elements, and 111,692 nodes.

Plaque

There are two types of plaque generated in coronary artery, viz., hypo-cellular and calcified plaque. The hypo-cellular type was used for the modeling of plaque. Geometrical parameters used for the plaque are as follows. Length of plaque = 10 mm, outer diameter of plaque = 4 mm, and thickness of plaque = 1 mm. Element type used for modeling of plaque in ABAQUS was C3D8R (linear 8-noded solid element). The FE model of the plaque consisted of 21,030 elements and 125,390 nodes

Non-linear bending analysis

The bending of coronary stent implant has both limitations and advantages. On one side, bending of coronary stent produces elevated stresses on coronary artery; due to these high stresses vessel damage might occur. On other side, the coronary stent bending can facilitate to keep coronary stent steady against the force of pulsate movement of blood. Hence the bending analysis in coronary stent implant can be extremely helpful to alter design of coronary stents. In this article only cardiovascular stent is considered for bending analysis.

Materials properties

In this study, the assumption was made that the PEEK 450G biomaterial was homogenous and isotropic characterized by four material properties, i.e., Modulus of elasticity, Density, Poisson's ratio and Yield stress. The mechanical properties of PEEK 450G biomaterial are represented in the Table 1^[15].

Loading and constraints

Based on numerical data for biomedical stent using PEEK 450G biomaterial the deformation of the stent in terms of bending which is dealt as four different loading cases as shown in Table 2^[16] and also the one end of the stent is fixed in all DOF.

Analysis

Finite element analysis solver ABAQUS V6.10 was used to find out von-mises stresses of PEEK 450G stent at various loading cases, i.e., 1 mm displacement in Y direction, 2 mm displacement in Y direction with 0.1

Table 2. Loading cases^[16]

Loading cases	Displacement-Y in mm	Displacement-Z in mm
Case-1	1	0
Case-2	2	0.1 radian
Case-3	2	0.15 radian
Case-4	2	0.25 radian

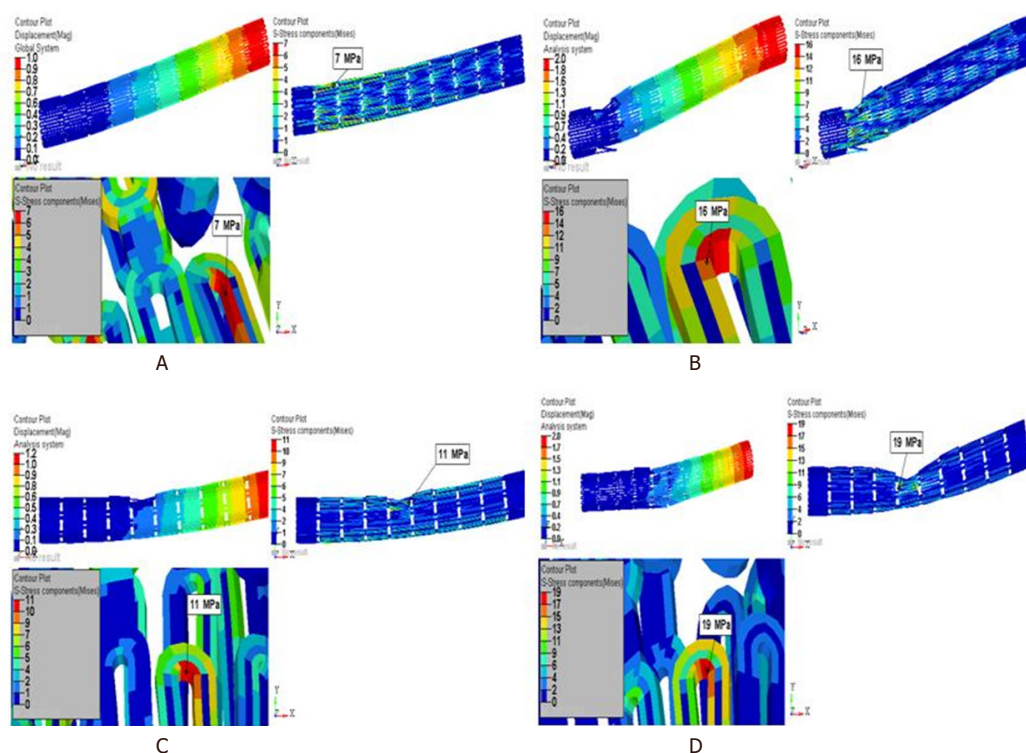


Figure 3. Von-mises stress distribution of PEEK 450G stent when subjected to (A) 1 mm displacement (B) 2 mm displacement with 0.1 radian rotation (C) 0.15 radian rotations, (D) 0.25 radian rotations. PEEK: polyether ether ketone

radian rotation in Z direction, 0.15 radian rotation in Z direction and 0.25 radian rotation in Z direction. [Figure 3](#) illustrates the analysis of PEEK 450G biomaterial for the above represented loading cases.

Non-linear pressure analysis

Here, coronary stent, balloon and vessel with plaque was considered to carry out non-linear pressure analysis by using commercially available ABAQUS 6.10 software, and to determine radial displacement, von mises stresses and plastic strain of coronary stent when it is subjected to different loading conditions i.e., 0.8 MPa, 1 MPa, and 1.2 MPa. [Figure 2](#) illustrates the FE meshed model of stent, vessel, balloon and plaque was selected for simulation.

Constitutive stent material behavior

The stress strain performance determine from the Ogden model is shown in [Figure 4^{\[17\]}](#) with parameters specified in [Table 3](#).

Intima vessel wall was modeled as a single homogeneous hyper elastic layer. Intima vessel layer is stiffer than other layers like media and adventitia, and have only intima vessel is considered for the analysis. Isotropic material properties were used and described by third-order Mooney-Revin hyperelastic model^[18]. The third-order strain energy potential for Ogden model is given by the equation:

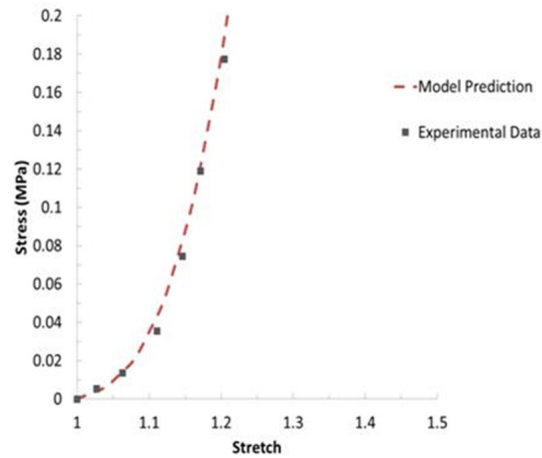


Figure 4. Stress-stretch curve for intima vessel^[13]

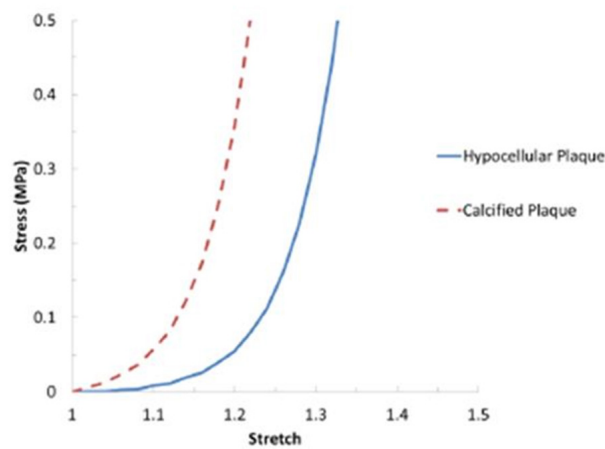


Figure 5. Stress-stretch curve for hypo-cellular and calcified plaque^[13]

Table 3. Ogden model parameters of vessel

Material	ρ (kg/mm ³)	μ_1	μ_2	μ_3	α_1	α_2	α_3	D_1
Intima	1.07×10^{-6}	-7.04	4.23	2.85	24.48	25.00	23.54	8.95×10^{-7}

$$W = C_{10} (I_1 - 3) + C_{01} (I_2 - 3) + C_{20} (I_1 - 3)^2 + C_{11} (I_1 - 3) (I_2 - 3) + C_{30} (I_1 - 3)^3 \quad (1)$$

Where, W = Strain energy density function; I_1 , I_2 and I_3 = the strain energy invariants; C_{ij} = hyperelastic constants.

The stress-strain behaviour of hypo-cellular and calcified plaque show that, hypo-cellular plaque is less resistance to stretch or deformation than calcified plaque as shown in Figure 5^[17]. Hence, in this work hypo-cellular plaque was considered for simulation.

Like blood vessel, here also the similar Ogden hyper elastic model was used, but with first order (i.e., $i = 1$) and the values are specified in Table 4^[17].

Polyurethane rubber material was used for modelling of balloon, by using Mooney-Revin hyperelastic strain-energy potential, W and is given by the equations:

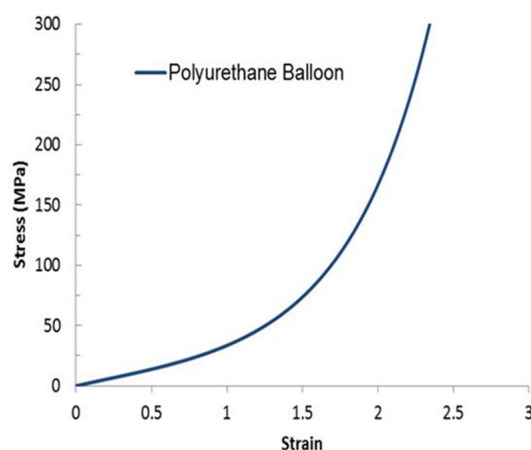


Figure 6. Stress-strain curve for polyurethane rubber balloon^[13]

Table 4. Values of the Ogden model parameters for hypo-cellular plaque

Material	ρ (kg/mm ³)	μ_1	α_1	D_1
Hypo-cellular Plaque	1.45×10^{-6}	0.093	8.17	4.30×10^{-7}

Table 5. Values of the Ogden model parameters for hypo-cellular plaque

Material	ρ (kg/mm ³)	C_{10}	C_{01}	D_1
Polyurethane	1.07×10^{-6}	1.03176	3.69266	0

$$W = C_{10} (I_1 - 3) + C_{01} (I_2 - 3) + 1/D_1 (J - 3) \quad (2)$$

$$I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \quad (3)$$

$$I_2 = \lambda_1^2 \lambda_2^2 + \lambda_1^2 \lambda_3^2 + \lambda_2^2 \lambda_3^2 \quad (4)$$

$$I_3 = \lambda_1^2 \lambda_2^2 \lambda_3^2 \quad (5)$$

Where, C_{10} , C_{01} and D_1 = model co-efficient; J = volumetric stretch; $\lambda_1 \lambda_2 \lambda_3$ = stretches in 3 principal directions.

The polyurethane material is incompressible and was defined by a non-linear first order hyper-elastic Mooney Revlin model^[17]. The stress strain curve for polyurethane rubber balloon is shown in Figure 6, and values of corresponding parameters illustrated in Table 5.

Loading and constraints

To simulate the inflation process of stent, a pressure was applied to the inner surface of the balloon. The pressure applied was 0.8 MPa, 1.0 MPa, and 1.2 MPa. In simulation, the balloon has fixed in all degrees of freedom at left end, the balloon were fixed in all degrees of freedom at right end, preventing axial movement of balloon to slide in the artery. Contacts between the balloon and stent, plaque and stent, artery and plaque, were modeled as face-to-face solid contacts, with a frictionless movement under common interaction.

There was no direct contact among any surfaces of the stent model at the beginning of the simulation. When pressure was applied at internal surface of the rubber balloon, first contact between stent and balloon was recognized, then between stent and plaque, and finally between plaque and vessel. In stent expansion, contact between stent and vessel was also noticed.

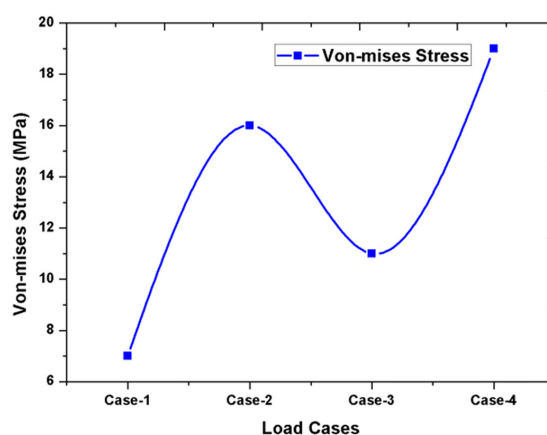
FIGURES

Figure 7. Relation among von-mises stresses and different load cases

Finite element simulation

Explicit solver (ABAQUS 6.10) was used to carry out simulations, which was used to produce the expansion of the balloon. Considering artery and plaque for the non linear pressure analysis of stent expansion, for the stent inflation balloon was used as a medium for which various pressure load cases was performed to know the mechanical behaviour of artery and stent, such as radial displacement of artery and stent, von-mises stresses sharing of artery and stent and plastic strains at plaque and non-plaque regions.

RESULTS

Many researchers elaborated in their work, Co-Cr L605 alloy stent implant has higher modulus of elasticity; owing to higher level of stresses developing in stent expansion which affects the coronary artery during stent expansion, hence chances of vessel damage is very high. To overcome these problems, the alternate material is essential for the cardiovascular stent implants. The results of PEEK450G stent implant has discussed in following two methods.

Non-linear bending analysis

Von-mises stresses

The results of non-linear bending analysis of coronary stent by using PEEK 450G as shown in Figure 3. It is observed that the stresses developed in case of PEEK 450G stent when subjected to different loading cases, i.e., 1 mm displacement, 2 mm displacement with 0.1 radian rotation, 0.15 radian rotation and 0.25 radian rotation are 7 MPa, 16 MPa, 11 MPa and 19 MPa as shown in Figure 7. The stresses generated in PEEK 450G is very lesser and well below the yield function of the material, i.e., 100 MPa^[15]. The bending of coronary stent causes high stresses on artery may injure the coronary artery^[14]. Hence, PEEK 450G has an ideal material in terms of flexibility and stability, also recommended for future coronary stent implants.

Non-linear pressure analysis

Radial displacement

The results obtained for PEEK 450G stent in terms of radial displacement, when subjected to 0.8 MPa pressure load case as shown in Figure 8. Radial displacement of PEEK 450G stent against the expanding pressure at non-plaque region as shown in Figure 9. As can be seen in Figure 9, maximum radial displacement is observed for the maximum pressure of 1.2 MPa. It is observing that the radial displacement of PEEK 450G is 1.4 mm for the pressure load of 1.2 MPa at non-plaque. Figure 10 represents the relation between radial displacement and pressure at plaque region. The radial displacement of PEEK 450G is

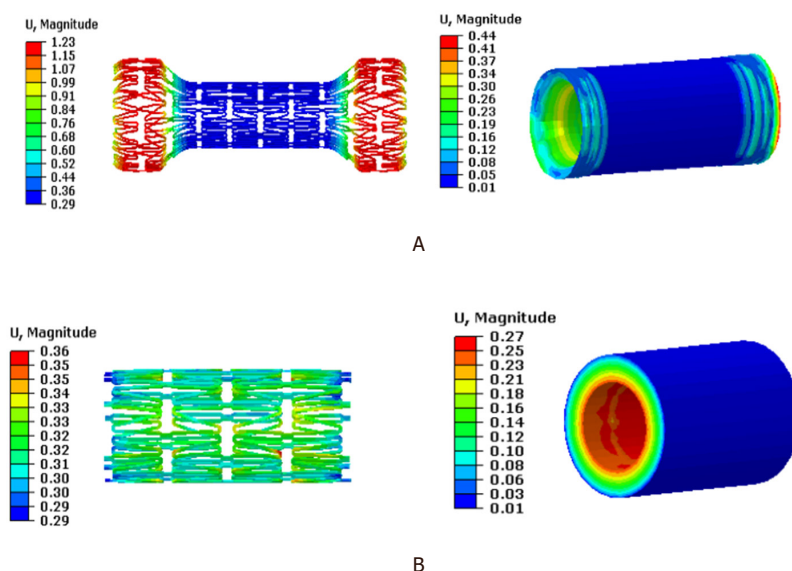


Figure 8. Radial displacement of PEEK 450G stent (Left) with artery (Right) at non-plaque (A) and plaque (B) regions when subjected to 0.8 MPa pressure load. PEEK: polyether ether ketone

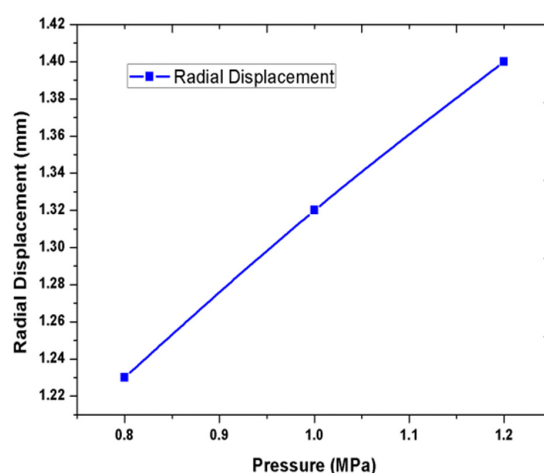


Figure 9. The relation between radial displacement and pressure at non-plaque region

0.45 mm at plaque region. Higher the radial displacement, higher will be the blood flow rate. The radial displacement of PEEK 450G is better both in plaque and non-plaque regions. Hence, PEEK 450G initiates is a better material for coronary stent implants.

DISCUSSION

Von-mises stress

The results obtained for PEEK 450G stent in terms of von-mises stress, when subjected to 0.8 MPa pressure load case as shown in Figure 11. Von-mises stresses of PEEK 450G stent against the inflating pressure at non-plaque region as shown in Figure 12. As observe in Figure 12, maximum von-mises stress observed for the maximum pressure of 1.2 MPa. It is noticed that von-mises stresses for PEEK 450G is 110 MPa for the pressure load of 1.2 MPa at non-plaque. Figure 13 represents the relation between von-mises stress and pressure at plaque region. The von-mises stress for PEEK 450G is 97 MPa at plaque region. Higher the von-mises stress

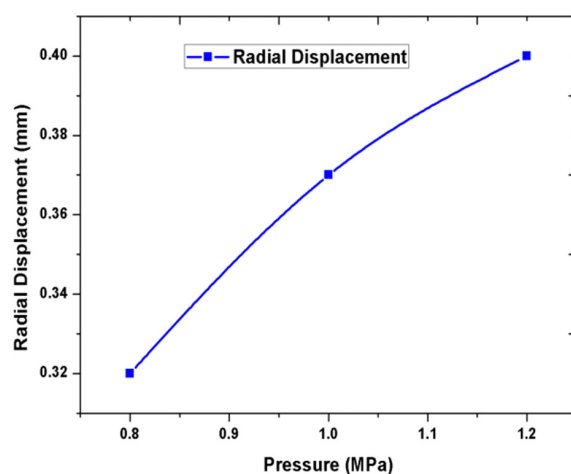


Figure 10. The relation between radial displacement and pressure at plaque region

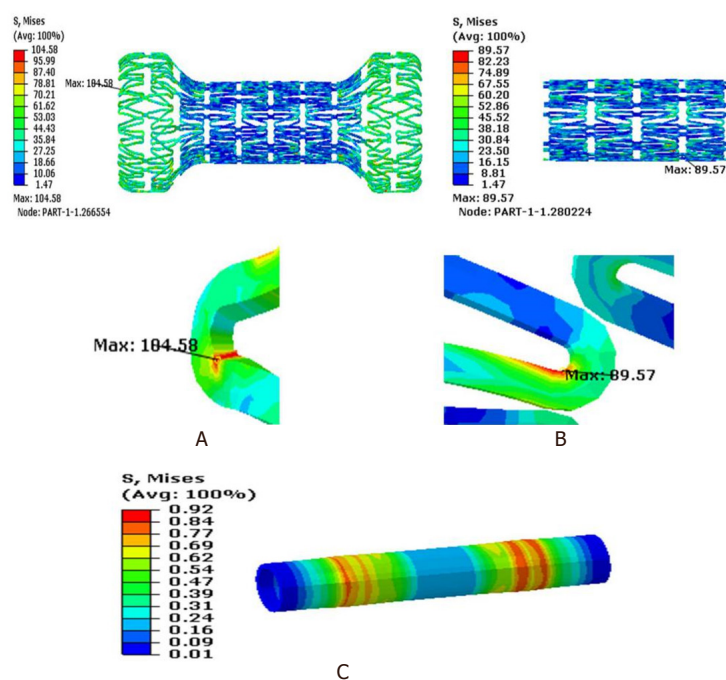


Figure 11. Von-mises stress of PEEK 450G stent at (A) non-plaque region (Left) and (B) plaque region (Right) with (C) artery subjected to 0.8 MPa pressure. PEEK: polyether ether ketone

in stent, the stiffness of the stent being high, but which may damage the coronary artery^[14]. The von-mises stress of PEEK 450G is superior in both plaque and non-plaque regions and also flexible to expand. Thus, PEEK 450G is a better material for coronary stent implants.

Plastic strain

The results obtained for PEEK 450G stent in terms of plastic-strain, when subjected to 0.8 MPa pressure load case as shown in Figure 14. Plastic-strain of the PEEK 450G stent against the inflating pressure at non-plaque region as shown in Figure 15. As observe in Figure 15, maximum plastic-strain observed for the maximum pressure of 1.2 MPa. It is noticed that plastic-strain for PEEK 450G is 61% for the pressure load of 1.2 MPa at non-plaque. Figure 16 represents the relation between plastic-strain and pressure at plaque

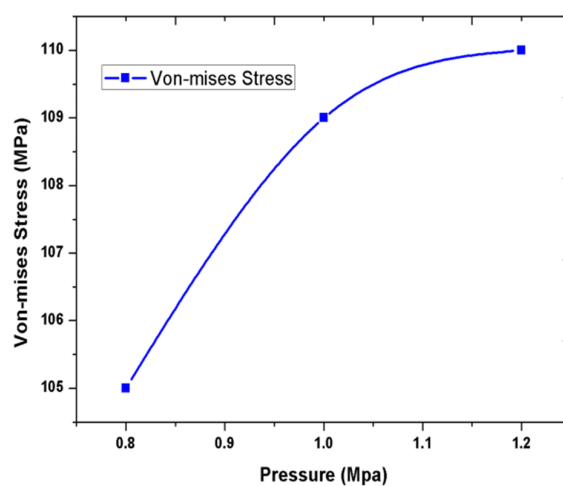


Figure 12. The relation among von-mises stress and pressure at non-plaque region

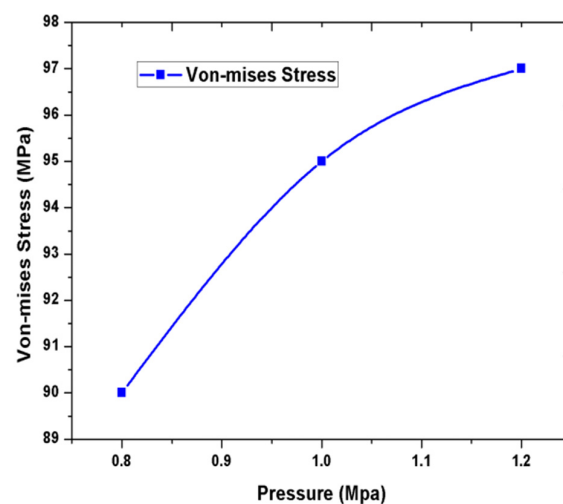


Figure 13. The relation among von-mises stress and pressure at plaque region

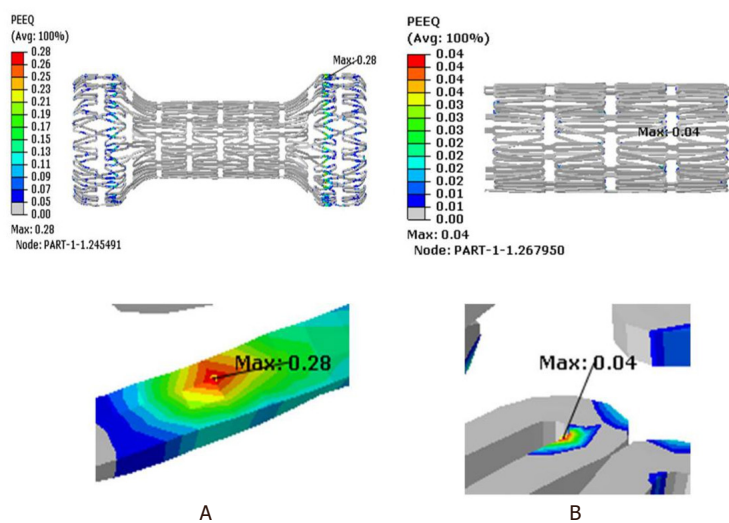


Figure 14. Plastic strain of PEEK 450G stent at (A) non-plaque region (Left) and (B) plaque region (Right) subjected to 0.8 MPa pressure. PEEK: polyether ether ketone

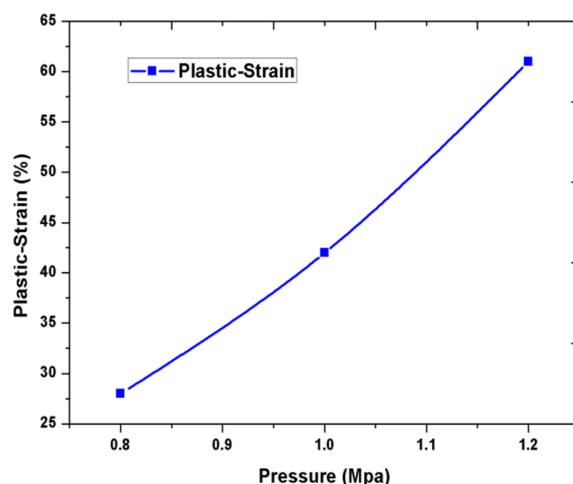


Figure 15. The relation between plastic strain and pressure at non-plaque region

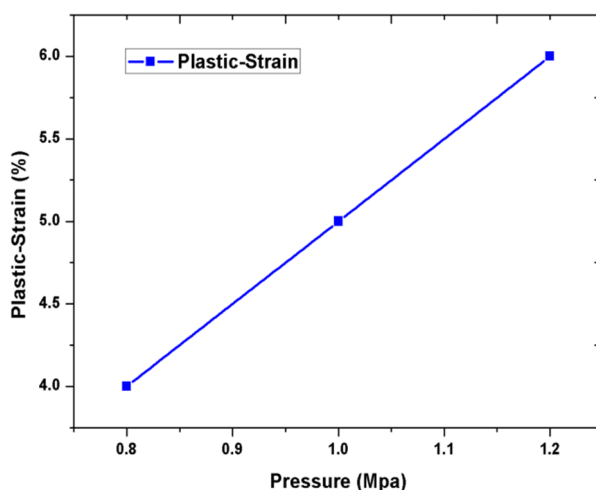


Figure 16. The relation between plastic strain and pressure at plaque region

region. The plastic-strain for PEEK 450G is 6% at plaque region. The plastic strain of PEEK 450G is higher both in plaque and non-plaque regions, because of softness, material failure begins at yield point so that material may soften and get distorted, finally material fracture may occur. The material starts yielding as the strain rate increases. Hence, the strain rate needs to be minimized by suitably varying its chemical composition.

In this work, 3D modeling of PEEK 450G stent was done by using CATIA V5 and finite element analysis of cardiovascular stent implant were carried out by using commercially available ABAQUS 6.0 software to evaluate the radial displacement, stress distribution, and plastic strain in the proximal area of PEEK 450G biomaterial under pressure load conditions of 0.8, 1.0, and 1.2 MPa. It was clear from FE simulation, both in non-linear bending analysis and non-linear pressure analysis, that PEEK 450G stent exhibits very good radial expansion and lowest stress concentration and also which is well below the yield level (100 MPa), however plastic strain is high because of softness, the strain rate needs to be minimized by suitably varying its chemical composition. Hence, blood circulation will be appropriate and also chances of vessel damage may be reduced more by using PEEK 450G. The FE analysis results showed that PEEK 450G is a best alternate candidate biomaterial suitable for cardiovascular stent implants.

DECLARATIONS

Authors' contributions

Formulated the problem, designed model and performed FEM analysis: Kumar V

Wrote the first draft of the manuscript: Kumar V, Ramesha CM, Sharanraj V

Discussed the results and implications as well as commented on the manuscript at all stages: Kumar V, Ramesha CM, Sharanraj V

Read and approved the final manuscript: Kumar V, Ramesha CM, Sharanraj V

Availability of data and materials

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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.

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Original Article

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Outcomes of long left coronary endarterectomy in patients with diffuse coronary artery disease

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Abstract

Aim: Historically the outcome of left coronary artery endarterectomy (LCAE) has been associated with increased morbidity and mortality when surgeons performed it with coronary artery bypass grafting (CABG). We aim to review outcomes after open LCAE-CABG in patients managed with aggressive dual antiplatelet therapy.

Methods: From 1999 to 2007 open LCAE with CABG was performed in 87 patients. We compared the short and long-term outcomes of 75 propensity-matched conventional CABG patients. Both groups were operated on by a single surgeon.

Results: Sixty-six percent (66%; $n = 58/87$) of LCAE group had diffuse atheroma in Left anterior descending artery (LAD); 31% ($n = 27/87$) involved both LAD and branches of the circumflex artery (Cx); 3% ($n = 3/87$) involved the Cx in isolation. Cross clamp time (43.29 *vs.* 59.04, $P = 0.019$) and bypass time (57.29 *vs.* 74.04, $P = 0.007$) were significantly higher in the LCAE group. There was no significant difference in early (1% *vs.* 1.3%) and late mortality (4% *vs.* 4.5% at 10 years). The hospital length of stay (5.58 *vs.* 6.67, $P = 0.03$), was higher in the LCAE group when compared with the CABG group. The freedom from angina and long-term survival were not significantly different between the two groups.

Conclusion: Patients undergoing CABG with Left-sided coronary endarterectomy had increased cross-clamp and bypass times with prolonged stay in hospital and increased blood transfusion rates. The mortality, morbidity, long-term survival and freedom from angina are not different when compared to CABG alone. The use of retrograde blood cardioplegia and aggressive antiplatelets may have contributed to the excellent outcome.



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Keywords: Coronary endarterectomy, coronary artery disease

INTRODUCTION

Primary coronary endarterectomy without coronary artery bypass grafting (CABG) was first introduced in 1957 by Bailey for the treatment of acute myocardial infarction^[1-3]. Subsequently, endarterectomy was combined with CABG alone, and later by vein patch and left internal mammary (LIMA) bypass of the left anterior descending artery (LAD)^[4-8]. Initial published reports showed a high incidence of perioperative mortality and ischemia^[9-13]. This led to reluctance in performing coronary endarterectomy. The adverse outcomes have resulted in some patients with severe and diffuse left-sided disease denied complete surgical revascularization. The use of dual antiplatelet therapy (aspirin and clopidogrel) has been shown to improve graft patency. Which led reduce major adverse cardiovascular events. The combination of both may improve survival following CABG^[14,15]. We aim to assess the early and late results of left coronary endarterectomy (LCAE). Which we used as an essential step in CABG. We also combined surgery with dual antiplatelet therapy post-op (aspirin and clopidogrel).

METHODS

We included Patients with no option for percutaneous coronary intervention (PCI) and were rejected for conventional CABG. The rejection was because angiographically patients had atheroma involving < 80% of the length of the coronary artery. These patients underwent CABG with LCAE at a university teaching hospital by a single surgeon from February 1999 to September 2007. We identified the patients from a prospectively collected database. We compared this group with a propensity-matched cohort (to allow matching patients characteristics and risk profile) from the same period operated on by the same surgeon in the same hospital. Information regarding pre-operative status was gathered from the clinical database. Also, the clinical notes were reviewed retrospectively for these patients. Post-operative data were obtained from the clinical database, clinic letters and GP surgery data. If the clinical records were not adequate, the patient's general practitioner was contacted by phone or e-mail. [Table 1](#) summarizes the pre-operative patient demographics of the study patients. The mean follow-up of the patients was for five years (Range 0-10 years).

LCAE was performed on occluded or nearly occluded vessels with multiple and long distal stenoses. Patterns of atherosclerosis were identified pre-operatively, but the decision to perform endarterectomy was made intra-operatively. The surgeon decided on technical consideration in combination with angiographic features. Off-pump CABG cases were included in both the groups (CABG = 28/75, LCAE = 13/87). The methods of myocardial protection techniques were comparable in both groups. Core temperatures were maintained between 30 degrees to 32 degrees during cardiopulmonary bypass.

All coronary endarterectomies were performed manually. A Watson-Cheyne dissector was used to develop a plane between tunica media and the core of the atheromatous plaque. The site for arteriotomy was chosen, and the decision to do endarterectomy was made if the artery was almost entirely or wholly occluded, or if the vessel wall contained heavily calcified plaques. Arteriotomy was extended as required (2.5-9 cm) and Watson-Cheyne dissector used to develop the plane, and gentle graduated traction was used to tease off the atheromatous core from the distal end of the vessel [\[Figure 1\]](#). If the distal end of the atheroma was not tapered, then the arteriotomy was extended until a satisfactory result was obtained, but no attempt was made to extract the atheromatous core from the individual branches of the artery. A vein patch was used in all the cases. The conduit was anastomosed end to side to the vein patch in all the cases.



Figure 1. endarterectomy

Table 1. Patient demographics in control and study population

Variable	CABG + LCAE (n = 87)	CABG (n = 75)	P value
Age	67 (37-85)	64(43-84)	0.06
Sex	M 68, F 19	M 62, F 13	0.55
Diabetes Mellitus	8/87	16/75	0.04
Hypertension	48/87	35/75	0.34
Parsonnet score	8(0-43)	6.9 (0-27)	0.10
Timing of Surgery			
Elective	27	11	0.01
Urgent inpatient	60	62	0.02
Emergency	0	2	0.21
Salvage	2	0	0.49

CABG: coronary artery bypass grafts; LCAE: left coronary artery endarterectomy

As part of our routine clinical practice, all patients undergoing CABG receive dual antiplatelet therapy (DAPT) post-operatively. Aspirin 300mg is given at 4-6 h post-operatively and 150 mg/day after that which was recommended to continue lifelong. In addition to aspirin, patients received clopidogrel 300 mg at 4-6 h post-operatively and 75 mg/day after that, which was recommended to continue for 1-year. In both groups, DAPT was administered if the bleeding was < 150 mL/h for the first 4 h postoperatively.

Statistical analysis

Statistical significance was designed to test the all or none hypothesis that use of concomitant coronary endarterectomy will not affect the outcome of Coronary artery bypass grafting. Statistical significance was obtained by a *P*-value < 0.05. Nominal data were analyzed using the Fisher test and interval data using the student *t*-test. Actuarial survival curves were calculated using the Kaplan Meier survival analysis. All statistical analysis was done using the GraphPad Prism statistical package.

RESULTS

The patient demographics were similar in both the groups as presented in [Table 1](#), except for the incidence of Diabetes Mellitus in the CABG group and increased elective surgery numbers in the LCAE group.

Table 2. Operative data

Variable	CABG + CE (n = 87)	CABG (n = 75)	P value
Number of grafts			
One	4	4	1.00
Two	9	16	0.08
Three	42	44	0.20
Four	16	20	0.25
Five	1	1	1.00
Arterial grafts	1.32 (0-4)	1.28 (0-4)	0.72
Vein grafts	1.66 (0-4)	1.72 (0-3)	0.71
IMA used	89%	94%	0.31
Cross clamp time (min)	60.5	45.5	0.012
Bypass time (min)	77.2	62	0.018
Coronary endarterectomy + vein patch			
LAD only	58	0	
LAD + Cx	26	0	
Cx only	3	0	

CABG: coronary artery bypass grafts; Cx: circumflex; IMA: internal mammary artery; LAD: left anterior descending; LCAE: left coronary artery endarterectomy

Operative data are presented in Table 2. The number of grafts was similar in both groups. The additional time to perform the grafts, including the endarterectomy, resulted in longer cross-clamp time (60.5 min *vs.* 45.5 min) and longer bypass times (77.2 min *vs.* 62 min). LIMA use was slightly higher in the CABG group (94% *vs.* 89%). Of the 87 with diffuse coronary atheroma, the lesion distribution was, 58 patients had isolated LAD endarterectomy, three patients had Cx endarterectomy, and 26 patients had both Cx and LAD endarterectomy. The number of arterial and vein grafts was similar in both groups of patients. All patients undergoing endarterectomy received a vein patch.

Peri-operative complications

The hospital complications observed are presented in Table 3. There were two patients with low cardiac output (2.3%) in the LCAE group, and one patient (1.3%) had low cardiac output in the immediate post-op period in the CABG group. The rate of postoperative bleeding, prolonged ventilation, post-operative arrhythmia's, non-fatal strokes, TIA, renal impairment, chest infection and wound infection were similar in both groups.

Mortality

Each group had one death within 30 days of surgery. The patient who died in the LCAE group was a 62-year-old female who had a salvage CABG with endarterectomy on cardiopulmonary bypass, but developed vascular embolic phenomenon, stroke and GI bleed leading to death. The death in the CABG group was a 73-year-old male who had an urgent inpatient CABG which was complicated by a post-op chest infection, renal failure, prolonged intubation and death due to respiratory failure.

Hospital resource utilization

The hospital resource utilization data are presented in Table 4. The period of mechanical ventilation was the same in both groups. The patients with coronary endarterectomy had an increased period of ICU stay 0.37 days (0-14) *vs.* 0.13 days (0-30) in the CABG group. The blood transfusion was also higher in the Coronary endarterectomy group with an average infusion of 458 mL (0-4,134 mL) per patient, as compared to 308 mL (0-2,137 mL) in the CABG group. The hospital length of stay for endarterectomy patients was longer compared to those undergoing CABG alone.

Actuarial survival and follow up

There was no significant difference in actuarial survival between the two groups, as shown in Figure 1. After a mean follow-up of 5 years, there were four deaths in the LCAE group when compared to 3 deaths

Table 3. Perioperative complication

Characteristics	CABG + CE (n = 87)	CABG (n = 75)	P value
Deaths			
30 - day	1 (1.1%)	1 (1.3%)	1
Late	4 (4.5%)	3 (4%)	0.7
Complications			
Low cardiac output	2 (2.3%)	1 (1.3%)	0.6
Arrhythmia's	3 (3.4%)	2 (2.6%)	1
Permanent stroke	1 (1.1%)	0	1
TIA	3 (3.4%)	1 (1.3%)	1
Chest infection	1 (1.1%)	2 (2.6%)	0.5
Prolonged ventilation	4 (4.5%)	2 (2.6%)	0.3
Renal impairment	2 (2.3%)	1 (1.3%)	0.6
GI bleed	0	1 (1.3%)	1
Leg wound infection	1 (1.1%)	1 (1.3%)	1
Re-operation for bleeding	4 (4.5%)	1 (1.3%)	0.3

CABG: coronary artery bypass grafts; LCAE: left coronary artery endarterectomy; GI: gastrointestinal; TIA: transient ischaemic attack

Table 4. Hospital resource utilization

Category	CABG + CE (n = 87)	CABG (n = 75)
post-op ventilation hours	6.96 (2-32)	7.01 (2-15)
ICU stay in days	0.37 (0-14)	0.13 (0-3)
Length of hospital stay in days	6.67 (2-19)	5.58 (3-11)
Blood transfusion (mL)	458 (0-4,134)	308 (0-2,137)

CABG: coronary artery bypass grafts; ICU: intensive care unit; LCAE: left coronary artery endarterectomy

in the CABG group. The deaths in the LCAE group occurred after three years in 2 patients and four years in the other two patients. The three deaths in the CABG only group occurred after 2 years, 4 years and five years respectively. The one-year survival was 98.9%, and the 5-year survival was 95.5% in the LCAE group [Figure 2].

The freedom from angina was similar in both groups at the end of one year, as shown in Figure 3. There was no statistical difference in the incidence of angina in the post-op period at the end of 10 years follow up.

DISCUSSION

Despite the excellent results obtained with surgical revascularization, advances in the field of PCI coupled with its less invasive nature have made it the most common interventional treatment strategy for coronary artery disease^[16,17]. However, patients with complex and diffuse disease may be challenging to entirely surgically revascularize. Complete revascularisation and in particular arterial grafting to the left coronary system has a significant impact on long term survival of patients^[18,19]. It is, therefore, essential for surgeons to have an effective strategy for grafting patients with diffuse severe obstructive CAD to achieve complete revascularization. In the presence of diffuse disease, open endarterectomy is a surgical adjunct, which can produce good clinical results in these otherwise inoperable patients.

Various methods of coronary endarterectomy have been described. Initially, a closed traction method with primary closure of the arteriotomy was practised^[1]. Over time the procedure evolved to include patch closure of the arteriotomy combined with LIMA or saphenous vein bypass grafting^[6,7,20,21]. In this study, all endarterectomies were performed under direct vision, exposing the whole arterial lumen and side branches and arteriotomy closure with vein patch with subsequent end to side grafting was done in all patients. This has been an effective method, with only one patient requiring further revascularisation

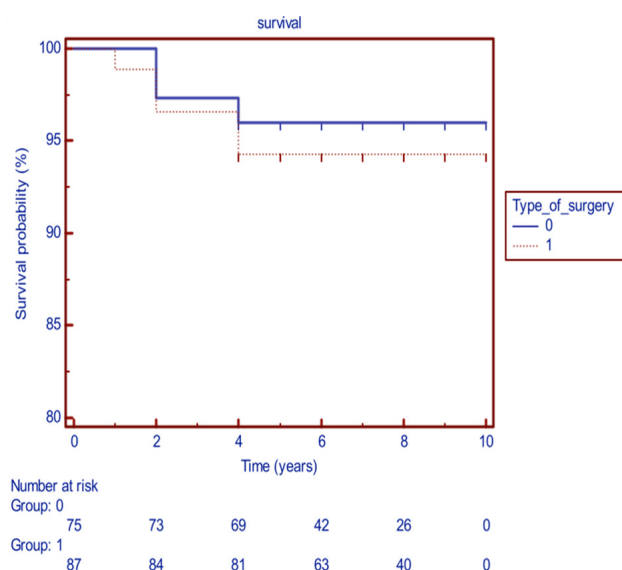


Figure 2. Survival in patients undergoing CABG only (group 0) or CABG combined with coronary endarterectomy (group 1)

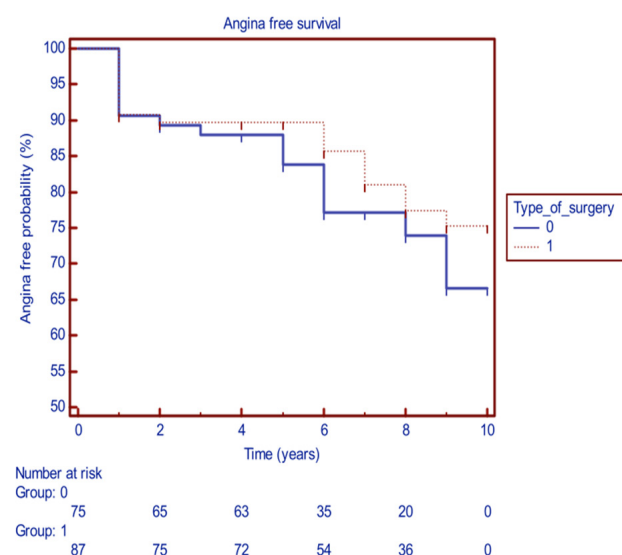


Figure 3. Freedom from angina in patients undergoing CABG only (group 0) or CABG combined with coronary endarterectomy (group 1)

in the immediate post-op period due to poor cardiac output. All patients in the LCAE group had diffuse severe coronary artery disease. The ability to achieve complete revascularization and where possible to use the left internal mammary artery (89% of patients in this study) to bypass the left coronary system has been shown to improve outcomes in patients with CAD. In the LCAE group, complete revascularization likely would not have been possible without the use of adjuvant coronary endarterectomy. Furthermore, in native coronary arteries with multiple sequential obstructive lesions, coronary endarterectomy coupled with vein patching and grafting allows the target vessel to be grafted with a single rather than numerous coronary anastomoses.

The higher incidence of perioperative myocardial ischemia in earlier studies involving technically excellent surgeons was not seen in this study^[5,9,15]. Two critical factors have likely contributed to improved outcomes in this compared to historical studies: firstly advances in techniques of myocardial protection, in particular,

the use of retrograde cardioplegia and secondly the use of aggressive post-op anticoagulation with dual antiplatelet therapy. All endarterectomy procedures were performed by an open method, and retrograde cardioplegia was used for flushing out all the debris immediately. During beating heart surgery, the endarterectomy was performed after application of a sling proximally, and retrograde flow used to flush the debris before vein patching and anastomosis. Early and midterm graft failure may be due to thrombosis of the graft itself or of the native vessel. Historically LCE was associated with an increased incidence of graft failure and perioperative myocardial infarction^[22,23]. This is likely due to exposure of the prothrombotic and platelet aggregating promoting components of the vessel wall following endarterectomy. Dual antiplatelet therapy, possibly by reducing early thrombotic events and platelet clumping, has been shown to improve graft patency in off-pump surgery, following acute coronary syndromes, when multiple vein grafts are used and in native vessel disease vessels with reduced runoff. This has translated into improved clinical outcomes in these groups. Besides, aggressive antiplatelet administration immediately post-op with aspirin and clopidogrel has been proven to be safe and effective after coronary artery surgery^[14,24-26]. We commenced all our patients on clopidogrel, and aspirin immediately following surgery and continued on Clopidogrel for 1-year post-op along with lifelong aspirin.

Ideally, angiographic confirmation of graft patency would have been preferred, but the freedom from angina in our study (91.8% at 1year and 79.4% at 10 years) along with a low incidence of perioperative ischaemic events indirectly shows that this strategy is a valuable additional benefit for these patients.

Our results, coupled with those from other centres, suggest that coronary endarterectomy can be accomplished safely and acceptably when applied in a particular way^[27,28]. In this study, we have shown comparable results of endarterectomy with the CABG only group when comparing long term survival and freedom from angina. Although numerically morbidity in the endarterectomy group was slightly higher than control, it did not have a lasting effect on long term outcome. We propose the use of left-sided endarterectomy as a safe adjunct in dealing with diffusely diseased coronary vessel disease, especially when long term prognosis is essential.

DECLARATIONS

Authors' contributions

Collected and analysed the data: Radhakrishnan K
Supervised and helped writing the manuscript: Galvin SD
Operating senior surgeon: El-Gamel A

Availability of data and materials

Data collected from King's college and Waikato dendrite data bases.

Financial support and sponsorship

None.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

The study has ethics approval from both king's College hospital local ethics committee and waikato hospital. All patients were consented for the study as per regulation.

Consent for publication

Not applicable.

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Review

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Acute mechanical complications in patients suffering from acute myocardial infarction

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Abstract

Acute mechanical complications following acute myocardial infarction have an incidence less than 1% in the era post coronary and systemic thrombolysis. However, the early mortality is still high even after surgical therapy, reaching 70%. Left ventricle free wall rupture, ventricular septal defect and papillary muscle rupture represent the most challenging complications after myocardial infarction. Prompt diagnosis, appropriate medical therapy and mechanical support, such as intra-aortic balloon pump and extracorporeal membrane oxygenation, and urgent or emergency surgical operation may favor to obtain encouraging results and acceptable long-term outcome.

Keywords: Acute myocardial infarction, cardiac rupture, acute mitral valve regurgitation, left ventricular free wall rupture, ventricular septal defect

INTRODUCTION

Mechanical complications of myocardial infarction are direct consequences of anatomic and pathological changes occurring in ischemic cardiac tissue. After a coronary occlusion, there is a lack in blood perfusion and in oxygen supply that cause functional, morphological and biochemical changes. Within the first 30 min from the occlusion, reversible changes happen: macroscopic and microscopic changes are not occurring yet, but myofibrils start to relax, and cells start to suffer. After 30 min, ischemic necrosis begins, and the irreversible damage occurs. After 2-4 h, complete necrosis of myocardial cells may occur,



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depending on the coronary collateral circulation, the severity and the time of coronary vessel occlusion and the individual demand for oxygen and nutrients. Loss of myocardial vitality is complete after 6-12 h from the onset of coronary occlusion. Within one week, macrophagic phagocytosis and irreversible collagen damage begin, and tissue becomes less resistant; in that period, myocardial tissue becomes more vulnerable and weaker and heart ruptures are more frequent^[1,2].

For the purpose of this review we analysed one of the 5 categories of complications of acute myocardial infarction (AMI) named as mechanical complications after AMI, such as ventricular free wall rupture, ventricular septal defect, papillary muscle rupture of mitral valve. The other 4 categories (electrical complication, inflammatory, ischemic and embolic complication) were excluded from this article.

Most of all mechanical complications of myocardial infarction require an urgent/emergent surgical therapy. Meanwhile, initial diagnosis should be rapid and initial medical therapy should be promptly started, to improve coronary perfusion, stabilise the haemodynamic condition and ameliorate the peripheral perfusion. Supplement oxygen, mechanical ventilatory support whether necessary, analgesic therapy, crystalloid infusion and inotropic agents represent usually the first medical approach. In very critical and challenging poor clinical and hemodynamic status, requirement of an intra-aortic balloon pump (IABP) or even a more advanced temporary circulatory support such as veno-arterial extracorporeal membrane oxygenation are valid options to manage and stabilise the patient^[3].

LEFT VENTRICULAR FREE WALL RUPTURE

Left ventricular free wall rupture (LVFWR) accounts for 15% after AMI and it is more frequent than septal or papillary muscle rupture^[4,5]. LVFWR may occur at any time after myocardial infarction, usually within the first 3-5 days but may happen up to 15 days^[6]. However, at least 25% of heart rupture occurs within the first 24 h. The short-term mortality remains very high even with rapid diagnosis and surgery. Data from one of the largest multicentre trials, the Global Registry of Acute Coronary Events study, report an incidence of LVFWR of 0.2% with an in-hospital mortality of 80%^[7]. In more recent retrospective studies and reviews, the early mortality was ranging between 14% to 35%, according to the initial haemodynamic status, the type of LVFWR and the type of surgical repair^[3,8-10].

Three types of cardiac rupture were classified as follows: acute, subacute, and chronic^[11]. The acute rupture (also named “blow-out” rupture) is characterized by a massive haemopericardium [Figure 1] and most of patients presented with electro-mechanical dissociation and sudden cardiac death. When the rupture area is smaller, the bleeding is stopped by clot formation or pericardial adhesions. This type is called also “oozing” rupture, and patients presented with hypotension and arrhythmias and clinical signs of pericardial tamponade.

The accuracy of diagnosis of subacute LVFWR requires a high degree of suspicion^[12]. Trans-thoracic echocardiogram is the first diagnostic tool as most of patients show pericardial effusion in addition to echogenic images, in an early period after AMI. Due to these findings, echocardiogram has a high level of sensitivity and specificity (more than 95%) for the LVFWR diagnosis^[13]. Furthermore, when pericardial effusion is more than 10 mm in patients with a recent diagnosis of AMI with ST elevation, the probability of 30-day mortality is as high as 43%^[14]. When pericardial effusion is associated with hypotension and bradycardia, rapid pericardiocentesis is useful to stabilize the patients before taking them for emergency surgical therapy.

Surgical repair of the rupture site is the gold and definitive treatment. Surgery may be performed in different ways and different techniques according to the hemodynamic status and the types of cardiac rupture. In a recent review, Matteucci *et al.*^[10] described the following surgical techniques: (1) linear closure

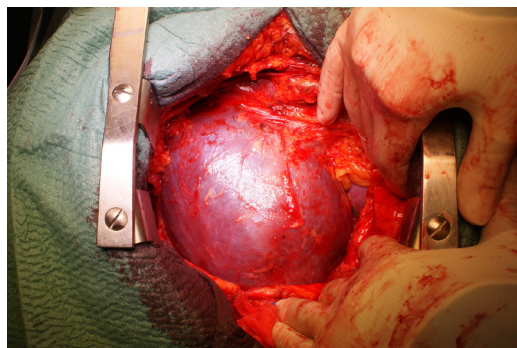


Figure 1. Massive hemopericardium following “blow-out” left ventricular free wall rupture

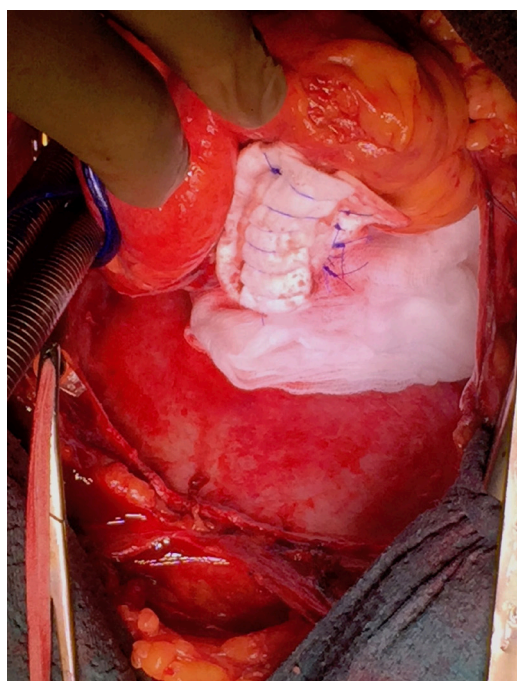


Figure 2. Linear closure of a “blow-out” left ventricular free wall rupture (inferior wall)

[Figure 2] or infarctectomy associated to closure of the defect with a prosthetic patch when the LVFWR is a blow-out type^[3]; (2) covering patch technique or sutureless technique (named also “patch and glue”) in case of oozing LVFWR [Figure 3]^[3,8]. Operations can be performed with or without cardiopulmonary bypass. Off-pump procedure is the technique of choice in case of oozing LVFWR and patients in stable conditions. Some authors have recently reported newer surgical strategies, usually performed in presence of oozing by using different materials such as ready-to-use haemostatic collagen sponges^[3,8,15] and newer acellular xenogeneic extracellular matrix patches [Figure 4]^[16].

Preoperative and postoperative insertion of IABP is advocated by some authors even when patients presented in stable haemodynamic status, with the aim to reduce the oxygen demand, the left ventricle wall tension and the left ventricle afterload^[17,18]. This approach may reduce the risk of re-rupture during the early postoperative period. In the last 20 years, ECMO has emerged as a rescue tool to warrant a rapid haemodynamic stability in such patients presented with acute cardiogenic shock or even cardiac arrest^[19,20]. ECMO may be used also to stabilize patients affected by LVFWR in referring hospitals^[21,22]. However, there is

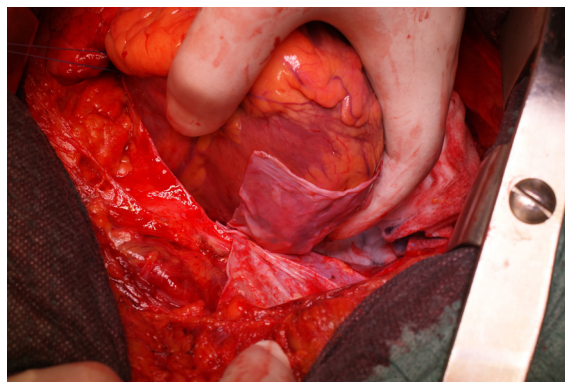


Figure 3. Sutureless technique with autologous pericardial patch in “oozing” left ventricular free wall rupture

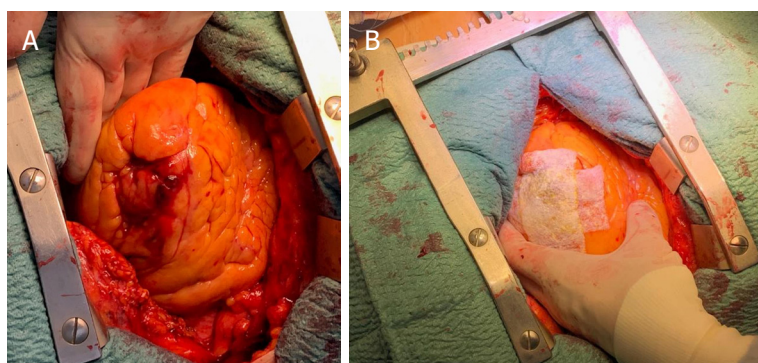


Figure 4. Oozing anterior left ventricular free wall rupture (A); ready-to-use haemostatic collagen sponges (B)

Table 1. Early and long-term outcome of left ventricular free wall rupture

Authors	Patients	In-hospital mortality	Long-term survival
Okamura <i>et al.</i> ^[8]	42	6 (17%)	5-year: 68.6%; 10-year: 62.9%
Formica <i>et al.</i> ^[3]	35	12 (34.3%)	5-year: 80.9%; 10-year: 74.7%
Sakaguchi <i>et al.</i> ^[23]	32	5 (15.6%)	5-year: 74%
Zoffoli <i>et al.</i> ^[24]	25	3 (12%)	-
McMullan <i>et al.</i> ^[25]	18	11 (61%)	-

a lack of evidence of any benefit of preoperative and perioperative ECMO in patients affected from LVFWR due to the very low numbers of reports regarding this specific topic.

In-hospital mortality is extremely variable, ranging from 12% to 61% among different Institutions^[3,8,23-25]. Despite the relative high in-hospital mortality, midterm and long term survival show encouraging outcome [Table 1].

VENTRICULAR SEPTAL DEFECT

The incidence of VSD has been estimated between 1% and 2% of all patients suffering from acute myocardial infarctions even if the advent of reperfusion therapy has decreased this value below 0.5%. Despite the low incidence, the early mortality is still high, reaching about 60%-70%. Without surgical treatment, only about 10% of patients survives after 3 months^[26].

Risk factors for VSD include advanced age, hypertension, absence of angina, extensive myocardial infarction and single vessel disease^[27,28].

The acute rupture occurs 3-7 days after a wide transmural infarction but may occur rarely after 2 weeks^[28,29]. VSD results in a left-to-right shunt which is the main cause of low cardiac output, decreased urine output, shortness of breath, altered mental status and finally cardiogenic shock^[30]. At physical examination, a harsh and loud pan-systolic murmur at the left lower sternal border is present in over 90% of cases. A palpable thrill can be detected in up to 50% of patients.

VSDs has been categorized in two other categories: simple and complex. Simple rupture is a discrete lesion, with defect located at a similar level in both ventricles. That is typical pattern of anterior myocardial infarction. Inferior myocardial infarction usually is associate to a complex type^[31,32].

In both cases, the defect may vary in size from some mm to more than 15 mm. The size of defect may affect the magnitude of left-to-right shunting, and therefore influencing the clinical presentation and the likelihood of survival. Trans-thoracic and transesophageal echocardiography with Doppler imaging is considered a high sensitive and specific diagnostic tool for immediate diagnosis.

Medical therapy is usually managed with the use of vasodilators, which reduce afterload and decrease left ventricular pressure and the left to right shunt, with inotropic agents, which may increase the cardiac output, diuretics, and IABP. Use of preoperative ECMO was reported in patients with refractory cardiogenic shock^[33,34]. Some authors have reported the use of the Impella to achieve haemodynamic stabilization with acceptable results. However, the use of Impella is limited in selected patients and only few experiences with few patients were published so far^[35-37].

Timing of surgery is still debating. Recently, Papalexopoulou and co-workers published a review reporting that early surgery is advocated if VSD size is more than 15 mm and an instable haemodynamic. While delayed surgery might be performed up to 3 or 4 weeks in case of clear hemodynamic clinical status. Emergency surgery should be performed in patients with refractory cardiogenic shock and rapid deterioration^[38]. A review form the Society of Thoracic Surgeon National Database identifies that mortality of patients who underwent surgery within 7 days was approaching to 54% compared to patients who underwent surgery after 7 days. In this latter clinical scenario, mortality was 18%^[39]. Early mortality is affected also from the VSD location. In patients with basal or inferior VSD mortality is as high as 70%^[34]. Apical or anterior VSD are affected from a lower early mortality (30%)^[27].

Right ventricular dysfunction is an issue to be considered in VSD. The right ventricular dysfunction maybe consequence of right ventricular infarction and right ventricle acute overload depending on the magnificence of the left-to-right shunt.

Multiple surgical techniques have been described [Figure 5]. Apical amputation is the simpler in patients with apical defects. Other techniques involve infarct exclusion and defect closure with a patch (biological or synthetic) using both stitches or glues^[40,41]. Despite continuous advances in surgical approaches, operative mortality remains high (ranging from 20% to 71.4%), with no clear differences between different techniques^[41-43]. Female gender and depressed left ventricular dysfunction at admission are linked with a high hospital death^[43].

Table 2 reports the in-hospital mortality rates and long-term survival percentages of studies with relatively large number of patients.

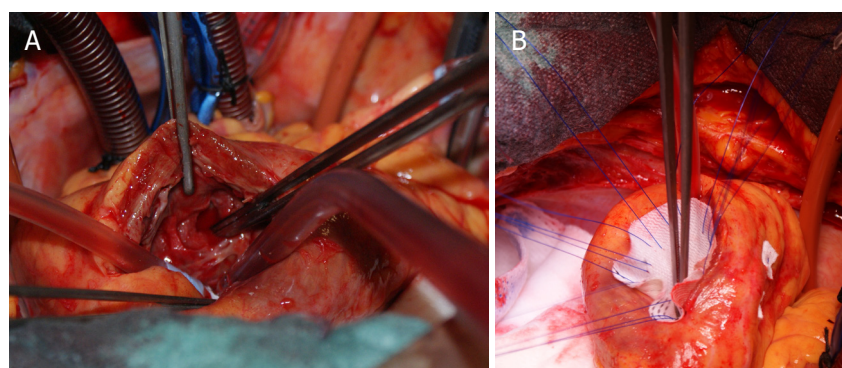


Figure 5. Apical ventricular septal defect (A); prosthetic patch for septal defect closure (B)

Table 2. Early and long-term outcome of ventricular septal defect

Authors	Patients	In-hospital mortality	Long-term survival
Pojar <i>et al.</i> ^[44]	39	14 (36%)	-
Okamoto <i>et al.</i> ^[45]	21	5 (23.8%)	3-year: 70.8%; 8-year: 57.9%
Liebelt <i>et al.</i> ^[42]	14	10 (71.4%)	-
Takahashi <i>et al.</i> ^[46]	52	19 (36%)	5-year: 75%; 10-year: 31%
Papadopoulos <i>et al.</i> ^[47]	32	10 (31.2%)	5-year: 79%; 10-year: 51%
Prêtre <i>et al.</i> ^[48]	54	14 (26%)	5-year: 65%; 10-year: 40%

Percutaneous closure of the VSD remains an attractive alternative to surgical repair mainly in extremely high-risk patients with reasonable results. Despite the in-hospital mortality is graver from a relatively high incidence (from 20% up to 46%), the patients who survived to discharged had a very acceptable long-term survival^[43,49].

PAPILLARY MUSCLE RUPTURE OF MITRAL VALVE

Papillary muscle rupture (PMR) is a rare entity and occurs in less than 0.5% of patients with acute myocardial infarction^[50]. Timing of rupture is ranging between 2 and 7 days, but 80% occurs in 7 days.

Mortality may be as high as 50% in the first 24 h, and rises up to 90% within the first week^[51].

Anterior papillary muscles receive a dual blood coronary supply, while posterior papillary muscle receives blood from the only right coronary artery. Due to this anatomical difference, PMR is most common after an inferior acute myocardial infarction, because the posteromedial papillary muscle is most often involved.

Doppler transthoracic and transesophageal echocardiography is the gold diagnostic tool. Transesophageal echocardiography has a very high diagnostic accuracy approaching to 100%^[52] for the evidence of a tear in papillary tissue and the flail of mitral valve leaflet leading to severe mitral regurgitation [Figure 6].

Emergent or urgent surgery is the only therapy for PMR, despite operative mortality rises up to 20%-39%^[53-55]. Medical therapy before surgery aims to reach a hemodynamic stability and includes aggressive afterload reduction to decrease the huge regurgitant fraction by using nitrates, sodium nitroprusside, diuretics, IABP. The use of ECMO was also reported.



Figure 6. Transesophageal echocardiographic evidence of papillary muscle rupture

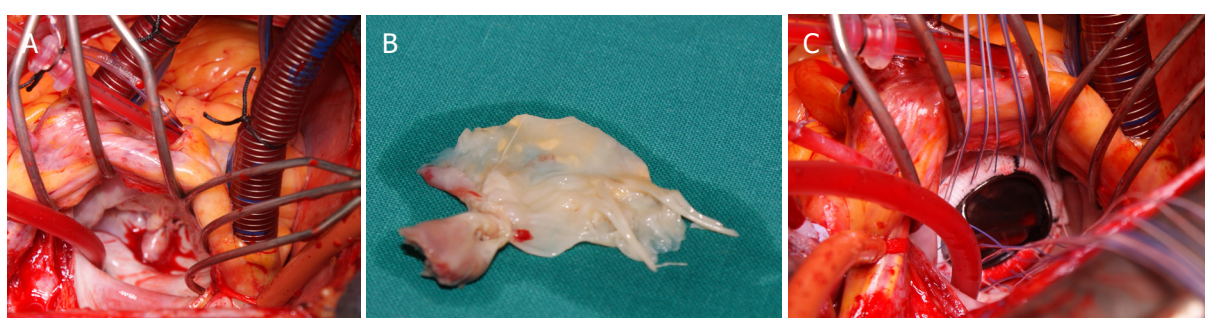


Figure 7. Surgical view of papillary position (A); posterior mitral leaflet and portion of papillary muscle (B); bi-leaflet prosthetic mechanical valve in mitral position (C)

Table 3. Early and long-term outcome of papillary muscle rupture

Authors	Patients	In-hospital mortality	Long-term survival
Bouma <i>et al.</i> ^[54]	48	2 (4.2%)	-
Schroeter <i>et al.</i> ^[55]	28	11 (39.3%)	-
Tavakoli <i>et al.</i> ^[56]	21	4 (19%)	5-year: 68%; 10-year: 56%
Russo <i>et al.</i> ^[57]	54	10 (18.5%)	5-year: 79%; 10-year: 55%

Surgical technique depends upon the location and the type of rupture. In case of partial PMR, surgeons usually attempt for a repair of mitral valve. However, a surgical repair of the papillary muscle head is really rare. In case of complete PMR, mitral valve replacement becomes the treatment of choice [Figure 7^{\[53,54\]}](#).

Long-term survival of patients who underwent mitral valve replacement for PMR is very acceptable, with a 5-year survival rate ranging between 60%-70%^[56,57].

[Table 3](#) reports the in-hospital mortality rates and long-term survival percentages of studies with relatively large number of patients.

CONCLUSION

Mechanical complications after acute myocardial infarction are still affected by high 30-day mortality and poor long-term survival. The incidence of such complications is reduced in the post thrombolysis era, but the perioperative surgery continues to be relatively high. The diagnosis should be as prompt as possible,

since in case of acute cardiogenic shock the surgery should be performed very early to increase the perioperative survival. Rescue tool as ECMO is an emerged therapy to stabile patients and even allowing the stabilization in referring hospital.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed literature search and interpretation: Formica F, Mariani S, D'Alessandro S

Availability of data and materials

Not applicable.

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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.

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Review

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The frontier in Cardiac Surgery is intellectual

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Abstract

Cardiac Surgery is a Specialty undergoing profound changes. Since its inception, innovation has been at the forefront of activities seeking for the best of the patients. The introduction of the heart-lung machine in clinical practice in the 1950s of the twentieth century allowed for the correction of intra- and extracardiac defects. The recent past two decades have seen the progressive incorporation of transcatheter therapies to treat a variety of heart defects. Multimodality imaging approach has become a fundamental tool in the pre- intra- and postoperative assessment of patients. The future in Cardiac Surgery contemplates redesigning training programs with the mandatory acquisition of catheter skills, the knowledge of the different imaging modalities and allocation of resources and timing for basic and translational research.

Keywords: Cardiac Surgery, innovation, frontier, intellectual component, transcatheter therapies, multimodality imaging

INTRODUCTION

Cardiac Surgery is a well-established, mature, surgical specialty. It has been considered a young specialty as in the way we practice, the first reported cases of major cardiac operations are dated back in 1953 when John Gibbon from Philadelphia first performed the intracardiac correction of an atrial septal defect using the heart-lung machine (HLM)^[1,2]. The classical historical vignette of Ludwig Rehn repairing a stab wound of the heart in 1896^[3] is generally considered as the pioneering surgical event which cleared the path towards a more advanced approach to the surgical treatment of cardiac diseases. After four decades of laboratory research, it became clear that the correction of intracardiac defects could be possible in the middle of the 20th century. The courageous attempts of Gibbon^[1] with the HLM, Lillehei^[4] with cross-



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circulation and DeWall^[5] in the beginning of the fifties of the twentieth century have to be seen with the perspective of time as ground-breaking events that could probably be difficult to replicate in current times.

Cardiac Surgery has undergone impactful changes since it became a medical specialty. It has been associated with the development of specialties within the chest and related to the cardiovascular surgery. The name has also undergone changes and different terms have been used over time. Cardiac Surgery, cardiovascular surgery, cardiothoracic surgery, have been associated to innovation. The approach chosen by Gibbon^[1] was innovative at that time. The idea of oxygenating the blood while circulating through artificial tubes out of the body was an outstanding example of innovation. This followed the original studies of Max von Frey at the Carl Ludwig Leipzig Physiological Institute at the end of the 1890s^[6], who also innovated at that time by developing a system for blood oxygenation. Since its inception as an independent specialty, inclusive of name changing, Cardiac Surgery has also been associated to education. Education is the foundation of the individual and the society. Education in Cardiac Surgery is a dynamic process and is considered as one of the most important activities in the Specialty. This has been highlighted over the years by many. Every department, every scientific society allocates resources and budget to cope with the needs of those who will deliver high-quality services and is highlighted in their respective mottos^[7,8].

As time goes by, it is evident that our Specialty changes. What it was done in the early developmental phases, does not apply in current times. The introduction of the HLM represented a breakthrough in surgery as it allowed to repair or palliate a number of previously unaddressed pathologies. Over the past 70 years, we witnessed the evolution of the techniques, the consolidation of the specialty, the improvement in clinical results, thus resulting in the benefit of the community. This has been extensively recorded in the literature^[9-13].

There have been substantial changes over time in the perception and action in the Specialty. Coronary artery surgery continues to be the gold standard in the treatment of triple-vessel disease, with or without left main lesions^[14]. Despite the advent of the newer transcatheter techniques that are later discussed, mitral valve repair for degenerative disease^[15], surgery for infective endocarditis^[16], aortic valve repair^[17], obstructive cardiomyopathy^[18] and aortic surgery^[19], to name just a few, are well-established and routine procedures all over. On the contrary, cardiac pacing, a surgical activity for decades^[20], including all sorts of pacemakers and implantable cardioverter defibrillators^[21] was slowly taken over by cardiologists almost everywhere. The improvement and miniaturization of hardware, the easiness of implantation and other factors, progressively shifted pacing towards Cardiology.

Cardiac Surgery currently faces new challenges. This is well known, as challenges are always out there and are the foundation for development. This means Cardiac Surgery is continuously evolving as the surgeons do. It is not to be forgotten that surgery, despite its etymology, is not a manual activity, it is an intellectual activity. The surgeon does a manual work, surgery -from the Greek, χεῖρ *cheîr* “hand” ἔργον *érgon* “work”^[22], which is intellectually driven. Here we tried to understand which are the current challenges in Cardiac Surgery, where the frontiers for future development are and what we need to do to overcome difficulties in an adaptive process.

THE CHALLENGES AND THE FRONTIER

Adaptive changes result from challenges. Darwin understood that species undergo adaptive changes as a response to environmental challenges^[23], a theory that has also been challenged. The technological breakthrough of the HLM was challenged by a number of technological and biological unprecedented problems related to the new interactions between machines and the human body. New materials and new technology resulted in sophisticated devices incorporating better pump heads, biocompatible surfaces for cardiopulmonary bypass tubing aiming at reducing inflammatory response^[24], multiple in-line sensors for

better safety, more effective and less traumatic oxygenators with superior performance^[25], in-line filters of all kinds^[26,27], hemoabsorbers^[28] and other complements that allowed safer conduct of cardiopulmonary bypass for repair of cardiac disease.

The challenges in Cardiac Surgery are like the nine-headed Hydra^[29]. As highlighted by De Paulis^[30], new technologies, professional issues, educational differences, the changing profile of the cardiac patient, the increased complexity, the trend towards reducing the surgical access and the booming of transcatheter techniques and technologies over the past couple of decades, have designed a complex scenario that can only be addressed with imagination and adaptive willingness.

Where does the Frontier lie? Although individual opinions can be challenged by many, the frontier is related to our understanding of what we used to neglect.

IMAGING

Imaging has evolved in the years from a diagnostic tool to an alternative to direct vision; today imaging is critical in all phases of patient handling: from diagnosis, to stratification, indication, planning, intraprocedural guidance and follow-up. Intraprocedural imaging is the eye of the modern surgeon for guidance of endovascular procedures.

The impact of conventional imaging

The developments in imaging over the past three decades have substantially modified our approach to the disease and the patient. What currently are conventional plain X-rays were impactful over hundred years ago as radiology allowed the physicians to see things that no one could imagine^[31]. Diagnosis improved, accuracy allowed for better delivery of care. Arteriography^[32,33] allowed surgeons to identify and understand the anatomy of lesions amenable for surgical therapy. And this applied to each and every vessel in the body.

The ultrasound - echocardiography - quality

In the field of Cardiology and Cardiac Surgery, echocardiography developed in a way that it currently plays the same role EKG used to play for decades, namely a foundational examination to support a clinical diagnosis. The introduction of M-mode echocardiography in 1954 by Edler and Hertz^[34] represented a breakthrough in diagnosis in Cardiology. Later, two-dimensional echocardiography improved the ability to make the interpretation of cardiac valvular anatomy for better characterization of structural competence. The introduction of the Doppler effect in echocardiography, by Hatle^[35,36] paved the way for echocardiography to become the major diagnostic tool in future Cardiology and Cardiac Surgery. Further refinements like transesophageal echocardiography in 1976^[37] and more recently the 3D-echocardiography^[38] anatomical description have confirmed the critical role of echocardiography in the assessment of valve disease and cardiac function. Even 3D echocardiography has evolved in rapid steps to full volume with best frame rate without and even with color Doppler sonography. Computer progress made it possible that we can see double volumes from the same data set, visualizing the mitral valve from the left atrial side and from the left ventricle side at the same time, without stitch artifacts. Machine learning is helpful for performing anatomical models from the 3D data set and do multiple measurements by itself with a fantastic reproducibility.

Echocardiography has also played a significant role in improving the quality of Cardiac Surgery. Surgeons and cardiologists have improved their standards by incorporating echocardiography in their practices. Unfortunately, it took long for surgeons to adopt this tool as part of the daily activities. Surgeons understood the value of echocardiography, however, little was done for long time to include it in the training programmes. As echocardiography is not only a preoperative diagnostic tool but also a critical

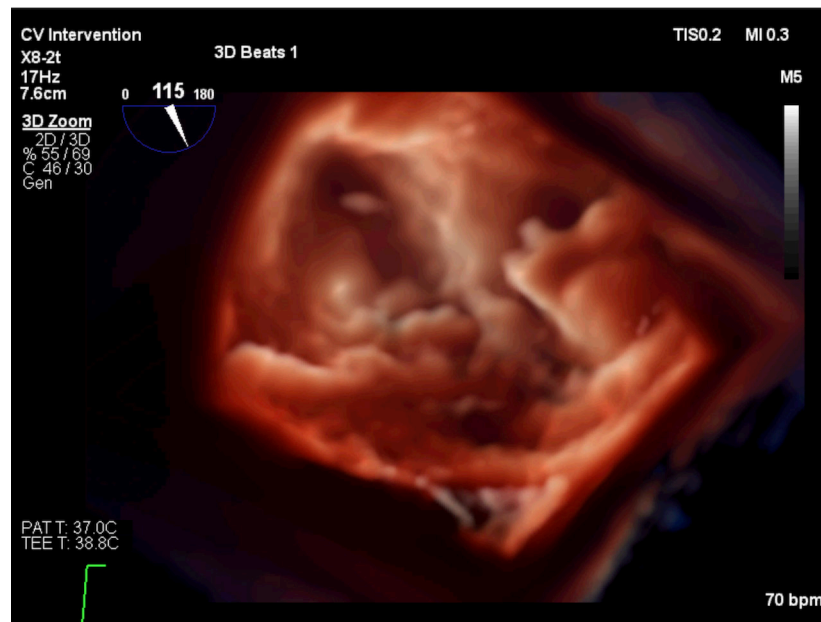


Figure 1. Preoperative image of mitral P3 segment flail in post-rheumatic valve

part of the intra- and postoperative anatomical and functional assessment^[39] and even as important a quality assurance instrumentation, its role in education must be reinforced. The ability to simulate interventions with computer technology pushed preoperative planning in a “*sine qua non*” position and will revolutionize a lot of future interventions, too. So, young surgeons need to become familiar with all such technologies not to miss future requirement before interventions. Implementation of computer technology and sophisticated postprocessing as for example true view and changes of light sources in the picture, will bring even more realistic anatomy to the treating physicians [Figures 1-3].

Advanced radiology

Computed tomography (CT) and magnetic resonance imaging (MRI) imaging changed the medical world as they allowed for a multiple view of the anatomy, for the identification of infracentimetric lesions, for better tissue characterization and the analysis of structural motion^[40,41]. These examinations are currently a fundamental part of medical practice and physicians frequently rely on them than on their own clinical judgment to design a pathway for therapy. Indeed, dramatic changes have already occurred in this field, including the introduction of promising new technologies such as coronary CT angiography^[42] and substantial reductions in reimbursement driven by cost-cutting and concerns regarding overuse. New technologies such as coronary fluid dynamics and physiology derived from CT^[43] and emission tomography^[44], offer the hope that we will soon gain outstanding and reliable imaging in coronary artery disease diagnosis: both anatomic and functional information will be obtained non invasively.

Multimodality and fusion imaging technologies

We live in an era of innovation and technological evolution, which is expressed at its best in the cardiovascular field. As already discussed, change and evolution are unstoppable. This inevitably influences the choices and future directions of a highly specialized discipline such as cardiac surgery. The advent of transcatheter technologies has progressively become a part of the armamentarium of available treatments, surely in a complementary way, merging with classical cardiac surgery and stimulating it for continuous improvement and refinement. The main advantage of percutaneous therapies is reducing the access and the invasiveness of the procedure, operating on the beating heart in patients at high surgical risk, avoiding

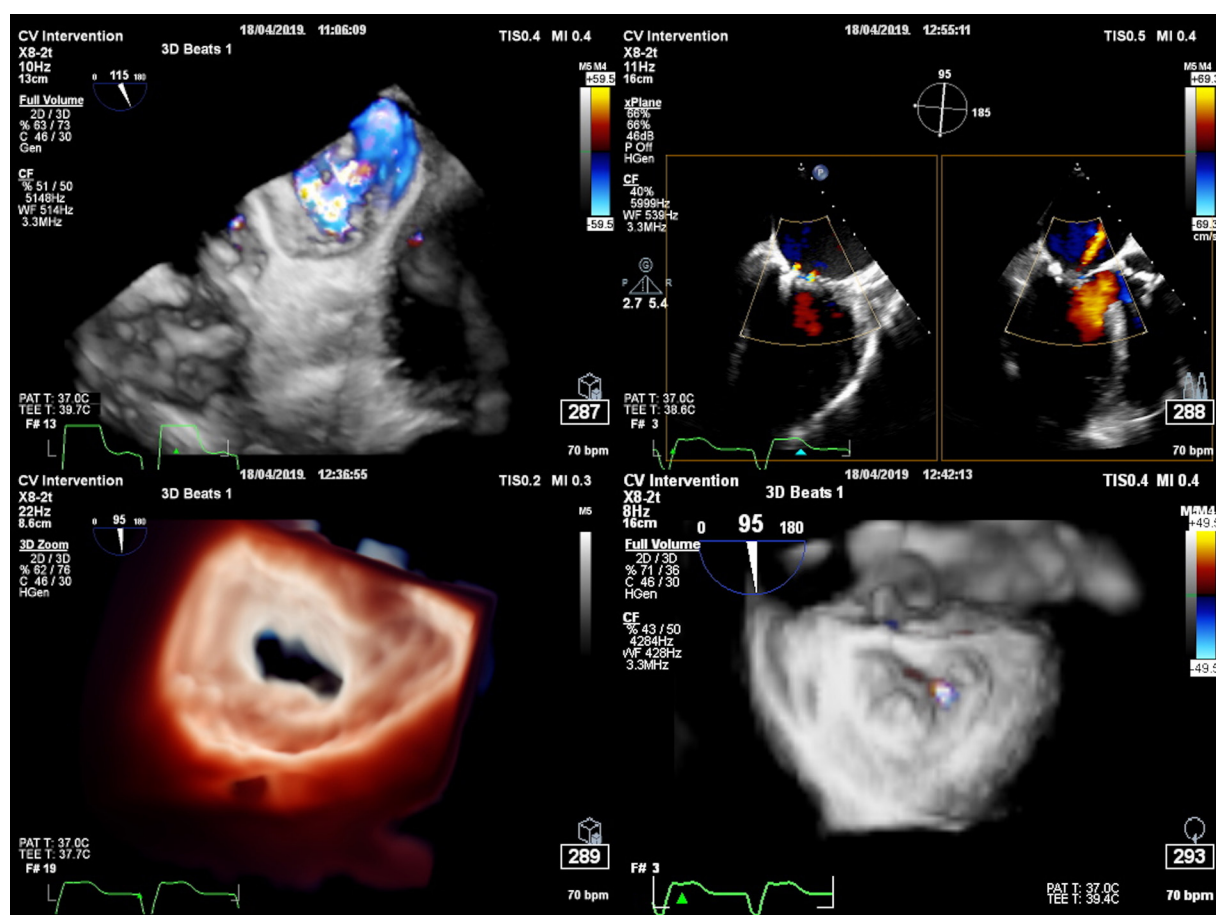


Figure 2. The same P3 lesion undergoing MitraClip™ procedure (left upper: severe MR before, right upper minimal MR after clipping, left lower diastolic frame in surgical view after clipping, medial commissure and right lower with minimal MR besides medial commissure in systolic view)

the burden of the extracorporeal circulation. This translates into new needs and new demands, in addition to the knowledge of transcatheter therapies, equipment and acquisition of wire skills. It then becomes mandatory to acquire knowledge of cardiovascular imaging, for the planning of procedures and for the guidance during interventions (intraprocedural imaging).

One of the most stimulating and enriching steps for future generations of cardiac surgeons will be the incorporation of imaging techniques in the surgical curriculum, the knowledge of the peculiarities of each image modality and how to proceed on a daily basis in clinical practice. One practical example could be understanding and navigating the right heart anatomy, using only the information provided by fluoroscopy, to guide interventional tricuspid procedures^[45] [Figure 4].

The idea that the field of multimodality imaging is far from the surgical domain and it is the exclusive competence of other professional figures of the heart team, such as the cardiologist echocardiographer, the interventional cardiologist (intraprocedural imaging and its modalities) or the radiologist (e.g., computerized tomography and cardiac magnetic resonance) is, unfortunately, well acknowledged. This way of thinking is restricting and risks confining the figure of the cardiac surgeon to that of the final recipient, a passive user, a handworker. The risk is to become insensitive to technological evolutions and new skills that could be acquired. Due to the technological refinement, cardiac CT is a routine part of assessment in transcatheter therapies^[46]. The sequential incorporation of increasingly sophisticated software has

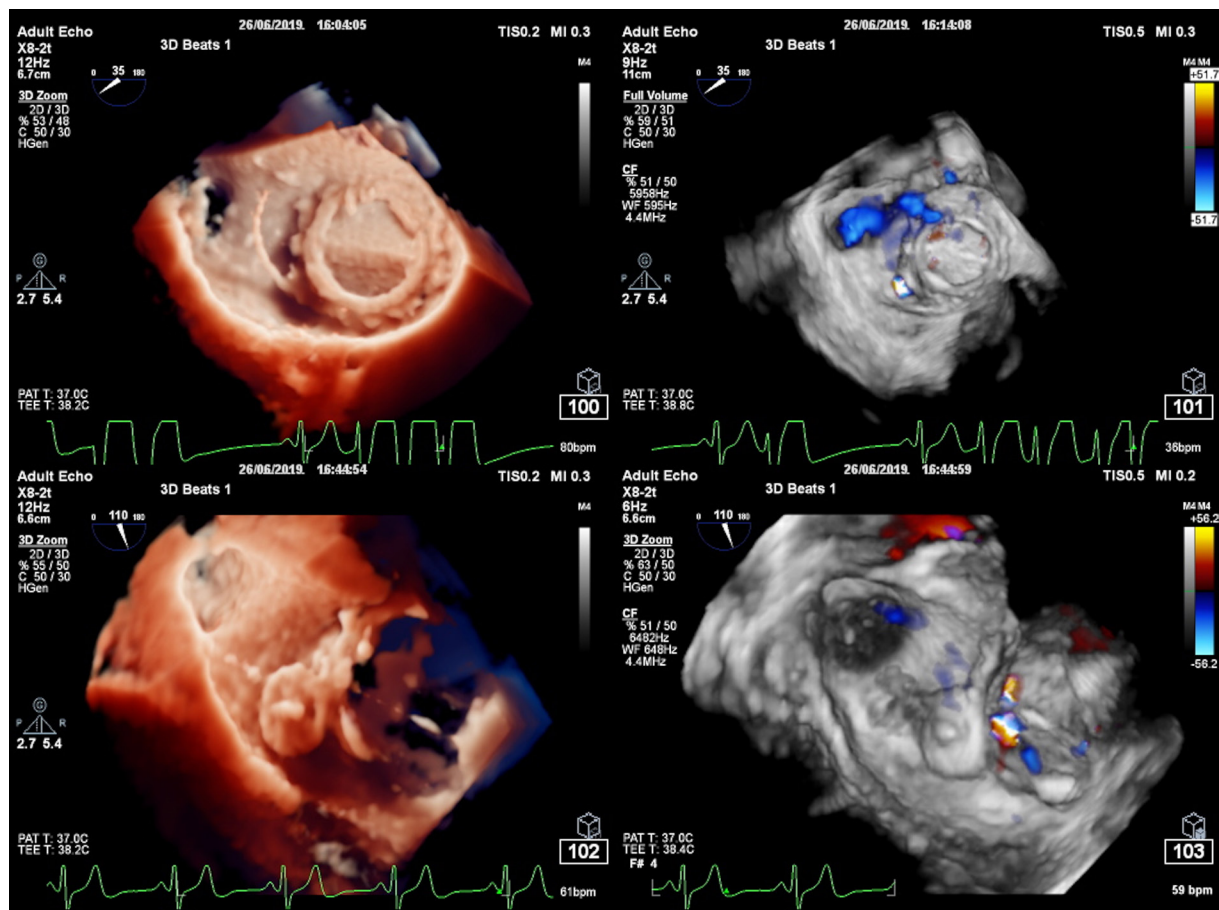


Figure 3. Picture of paravalvular leak (PVL) closure of a mechanical bileaflet On-X valve at 0800 with vascular plug amplatzer device. Left upper panel shows placement of the guidewire in the PVL, right upper the PVL at 0800 in systolic surgical view. Left lower end panel of procedure with amplatzer device in PVL and right lower no PVL left in color Doppler 3D systolic frame

allowed for pre-procedural planning resulting in near perfection at the time of device implantation. Learning to analyze a cardiac CT scan, measuring the size of the valve rings, the areas, the distance of the coronary arteries from the annulus, are skills acquired with great speed and precision by those who see and understand the cardiac structures with their own eyes in the daily practice. Surgical assessment of anatomical imaging prior to the procedures has become standard in most active centers and it is a mandatory step to achieve safety and effectiveness.

Especially to face complex interventional procedures, integrating in a multimodal way echocardiographic information and CT, fusing echocardiographic and angiographic intraprocedural images with last generation softwares - or CT and fluoroscopy [Figures 5 and 6] - should become part of the cultural background of future cardiac surgeons^[47]. Good surgeons of the future will be independent in the correct reading of the patient's imaging just like today they are good in interpreting the anatomy by direct vision: the one is not excluding the other... it is augmented reality, fusion of multimodality imaging inputs to enrich the information and achieve better results.

The progressive technological evolution has led the world of cardiac imaging to technical improvements considered unexpected only a few years ago. In a few years, there has been a move from two-dimensional diagnostic imaging, very operator-dependent, to the current highly sophisticated and integrative multimodality imaging approach^[48]. This is certainly a great innovation and the next frontier for those who

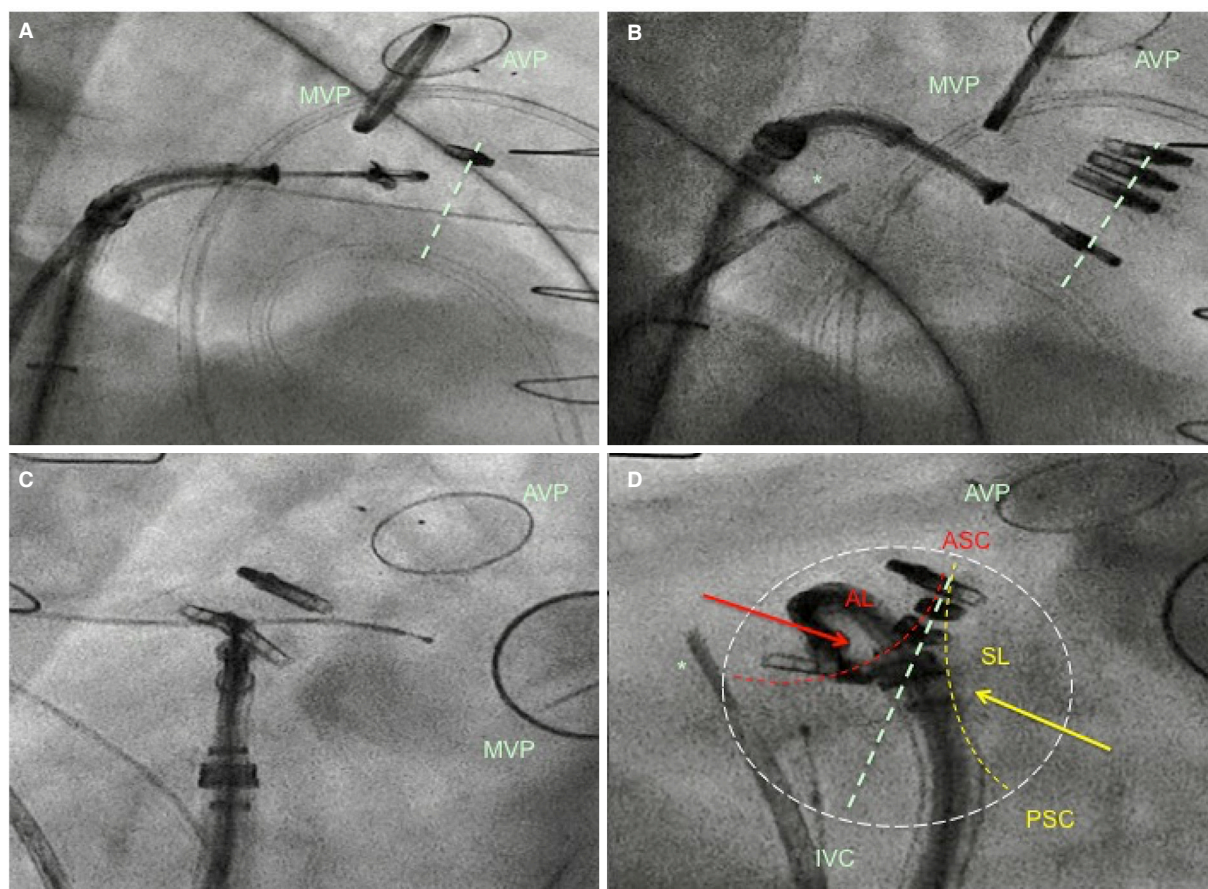


Figure 4. A and B: show two right fluoroscopic 2-chamber views during percutaneous tricuspid MitraClip™ procedure, useful to advance the catheters towards the tricuspid valve and to calculate the trajectories to address the targeted commissure. Light green dotted line traced in Panel A-B is the hypothetical line of coaptation between the anterior and the septal leaflet; C and D: show two en-face fluoro views during the same procedure, useful to guide catheter navigation within the valve area and to position the MitraClip™ in the antero-septal commissure (ASC), closest to the tricuspid annulus. AL is anterior leaflet (dotted line); PSC (yellow) posteroseptal commissure with SL septal leaflet (dotted line). Light green dotted line traced in Panel D is the line joining the aortic valve (ASC) and the inferior vena cava, reflecting the theoretical line of coaptation between the anterior and the septal leaflet. Arrows indicate traction forces on the leaflets done by the edge-to-edge percutaneous repair. The white dotted circle depicts the theoretical tricuspid ring. *Intracardiac echocardiography (reprinted with permission from Pozzoli *et al.* [47]). AVP: aortic valve prosthesis; MVP: mitral valve prosthesis

want to deal with cardiovascular therapies. Surgeons should more actively participate in this assessment and being able to further increasing their navigation skills.

THE CHANGING SCENARIO

Understanding the evolution

The advent of transcatheter/endovascular techniques has represented the latest major breakthrough in Cardiology, Cardiac and Vascular Surgery. From the first reported case of transcatheter aortic valve implantation in 2002 by Cribier *et al.* [49], there has been an unstoppable development in the treatment of valvular disease. The role of surgery in the treatment of the so-called structural heart disease - although popular a still somewhat confusing term [50] - is currently challenged by the community as more information is collected that supports the modification of the level of evidence for a given therapy [51]. One may argue that a large part of the evidence accumulated over the past couple of decades, not only in the field of structural heart disease, develops from sponsor-initiated studies and therefore there may be or there actually are a number of flaws and biases for obvious economic interests. On the other hand, investigator-initiated trials are less frequently produced as there are issues with regulatory bodies and financial support.

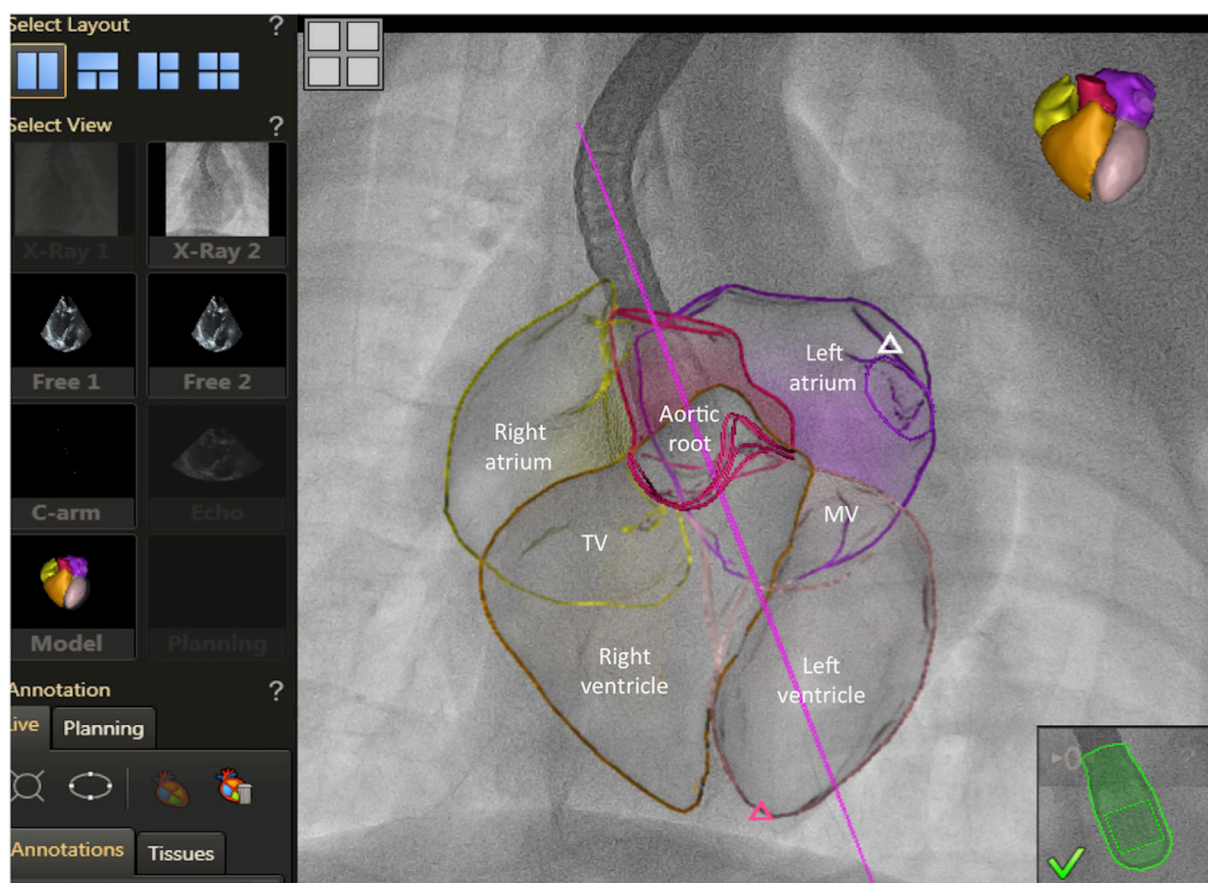


Figure 5. Echo/fluoro overlay of segmented 3D heart in fluoro space. TV: tricuspid valve; MV: mitral valve

Even with public funding, investigators may face problems with logistics and regulations that are a burden for someone with suboptimal structure for execution of research projects. The European Union regulations on research^[52] are an example of how complex and challenging the scenario becomes.

Transcatheter therapies came to stay and conventional surgery had to sustain the impact of this growing and currently accepted approach to treat heart and vascular disease. There has been a paradigm shift in therapeutic strategies; again, the example of transcatheter aortic valve implantation^[51] and transcatheter repair of mitral insufficiency^[53] shows that adaptive change is required and surgery and the surgeons must complete the initial efforts towards a full adoption of these technologies.

The same phenomenon has been observed in the field of Vascular Surgery. Once a fully open surgical specialty, vascular surgeons converted the Specialty in a combined surgical activity, for which wire skills became the routine. Thus, conventional surgery and transcatheter procedures are mixed and no differentiation within the team is made.

The intellectual component

From all of the above one can infer that challenges are to be positively accepted. Form follows function^[54] and this is why nature defined the evolution of species, as species undergo adaptive changes^[55]. A quick look into different species heart morphology allows to understand why the heart has different forms and sizes^[56].

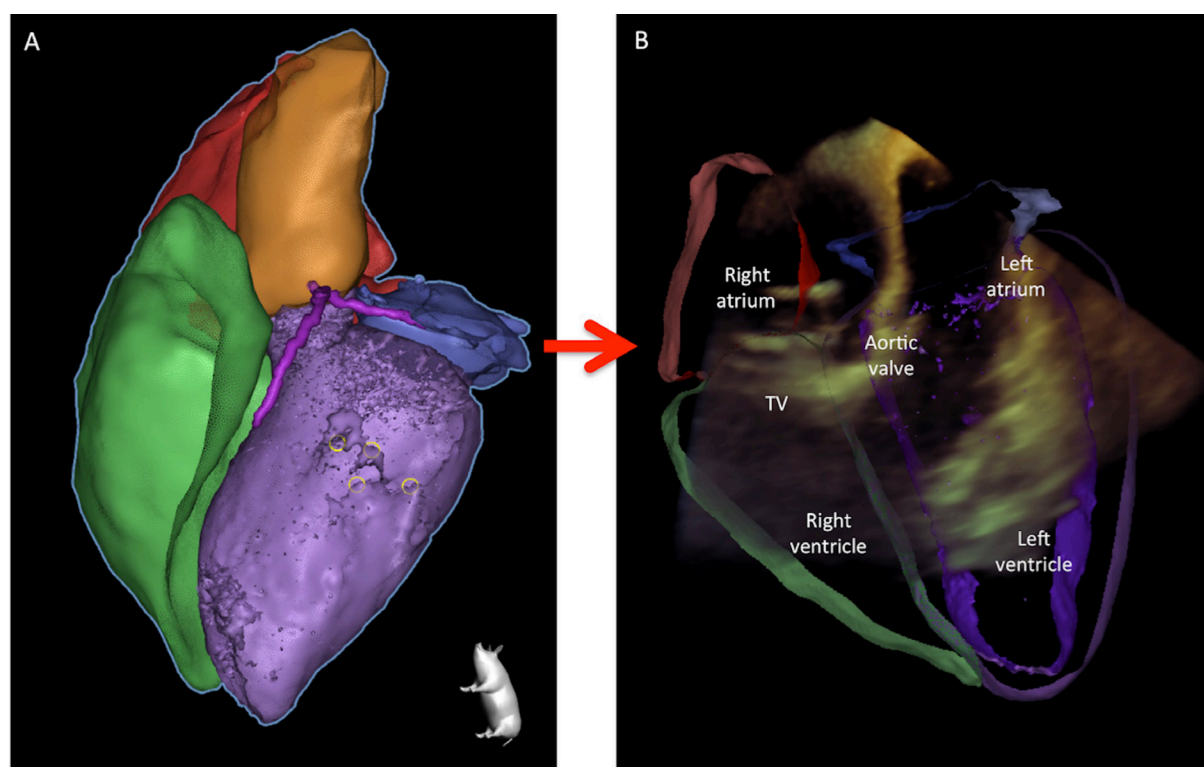


Figure 6. A glimpse into the future. Initial validation step of CT vs. Echo segmentation merged together in one image. TV: tricuspid valve

The key point is not to make the interpretation of information derived from clinical trials, or to learn how to work with a modern state-of-the-art technology or simply to perform a given procedure with success. Embracing technologies, analyzing data, acquiring hands-on proficiency in something are important in the daily medical practice. However, the most important is the intellectual changes required to understand a rapidly changing scenario and how to adapt to a fast-moving world that entails professional new opportunities.

The intellect is a unique characteristic of the human being. The intellect is the faculty of reasoning and understanding objectively, especially with regard to abstract matters^[57]. As it is supposed to be the most advanced in comparison with the remaining of the living species, it makes sense to understand that challenges in Cardiac Surgery do have a critical intellectual component.

THE FRONTIER

A frontier is defined as the extreme limit of understanding or achievement in a particular area^[58]. Therefore, this definition entails a substantial intellectual component. What seems clear is that all is about our understanding of the problem. The current scenario is that of technological evolution, combined with changing patterns in patient and environmental complexity, the availability of techniques allowing for a reduced access for treatment of structural heart diseases and changes in therapeutic paradigms through the collection of evidence.

The frontier is the ability to update our knowledge and skills and to implement responses that will allow to redesign and reorganize the surgical practice by expanding the surgical portfolio.

THE FUTURE

The future in Cardiac Surgery must contemplate impactful changes to allow the surgeon to continue its regular high-quality practice in cardiovascular disease. A number of issues to consider in this, already here, future can be summarized as follows:

1. Modification of the training process

- (1) Redesign of curriculum and syllabus;
- (2) Training in cardiovascular imaging;
- (3) Structured training in coronary angiography and interventional procedures;
- (4) Acquisition of specific skills for catheter-based interventions;
- (5) Mandatory simulation training;

2. Support innovation

- (1) Allocate departmental resources to enhance pooling of ideas;
- (2) Support departmental/institutional basic and clinical research (back to the basis);
- (3) Mandatory training in the animal laboratory;

CONCLUSION

Cardiac Surgery is a Specialty in continuous evolution. Since the introduction of the HLM, which allowed for the correction of intracardiac and extracardiac defects of all kinds, significant changes have been introduced in the surgical practice.

The current scenario, with the consolidation of transcatheter therapies in the treatment of structural heart disease, represents a challenge to conventional surgical practice. The evolution of Cardiac Surgery must include a redesign of the training of current and future specialist and a transformation of the daily practice. Conventional surgery and transcatheter procedures are to be routinely scheduled with no differentiation within the surgical team.

This needs an adaptive change, with an intellectual transformation to understand the importance of incorporating a different type of training and practice.

DECLARATIONS

Authors' contributions

Researched data for the article, discussed its content, and wrote, reviewed, and edited the manuscript: Mestres CA

Researched data for the article, and wrote, reviewed, and edited the manuscript: Pozzoli A

Discussed the content and reviewed and edited the manuscript before submission: Taramasso M, Zuber M, Maisano F

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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.

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Commentary

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Percutaneous edge-to-edge mitral valve repair for secondary mitral regurgitation: perspectives on COAPT and MITRA-FR trials

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Mitral valve regurgitation (MR) is classified as primary or secondary depending upon pathophysiology. Primary MR arises from degenerative disease of the valve leaflets whereas secondary MR is due to dilatation of left ventricle leading to distortion of valve architecture^[1]. Therapy for primary MR is established: valve repair (surgery or percutaneous), or valve replacement when repair is not possible^[2,3]. In contrast, therapy for secondary MR is still a matter of debate^[4]. It is universally agreed that treatment for left ventricular dilatation is foremost by guideline directed medical therapy (GDMT) and cardiac resynchronization therapy^[3]. Whether to treat the MR mechanically constitutes the dilemma. This intriguing conundrum becomes even more interesting with publication of two similar trials with opposite results^[5,6]. If reproducibility is the foundation of settled new knowledge, then why did these two large randomized controlled trials have seemingly irreconcilable results?

Investigators of the COAPT (Cardiovascular Outcomes Assessment of the MitraClip Percutaneous Therapy for Heart Failure Patients with Functional Mitral Regurgitation, USA)^[6] and the MITRA-FR (Percutaneous Repair with the MitraClip Device for Severe Functional/Secondary Mitral Regurgitation, France)^[5] trials hoped to seek whether secondary MR was simply a marker of a diseased and dilated left ventricle or separately contributed to patient mortality and therapy via edge-to-edge percutaneous repair of mitral valve (MV) (MitraClip®) would be efficacious. Incidentally, results of the MITRA-FR trial support the former assertion while those of COAPT trial seem to corroborate the later.



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Table 1. Comparison between MITRA-FR and COAPT trials

	MITRA-FR	COAPT
Enrolled patients	307	614
Duration of follow-up	1-year	2-year
HF medications	Appropriate; titrations permitted after randomization	Maximized before randomizations
Severe MR (criteria by EROA)	> 0.2 cm ²	> 0.3 cm ²
LV dilatation (LVESD)	Unspecified	> 70 mm excluded
Mean EROA at baseline	0.31 cm ² (52% < 0.3 cm ²)	0.41 (41% ≥ 0.4 cm ²)
Mean LVEDV at baseline	252 mL	192 mL
NYHA stage III-IV	65%	58%
Post-procedure MR grade 3+/4+	< 10%	< 10%
Complications (procedural)	14.6%	8.5%
MR at 1-year (≥ 2 grade)	49.5%	31.0%
HF hospitalizations at 1-year	49%	38%
LV volume change	None	Decreased
Mortality (30-day)	3.3%	2.3%
Mortality (1-year)	24%	19%
Hazard ratio for all-cause mortality (95%CI)	1.11 (0.69-1.77) (No benefit)	0.62 (0.46-0.82) (38% risk reduction)

HF: heart failure; LV: left ventricular; MR: mitral valve regurgitation; EROA: effective regurgitant orifice area; LVESD: left ventricular end systolic diameter; LVEDV: left ventricular end diastolic volume; MITRA-FR: percutaneous repair with the mitralclip device for severe functional/secondary mitral regurgitation; COAPT: cardiovascular outcomes assessment of the mitralclip percutaneous therapy for heart failure patients with functional mitral regurgitation

COMPARE AND CONTRAST

The New England Journal of Medicine published articles detailing the results of these two trials with contradictory conclusions in the same 2018 issue. What followed were multiple viewpoints and reviews comparing and analyzing the trials apparent opposite results^[7-10]. We intend to review several of these interpretations as well as assess the methodologic and study population differences between these two studies [Table 1].

Underlying an informed analysis of MITRA-FR and COAPT trials is a new concept: “disproportionate”^[8] or “tertiary” MR^[7]. As explained by Packer *et al.*^[8] MR severity may or may not be proportional to left ventricular (LV) dilation, and also may or may not contribute to the disease process. In some patients the MR may become exaggerated or “out of proportion” (disproportionate MR) to their LV dilation. In these patients with disproportionately increased MR, the regurgitation may represent a causative agent for (or contributor to) worsening heart failure (HF), rather than an innocent “bystander”. In retrospect, by its inherent study design and inclusion criteria, the MITRA-FR trial enrolled all patients with secondary MR, irrespective of “proportionality” in degree of MR. In contrast, COAPT trial used more stringent selection criteria and included a greater percentage of patients with “disproportionate” MR. MITRA-FR trial enrolled an “all comers” patient population and was unable to show any benefit of percutaneous edge-to-edge repair of MV. Conversely, the COAPT trial was able to show remarkable clinical benefit of the procedure. Put another way, these two studies enrolled different populations using different study protocols and we are not surprised that the results were also different.

SPECIFIC SIMILARITIES AND DIFFERENCES

What were the differences in the COAPT and MITRA-FR study designs? In MITRA-FR, included subjects were required to have at least one prior HF hospitalization within 12 months and MR severity with effective regurgitant orifice area (EROA) > 20 mm² or MR regurgitation volume (RV) > 30 mL. B-natriuretic peptide (BNP) and left ventricular end systolic diameter (LVESD) were not specified. The primary endpoint was a composite of unplanned hospitalization for HF at 12 months or all-cause death. Whereas in COAPT,

study subjects were required to have at least one of three criteria: at least one hospitalization for HF within 12 months, BNP > 300 pg/mL or NT-proBNP > 1500 pg/mL, or MR severity with EROA > 30 mm² or RV > 45 mL. The primary outcome was HF hospitalizations within 24 months of follow-up^[10]. As a result, in MITRA-FR trial versus COAPT trial the average NT-proBNP, 12 months prior annual hospitalizations and LVEDV were approximately 3349 ng/L vs. 5558 pg/mL; 100% vs. 57%; and 135 mL/m² vs. 101 mL/m² respectively. Nishimura *et al.*^[11] in their accompanying editorial point out several features that could explain the differences: first, the strategy of medical management before and after enrollment differed. In COAPT trial, maximal HF treatment took place solely prior to randomization, therefore identifying patients truly refractory to medical therapy. After enrollment, further adjustments to medical therapy were not allowed. In MITRA-FR trial, medical therapy modifications were permitted before and after randomization. Second, the baseline valvular and ventricular characteristics were different. In COAPT, the MR was more severe compared to MITRA-FR (average EROA, 41 mm² vs. 31 mm²) but with a smaller LV size [left ventricular end diastolic volume index (LVEDVI) 101 mL/m² vs. 135 mL/m² & left ventricular end diastolic diameter, 62 mm vs. 69 mm]. Third, procedural skill may have contributed, as COAPT had more patients with multiple clips than MITRA-FR and possibly more experienced proceduralists since the study took place at a later date. Primary endpoint in COAPT trial which was HF hospitalization within 24 months of procedure was 35.8% in intervention vs. 67.9% in control arm ($P < 0.001$). Primary endpoint in MITRA-FR, which was death or HF hospitalization at 12 months was 54.6% in intervention group vs. 51.3% in control ($P = 0.53$).

After the studies and editorial were published, readers from all over the world submitted queries. Regarding COAPT trial, Crestanello *et al.*^[12] were curious about the echocardiographic methodology in calculation of EROA as for them (Simpson's method of discs) it would generate impractical numbers for both regurgitant volume and stroke volume. The authors explain this discrepancy due to difference in echocardiographic measurement techniques (Simpson's vs. Doppler)^[13]. To us, this emphasizes the inherent limitations of echocardiography, and questions validity of conclusion based on semiquantitative echocardiographic estimates. Drake *et al.*^[14] speculate that detailed knowledge of the preoperative transesophageal echocardiography could have introduced a bias to the interventionists' in COAPT trial leading to better outcomes. The authors agree with this observation emphasizing the value of preoperative advanced imaging and thus its use is representative of current medical practice and therefore should be included in the study protocol. Garbi *et al.*^[15] recognize that the "disproportionate"^[8] or "tertiary" MR^[7] along with reduced left atrial pressure observed in COAPT patients, both may have contributed to improved symptoms post-procedurally which the authors agree with as a possibility. Despite that the COAPT trial was company sponsored (Kalavrouziotis *et al.*^[16]), the authors posit an independence of medical decision making.

Godino *et al.*^[17] expressed concerns about the very frequent (> 20%) occurrence of mortality in both intervention and control groups in MITRA-FR trial. In response, the authors compared mortality at one-year in both the MITRA-FR and COAPT trials and found relatively similar mortality at one year^[18]. The study investigators also explain that the other major heart failure trials with lesser one year mortality were not comparable due to differences in study designs. For example, the PARADIGM-HF trial explicitly excluded "all hemodynamically significant mitral disease". Goliash *et al.*^[19] point out that the differing definition of severe MR between the COAPT (EROA 0.3 cm²) and the MITRA-FR (EROA 0.2 cm²) trials, reflecting American vs. European guidelines, could have contributed to the difference in results as well. Nevertheless, a sub-analysis controlling for EROA did not change the results. Silverio *et al.*^[20] posit that the lack of benefit observed in the MITRA FR trial could be due to relatively nonrestrictive study center-eligibility criteria, and inexperience on part of the proceduralist. The MITRA-FR authors point out that, despite the fact that they had only a five procedure per center minimal requirement, the COAPT had three subjects per center for active enrollment. Therefore, although COAPT trial ended up with more patients

per center, but criteria for eligibility was not much different than MITRA-FR trial. Lastly, as previously noted, the MITRA-FR authors do not deny that they may have included patients with heart failure too advanced to derive benefit^[18].

Intensive analysis of these two trials have led most practitioners to agree that the trials are “complimentary rather than contradictory” (Tang *et al.*^[21], Carabello *et al.*^[7]). Whether the diagnosis is “disproportionate MR” (Packer *et al.*^[8], Praz *et al.*^[9]) or “tertiary MR”^[7], consensus appears to be that patients with the combination of severe secondary MR (EROA > 0.3 cm²) with no more than moderate LV dilatation (LVEDVI < 96 mL/m²) potentially will benefit from percutaneous edge-to-edge repair (MitraClip[®]) procedure after optimal medical therapy, as shown in COAPT trial.

Furthermore, as pointed out by Praz *et al.*^[9], these two trials lead to the following conclusions regarding percutaneous therapy for secondary MR:

1. Extreme LV dilatation with less severe secondary MR (no benefit: MITRA-FR trial).
2. Moderate LV dilatation with more severe secondary MR (benefit: COAPT trial).
3. Extreme LV dilatation with more severe secondary MR (unknown benefit).

SURGICAL PERSPECTIVE

The American Association for Thoracic Surgery in 2016 revised their guideline recommendation for severe secondary MR to MV replacement rather than repair for patients with LVEDD > 65 mm^[1]. Surgical correction of secondary MR has been a topic of great interest and a matter of equipoise^[4]. The challenge has been that surgical anatomic correction has not necessarily translated into a lasting clinical benefit^[22]. Conceptually, the basis for surgical correction has been to produce a normal architecture, most often with placement of a mitral annuloplasty ring. Edge-to-edge percutaneous technique without any reinforcement with annular ring, seems unconvincing. Badhwar *et al.*^[23] posit that while MV replacement is best for symptom resolution, valve repair still has its “niche” role in pathoanatomically suitable patients with secondary MR. They suggest an entire pathoanatomic classification based on: (1) annular dilatation; (2) ejection fraction; and (3) leaflet tethering. Based on this they suggest “low surgical risk patients” may undergo CABG + mitral valve repair or replacement whereas “high surgical risk” may have catheter based transmitral valve replacement or repair. As a caveat, this hypothesis has not been tested yet in prospective randomized clinical trials. Definitive conclusions regarding the relative efficacy of other techniques for secondary MR including percutaneous neo-chord, percutaneous annuloplasty ring^[24], and percutaneous MV replacement await appropriate clinical studies. In the light of these evolving techniques. Future guidelines may increasingly rely on assessment of surgical risk, likelihood of successful anatomic correction and clinical benefit and feasibility.

CONCLUSION AND FUTURE STUDIES

Percutaneous edge-to-edge mitral valve repair for mitral regurgitation has been practiced in Europe since approved by the European Commission in 2008. It is estimated that 65% of percutaneous repair for MV in Europe are for secondary MR. In contrast, in North America, the United States Food and Drug Association approved this procedure in 2013 only for primary (degenerative) mitral regurgitation, consequently about 80% of all MitraClip procedures in US are for primary MR. Thus, Europeans overall have a much broader experience in MitraClip[®] procedures. This may be reflected in the approach taken by the MITRA-FR authors, namely that percutaneous MV repair would be more readily offered on a less stringent basis compared to the more medically refractory subjects included in the COAPT. For certain, percutaneous edge-to-edge repair is not for every patient with secondary MR. That said, there is one specific subset of patients that did show benefit. Identification of these patients and successful percutaneous valve repair will require meticulous medical optimization of heart failure, careful echocardiographic measurements and a

skilled interventional cardiologist. Once completed, RESHAPE-HF-2 (NCT02444338) should better define the role of transcatheter edge-to-edge repair of secondary MR. Finally, we eagerly await longer term studies that assess repair durability, compare different clip designs, and refine optimal medical management of patients before and after percutaneous repairs.

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Authors' contributions

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Deletion of retinoic acid-related orphan receptor gamma reduces body weight and hepatic lipids in mice by modulating the expression of lipid metabolism genes

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Abstract

Aim: Retinoic acid-related orphan receptor γ (ROR γ) functions as a ligand-dependent transcription factor and its loss has been shown to affect the circadian expression of lipid metabolism genes. However, its effect on body weight gain and hepatic lipids is not well understood. In this study, we investigated the impact of *Ror γ* gene deletion on changes in body weight and hepatic lipids.

Methods: Body weight and lipids were analyzed in the plasma and liver. Expression of lipid metabolism genes in the liver was evaluated in wild type and *Ror γ* knockout mice.

Results: We show that deletion of ROR γ results in reduced body weight and fewer lipids in the liver. Analysis of gene expression showed that deletion of *Ror γ* resulted in an overall lower expression of genes and transcription factors involved in lipid biosynthesis. We observed a decrease in the gene expression of cholesterol biosynthesis, efflux, and esterification but an increase in bile acid synthesis. There was a decrease in fatty acid and triglycerides biosynthesis genes and an increase in the fatty acid uptake genes. The decrease in the expression of lipid biosynthesis genes was accompanied by the decrease in the sterol response element binding protein (*Srebp*) genes. We observed an increase in



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the expression of peroxisome proliferator-activated receptor alpha (Ppara) and a decrease in the expression of acetyl-CoA carboxylase 2 (*Acc2*) genes.

Conclusion: Our data suggest that ROR γ regulates body weight and lipid metabolism genes and its modulation may be beneficial for the management of obesity and related lipid metabolic disorders.

Keywords: Lipid metabolism, triglycerides, retinoic acid-related orphan receptor γ , peroxisome proliferator-activated receptors, sterol response element binding proteins

INTRODUCTION

Obesity is a major risk factor that leads to the development of other metabolic disorders such as hyperlipidemia and hepatic steatosis. Ectopic accumulation of fat in the liver causes insulin resistance by activating various cellular stress and inflammatory signaling pathways^[1]. The liver plays an important role in lipid metabolism that involves several key proteins and transcription factors to maintain lipid homeostasis^[2]. Various cellular regulators that include several transcription factors control biosynthesis, oxidation, uptake, and secretion of lipids in the liver^[3]. Peroxisome proliferator-activated receptors (PPARs), which serve as hepatic lipid sensors, have been demonstrated to play a critical role in lipid metabolism by controlling the enzymes through various signaling events^[4,5]. Peroxisome proliferator-activated receptor α (PPAR α) is highly expressed in the liver and is primarily involved in the regulation of enzymes crucial for fatty acid oxidation. It also plays a role in regulating genes that control fatty acid elongation, desaturation and transport^[6]. On the other hand, peroxisome proliferator-activated receptor γ (PPAR γ), which is expressed at low levels in the liver^[7], controls the upregulation of a subset of the lipogenic genes that are involved in lipid synthesis and accumulation^[8]. Besides PPARs, the sterol response element binding proteins (SREBPs) that act as nutrient sensing transcription factors are also involved in the transcriptional control of the lipogenic gene expression^[9]. Sterol regulatory-element binding protein 1c (SREBP-1c) is a transcription factor that primarily upregulates the transcription of genes involved in fatty acid synthesis. In contrast, sterol regulatory-element binding protein 2 (SREBP-2) controls the activation of genes responsible mainly for cholesterol synthesis and uptake, as opposed to fatty acid synthesis. However, sterol regulatory-element binding protein 1a (SREBP-1a) leads to the activation of genes involved in both pathways^[10,11].

The transcriptional networks of orphan nuclear receptors govern the hepatic metabolic pathways^[12]. The retinoic acid-related orphan receptor γ (ROR γ), a member of the ROR subfamily of nuclear receptors, has been implicated in the control of a variety of physiological processes^[13]. ROR γ binds to specific ROR-responsive elements at its genomic targets to constitutively activate the gene expression in the absence of a ligand^[14]. Global ROR γ -deficient mice are born healthy and fertile but die within the first four months after birth due to high incidence of T-cell lymphomas in the thymus^[15]. The physiological functions of ROR γ , expressed in various peripheral tissues^[16], are still poorly understood. ROR γ has been shown to regulate the circadian expression of glucose and lipid metabolism genes^[17,18]. Pharmacological inhibition of ROR γ has been shown to reduce food intake and body weight gain^[19]. However, effect of *Ror γ* gene deletion on body weight and hepatic lipids is not well studied. Therefore, in this study, we investigated the effect of *Ror γ* gene deletion on body weight and hepatic lipids using a knockout (KO) mouse model. We also studied the expression of downstream lipid metabolism genes that are regulated directly or indirectly by ROR γ .

METHODS

Materials

Infinity cholesterol (catalog # TR13421) and Infinity triglyceride (catalog # TR22421) reagent kits were purchased from Thermo Scientific (Middletown, VA). TRIzolTM (catalog #15596018) was purchased from

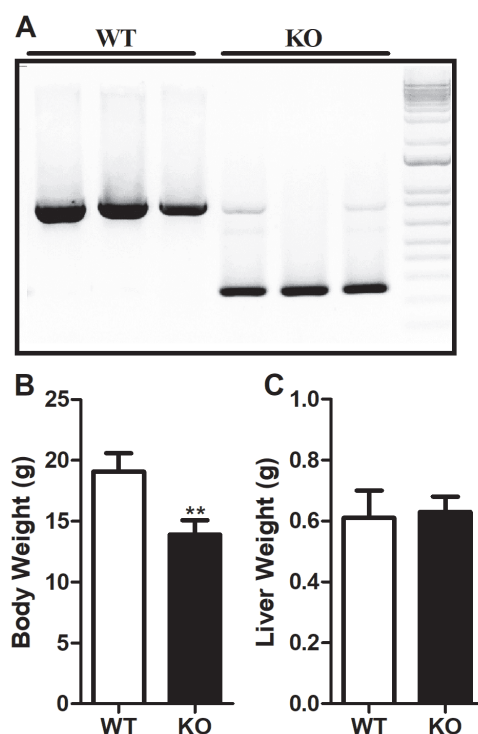


Figure 1. Effect of *RORγ* gene deletion on body and liver weights in mice. *RORγ* WT and KO mice ($n = 3$) were identified by genotyping the DNA isolated from tail tissues (A). Total body (B) and liver (C) weights of 8-week old chow diet fed WT and KO mice were measured. Values were plotted as mean \pm SD. P values were calculated using two-tailed Student's t test. ** $P < 0.01$. WT: wild type; KO: knockout; *RORγ*: retinoic acid-related orphan receptor γ

Life Technologies (Carlsbad, CA). Omniscript RT kit (catalog #205113) was from Qiagen (Germantown, MD). qPCRTM core kit for SYBR Green I (catalog #10-SN10-05) was purchased from Eurogentec (San Diego, CA). All other chemicals and solvents were obtained from Fisher Scientific through its local distributor in the Kingdom of Saudi Arabia.

Animals

Heterozygous *RORγ* mice were intercrossed to obtain *Rory*^{-/-} and wildtype (WT) littermate controls as described previously^[20]. Genotyping was performed by PCR analysis of tail DNA using 5'-TGGTAGTGTCACTATCTGTGTCCC-3' forward and 5'-CTTCAGAACTTATGTCAGGAACTCC-3' reverse primers to generate an 850-bp WT product and a 250-bp KO product to identify the WT and *Rory* KO mice [Figure 1A]. For this study, we used eight-week-old chow-diet-fed WT and *Rory* KO male mice ($n = 3$) on C57BL/6J background. All experiments were approved by Institutional Animal Care and Use Committee at King Faisal Specialist Hospital and Research Center.

Body weight and lipid analysis

Mice in WT and *Rory* KO groups were fasted overnight for 18 h, weighed using digital balance to measure total body weight and sacrificed to collect blood and livers. Liver weight of each animal was recorded. Blood was used to isolate plasma after centrifugation of the samples for 15 min at 2500 rpm using a tabletop centrifuge. Plasma and liver tissues were stored at -80 °C until further analysis. Hepatic lipids from WT and KO mice were extracted by Bligh and Dyer method^[21]. Total cholesterol and triglyceride levels in the plasma and liver tissues were measured using commercially available kits from Thermo Scientific as described previously^[22].

Table 1. List of primers used for the quantification of lipid metabolism genes

Gene	Forward primer	Reverse primer
Cholesterol metabolism genes		
<i>Hmgr</i>	AGCTTGCCCGAATTGTATGT	TCTGTTGTGAACCATGTGAC
<i>Hmgs</i>	TGGCTATAAAGCTGCGGAGG	GGTGAAAGAGCCAAAGGGGA
<i>Abca1</i>	GGACATGCACAAGGTCCTGA	CAGAAAATCCTGGAGCTTCA
<i>Abcg1</i>	GCTGTGCGTTTTGTGCTGTT	TGCAGCTCCAATCAGTAGGC
<i>Abcg5</i>	AGGGCCTCACATCAACAGAG	GCTGACGCTGTAGGACACAT
<i>Abcg8</i>	AGTGGTCAGTCCAACACTCT	GAGACCTCCAGGGTATCTTG
<i>Acat1</i>	CCAATGCCAGCACACTGAAC	TCTACGGCAGCATCAGCAAA
<i>Acat2</i>	TGTTGAAAGGTGGGCAGCAA	CAGGTAACATCCCATCCCGT
<i>Cyp7a</i>	CAACGGGTGATTCCATACC	ATTTCCCCATCAGTTTGCAG
Fatty acid metabolism genes		
<i>Cd36</i>	TGCACCACATATCTACCAA	TTGTAACCCACAAAGAGTTC
<i>Fabp1</i>	CAATAGGTCTGCCGAGGAC	CAGGGTGAACTCATTGCGGA
<i>Fas</i>	GGGTCTAGCCAGCAGAGTC	TCAGCCACTTGAGTGTCTC
<i>Acc1a</i>	GAGGAAGTTGGCTATCCAGT	CTTGAACCTGTCTGAAGAGG
<i>Acc2</i>	TGTCCCAGGAGGCTGCATTG	TGTGCAGGTCCAGTTTCTTG
<i>Scd1</i>	CCGAGACCCCTTAGATCGA	TAGCCTGTAAAGATTCTTG
Triglycerides metabolism genes		
<i>Dgat1</i>	GTTGAGCTCAGACAGTGGTT	TCAGCATCACCACACACCAA
<i>Dgat2</i>	AGCTGCAGGTCATCTCAGTA	CTGCAGGCCACTCCTAGCAC
<i>Mgat2</i>	GAGCAAAGCCCGTGTGTAGA	AAGGTCTGTAACCTGCGCTC
<i>Mttp</i>	CACACAAGTGGCTCTCATTAAAT	TGCCCCATCAAGAAACACT
<i>Vldlr</i>	GCCATATGAGAACATGCCGC	AGGACACGGGGATACACTGA
<i>Ldlr</i>	TGACTCAGACGAACAAGGCT	ATCTAGGCAATCTCGGTCTC
<i>Plin1</i>	TGCTGCACGTGGAGAGTAAG	AGCAGGGTTGGGCCCTTGTT
<i>Lpl</i>	TTTGGCTCCAGAGTTTGACC	TGTGTCTTCAGGGGTCTTA
<i>Apoc2</i>	CATGGGGTCTCGTTCTTCC	CTTAAGAGGGAGCCCTGCAC
Lipid metabolism transcription factor genes		
<i>Srebp1a</i>	CCATGGACGAGCTGGCCTTC	AGTTGGCACCTGGGCTGCTG
<i>Srebp1c</i>	GGAAGCTGTCGGGGTAGCGT	CATGTCTTCAAATGTGCAAT
<i>Srebp2</i>	GCGTTCTGGAGACCATGGAG	GAGCTACAAAGTTGCTCTGA
<i>Ppara</i>	GCGTACGGCAATGGCTTTAT	GAACGGCTTCCTCAGTTCT
<i>Pparg</i>	ATGGTGCCTTCGCTGATGCA	TGGCATCTCTGTGTCAACCA
Housekeeping gene		
<i>18 sRna</i>	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG

Analysis of gene expression by quantitative real time PCR

TRIzolTM was used to isolate total RNA from tissues as per the manufacturer's instructions. cDNA was synthesized using Omniscript RT kit from good quality RNA preparations with the purity of RNA having A_{260}/A_{280} ratio of more than 1.7. Quantitative PCR in each reaction was carried out in a total volume of 20 μ L, consisting of 5 μ L of 1:100 diluted first strand cDNA sample and 15 μ L of SYBR Green I PCR master mix solution. The reaction mixture was incubated for 10 min at 95 °C followed by 40 cycles of 15 s incubations at 95 °C and 60 s at 60 °C in a QuantStudioTM 6 Flex Real-Time PCR (Applied Biosystems). Data were analyzed and presented as arbitrary units that were normalized to the expression of 18 sRNA, according to the manufacturer's instructions. The primers were designed using Primer Express 3.0 (Applied Biosystems) and are shown in Table 1.

Statistical analysis

Data are presented as mean \pm SD. Statistical significance ($P < 0.05$) was determined using Student's *t* test (GraphPad Prism 5).

RESULTS

Effect of *Rory* gene deletion on body and liver weights

Age matched eight-week-old male *Rory* KO mice and littermate controls on chow diet were used to assess the effect of *Rory* gene deletion on total body and liver weights. *Rory* gene deletion resulted in a significant

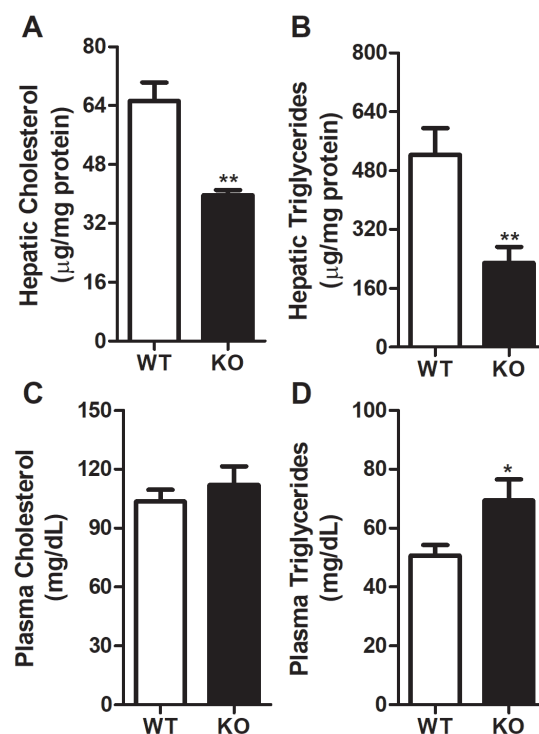


Figure 2. *RORγ* gene deletion reduces hepatic lipids and increases plasma triglycerides. Lipids from WT and KO mice livers ($n = 3$) were extracted by Bligh and Dyer method to measure cholesterol (A) and triglycerides (B). Total cholesterol (C) and triglycerides (D) were also measured in the plasma of these mice. Values were plotted as mean \pm SD. P values were calculated using two-tailed Student's t test. ** $P < 0.01$. WT: wild type; KO: knockout; *RORγ*: retinoic acid-related orphan receptor γ

decrease of 27% in total body weight compared to WT littermate control mice [Figure 1B]. However, no change was seen in the weight of livers in these mice [Figure 1C]. These results suggest that *RORγ* is important in maintaining the body weight in mice and its deficiency leads to reduced body weight gain.

***RORγ* KO mice decreases hepatic but not plasma lipids**

Next, we investigated whether change in body weight after *Rorγ* gene deletion also affects liver and plasma lipids. Our data indicate that there were significant decreases of 39% and 56% in the levels of liver cholesterol [Figure 2A] and triglycerides [Figure 2B], respectively, in *Rorγ* KO mice compared to WT littermates. However, we did not see any significant change in plasma cholesterol levels between WT and *Rorγ* KO mice [Figure 2C]. Contrary to decreased hepatic lipid levels, we observed an increase of ~37% in the levels of plasma triglycerides in KO mice compared to WT mice [Figure 2D]. These data suggest that *Rorγ* gene deletion affects only liver and not plasma cholesterol. On the other hand, both liver and plasma triglycerides are altered after the ablation of *Rorγ* gene.

Ablation of *Rorγ* activity affects expression of genes involved in lipid metabolism

To gain better understanding of how *Rorγ* activity regulates body weight and lipids, we looked at the changes in the expression of lipid metabolism genes after the deletion of *Rorγ* gene. We started by analyzing the changes in the genes that regulate cholesterol homeostasis in the liver. Coordinated expression of several genes regulate cellular cholesterol metabolism^[23,24]. We looked at the expression of cholesterol biosynthesis genes such as hydroxymethylglutaryl-CoA reductase (*Hmgr*) and hydroxymethylglutaryl-CoA synthase (*Hmgs*). The expression of rate-limiting enzyme gene, *Hmgr*, was not affected by the deletion of *Rorγ* in the liver [Figure 3A]. However, we observed a decrease of 63% in the expression of *Hmgs* in the livers of *Rorγ* KO mice compared to WT littermates [Figure 3B]. Next, we looked at the expression of genes

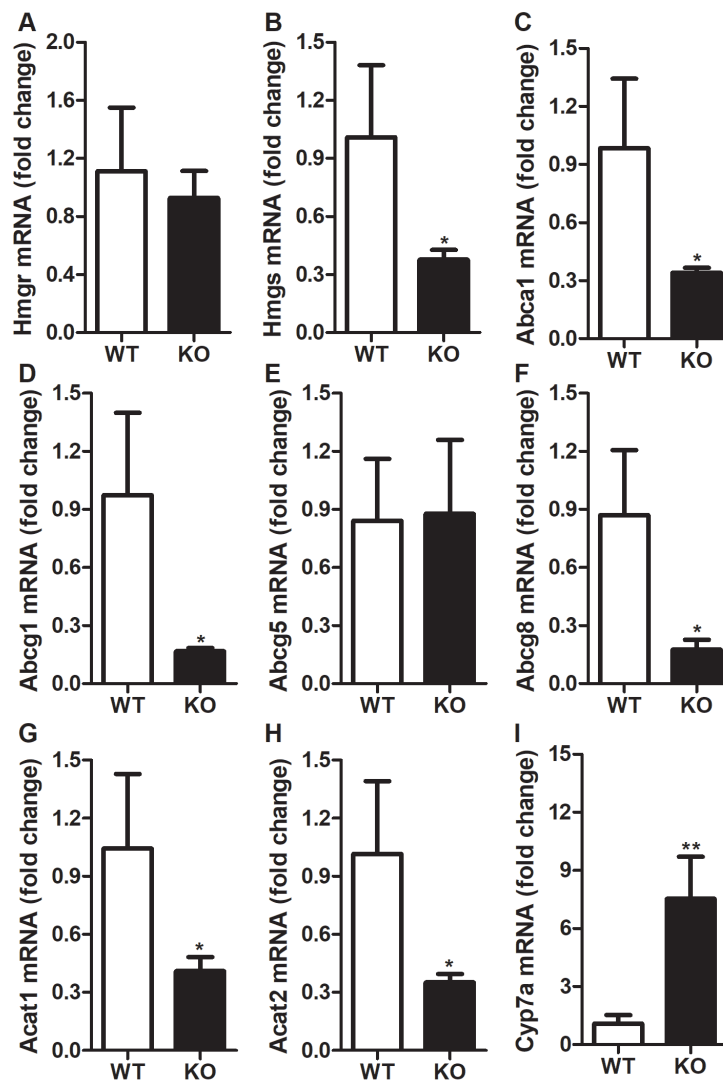


Figure 3. Changes in hepatic expression of cholesterol metabolism genes in *RORγ* knockout mice. Total mRNA from the livers of WT and KO mice were used to determine the expression of cholesterol metabolism genes. Relative changes in the mRNA expression were calculated based on the expression of 18 sRNA. Values were plotted as mean \pm SD. *P* values were calculated using two-tailed Student's *t* test. **P* < 0.05 and ***P* < 0.01. WT: wild type; KO: knockout; *RORγ*: retinoic acid-related orphan receptor γ ; *Hmgr*: hydroxymethylglutaryl-CoA reductase; *Hmgs*: hydroxymethylglutaryl-CoA synthase; *Abca1*: ATP-binding cassette transporter A1; *Abcg1*: ATP-binding cassette transporter G1; *Abcg5*: ATP-binding cassette transporter G5; *Abcg8*: ATP-binding cassette transporter G8; *Acat1*: acetyl-CoA acetyltransferase 1; *Acat2*: acetyl-CoA acetyltransferase 2; *Cyp7a*: cholesterol 7 α -hydroxylase

involved in the efflux of cholesterol in the liver. There was a decrease of 65%-83% in the expression of ATP-binding cassette transporter A1 (*Abca1*), G1 (*Abcg1*), and G8 (*Abcg8*) but not G5 (*Abcg5*) [Figure 3C-F]. Besides the decrease in cholesterol biosynthesis and efflux genes, we also observed a 61%-65% decrease in the expression of acetyl-CoA acetyltransferase 1 (*Acat1*) and acetyl-CoA acetyltransferase 2 (*Acat2*) genes that are involved in the esterification of cholesterol for storage and lipoprotein secretion [Figure 3G and H]. On the other hand, expression of cholesterol 7 α -hydroxylase (*Cyp7a*) involved in the biosynthesis of bile acids was increased by ~6 folds in the livers of *Rorγ* KO mice [Figure 3I]. Taken together, these results demonstrate that ablation of *Rorγ* activity results in decreased expression of genes involved in cholesterol biosynthesis, efflux, and storage but increased expression of genes involved in bile acid biosynthesis.

We then looked at the changes in the expression of fatty acid metabolism genes. Uptake of long-chain fatty acids by the liver is dependent on fatty acid transporters such as cluster of differentiation 36 (Cd36).

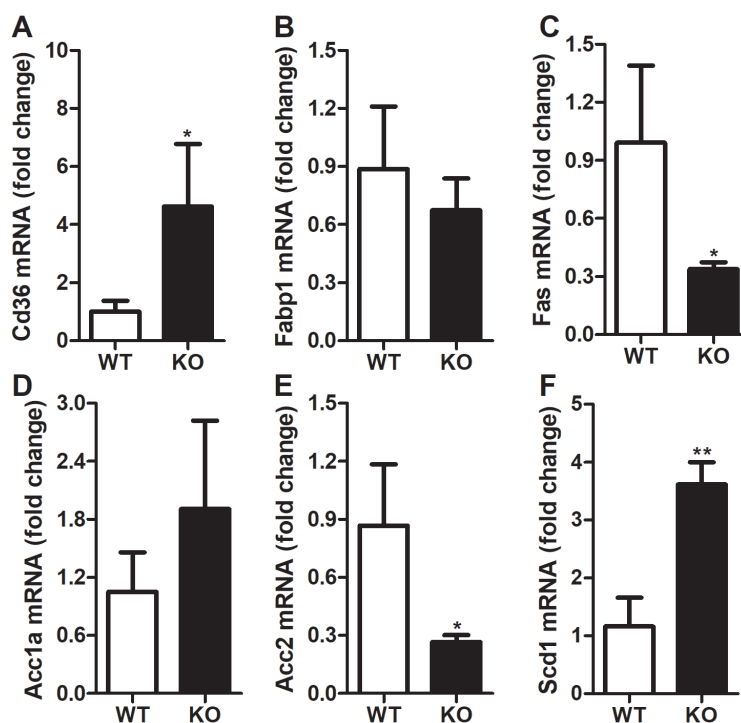


Figure 4. Changes in hepatic expression of fatty acid metabolism genes in *RORγ* knockout mice. Total mRNA from the livers of WT and KO mice were used to determine the expression of fatty acid metabolism genes. Relative changes in the mRNA expression were calculated based on the expression of 18 sRNA. Values were plotted as mean \pm SD. *P* values were calculated using two-tailed Student's *t* test. **P* < 0.05 and ***P* < 0.01. WT: wild type; KO: knockout; *RORγ*: retinoic acid-related orphan receptor γ ; *Cd36*: cluster of differentiation 36; *Fabp1*: fatty acid binding protein 1; *Fas*: fatty acid synthase; *Acc1a*: acetyl-CoA carboxylase 1a; *Acc2*: acetyl-CoA carboxylase 2; *Scd1*: stearoyl-coenzyme A desaturase-1

There was a ~4.5-fold increase in the expression of *Cd36* in the livers of KO mice compared to WT mice [Figure 4A]. However, we did not see any change in the expression of fatty acid binding protein 1 (*Fabp1*) in the livers of *Rorγ* KO mice [Figure 4B]. Next, we looked at the expression of fatty acid synthesis genes and found that deletion of *Rorγ* decreased the expression of fatty acid synthase (*Fas*) and acetyl-CoA carboxylase 2 (*Acc2*) by 66% [Figure 4C] and 70% [Figure 4E], respectively, but had no significant effect on the expression of acetyl-CoA carboxylase 1a (*Acc1a*) [Figure 4D]. Interestingly, expression of stearoyl-coenzyme A desaturase-1 (*Scd1*), which catalyzes the conversion of saturated fatty acids to monounsaturated fatty acids, was increased by ~3 folds in the livers of *Rorγ* KO mice [Figure 4F]. These results suggest that deletion of *Rorγ* increases the expression of genes involved in fatty acids uptake and desaturation but decreases the expression of genes involved in their biosynthesis.

We further investigated the effect of *Rorγ* gene deletion on the expression of genes involved in triglycerides metabolism. There was a significant reduction of 77% in the expression of diacylglycerol O-acyltransferase 2 (*Dgat2*) gene [Figure 5B] in contrast to a small but not significant increase in the expression of *Dgat1* gene [Figure 5A], and no change in the expression of acyl CoA: monoacylglycerol acyltransferase 2 (*Mgat2*) gene [Figure 5C] in the livers of *Rorγ* KO mice as compared to WT mice. *Dgat2* is mainly expressed and involved in the synthesis of bulk of triglycerides in mouse liver in contrast to *Dgat1* and *Mgat2*, which are highly expressed in the small intestine^[25,26]. Our data also show that deletion of *Rorγ* gene did not affect the expression of microsomal triglyceride transfer protein (*Mttp*) gene, a key protein involved in the assembly and secretion of lipid rich lipoproteins^[27,28] [Figure 5D]. Very low-density lipoprotein receptor (*Vldlr*) and low-density lipoprotein receptor (*Ldlr*) are important proteins that regulate the uptake of lipid rich lipoproteins from the circulation. There was no significant difference in the expression of *Vldlr* between the

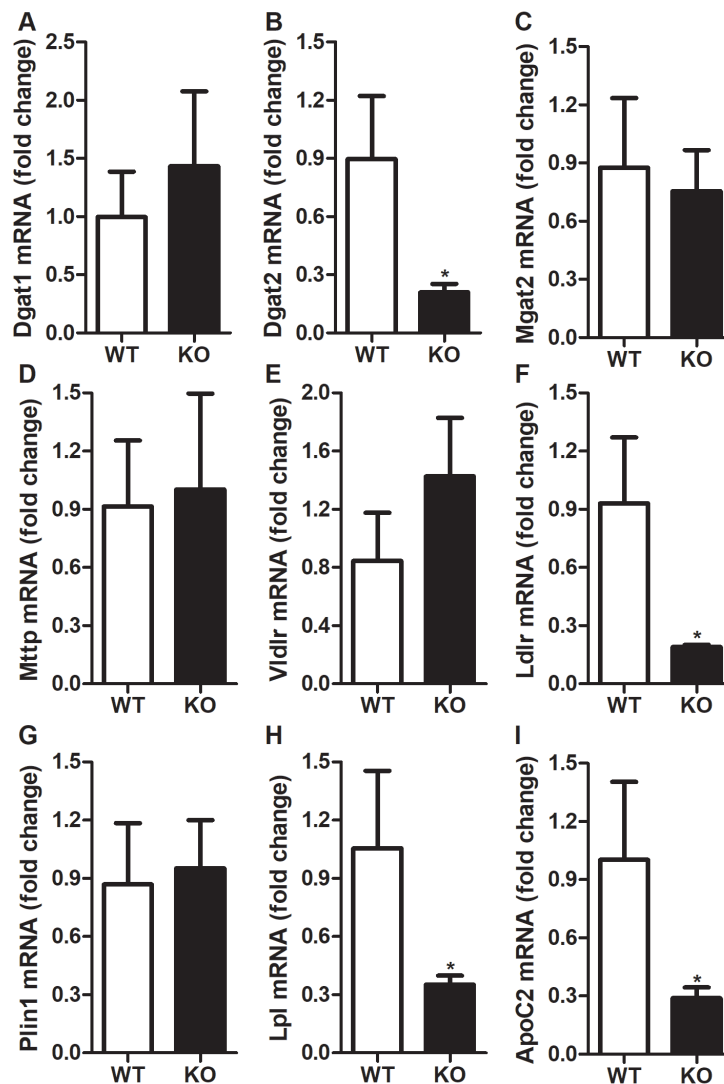


Figure 5. Changes in hepatic expression of triglycerides metabolism genes in *RORγ* knockout mice. Total mRNA from the livers of WT and KO mice were used to determine the expression of triglycerides metabolism genes. Relative changes in the mRNA expression were calculated based on the expression of 18 sRNA. Values were plotted as mean \pm SD. *P* values were calculated using two-tailed Student's *t* test. **P* < 0.05. WT: wild type; KO: knockout; *RORγ*: retinoic acid-related orphan receptor γ ; *Dgat1*: diacylglycerol O-acyltransferase 1; *Dgat2*: diacylglycerol O-acyltransferase 2; *Mgat2*: acyl CoA:monoacylglycerol acyltransferase 2; *Mttp*: microsomal triglyceride transfer protein; *Vldlr*: very low-density lipoprotein receptor; *Ldlr*: low-density lipoprotein receptor; *Plin1*: perilipin 1; *Lpl*: lipoprotein lipase; *ApoC2*: apolipoprotein C2

Rorγ KO and WT mice [Figure 5E] but we observed a 79% decrease in *Ldlr* gene expression in the livers of KO mice compared to WT mice [Figure 5F]. Furthermore, there was no difference in the expression of lipid droplet protein, perilipin 1 (*Plin1*), in the livers of *Rorγ* KO and WT mice [Figure 5G]. To understand the reasons for the increase in plasma triglycerides, we looked at the expression of lipoprotein lipase (*Lpl*) and apolipoprotein C2 (*ApoC2*), which are involved in the lipolysis of lipids in the circulation. Expression of both these genes was reduced by 67%-71% in *Rorγ* KO mice [Figure 5H, I]. These results suggest that expression of genes involved in triglycerides synthesis, uptake, and lipolysis is decreased in *Rorγ* KO mice leading to decreased triglycerides accumulation in the liver and increased triglycerides in the plasma.

To address whether alterations in cellular lipids is due to the changes in the expression of nuclear transcription factors, we looked at the expression of these genes in the liver. Our data indicate that the deletion of *Rorγ* reduced *Srebp2* gene expression by 80% in the livers [Figure 6A]. These data are consistent

with the lower hepatic cholesterol levels [Figure 2A] and decreased expression of downstream genes involved in cholesterol biosynthesis pathway [Figure 3]. Our results show that ablation of *Rory* activity in the liver leads to a 68% decrease in the expression of *Srebp1c* gene [Figure 6B], which is consistent with the reduced hepatic triglycerides [Figure 2B] and lower expression of the lipid synthesis genes [Figures 4 and 5]. Furthermore, we also noticed that expression of *Srebp1a* gene was significantly reduced by 62% in the livers of *Rory* KO mice compared to WT littermates [Figure 6C]. Next, we looked at the expression of *Ppara* and *Pparg* genes in the livers of *Rory* KO and WT mice. There was an around five-fold increase in the expression of *Ppara* gene, which is mainly involved in fatty acid oxidation [Figure 6D]. However, we observed a decrease of 74% in the expression of *Pparg* gene [Figure 6E]. These data suggest that expression of transcription factors that regulate lipid metabolism is modulated by ROR γ .

DISCUSSION

Our results identify a critical role of ROR γ in lipid metabolism. Here, we show that ablation of *Rory* gene decreases the body weight and reduces the accumulation of lipids in the liver. Interestingly, we did not see any significant change in the liver weight between the WT and *Rory* KO mice [Figure 1C]; however, we noticed a visible decrease in the content of abdominal fat mass in *Rory* KO mice (data not shown). This decrease in body weight and fat mass may be due to either reduced food intake or increased energy expenditure. It is known that activation of PPAR γ by agonists promote weight gain and fat accumulation mainly due to increased adipocyte differentiation and lipid storage^[29,30]. Our data indicate that *Rory* gene deletion results in lower expression of *Pparg* [Figure 6E], which may explain the decrease in body weight and fat mass. This finding is consistent with the repression of *Rory* activity by inverse agonist that lead to a reduction in fat mass and body weight in obese diabetic mice^[19].

A decrease in body weight and fat mass in *Rory* KO mice suggests that these mice do not store enough lipids in the adipocytes. Inability of adipocytes to store the excess fatty acids coming from the diet suggest that these fatty acids may be taken up by the liver for either oxidation or synthesis of lipids for storage in hepatic or extrahepatic tissues such as muscles. A significant increase in the hepatic expression of *Cd36* gene responsible for the uptake of long-chain fatty acids suggests that the livers from *Rory* KO mice may be taking up more fatty acids from the circulation [Figure 4A]. Takeda et al.^[17] also showed that expression of *Cd36* gene was upregulated at all the times during diurnal oscillations in the livers of *Rory* KO mice. Since both cholesterol and triglycerides levels were reduced in the livers of *Rory* KO mice [Figure 2A and B], we speculate that most of the fatty acids taken up by these mice are not utilized for lipid biosynthesis and storage. This was further supported by our observation that the expression of genes involved in lipid biosynthesis was lower in the livers from *Rory* KO mice [Figure 5B]. It is interesting to note that there was a reduction in the expression of *Acc2* in the livers of *Rory* KO mice. This enzyme is involved in the synthesis of malonyl-coA that plays an important role in regulating the oxidation of fatty acids in the mitochondria by inhibiting the carnitine/palmitoyl shuttle system^[31] in contrast to malonyl-coA that is generated by the ACC1 and utilized by Fas for the synthesis of fatty acids in the cytosol^[32]. Therefore, lower levels of *Acc2* gene expression suggests that the oxidation of fatty acids may be enhanced in the livers of *Rory* KO mice, which is consistent with the inhibition of *Rory* activity by inverse agonist that leads to enhanced fatty acid oxidation^[19]. Hence, besides reduced lipid biosynthesis, increased fatty acid oxidation may also contribute to reduced accumulation of triglycerides in the livers of *Rory* KO mice.

Contrary to lower hepatic lipids, we observed an increase in the levels of triglycerides in the plasma of *Rory* KO mice. On the other hand, Takeda et al.^[17] reported a decrease in the levels of plasma lipids in *Rory* KO mice. The discrepancy in results may be due to the feeding of high fat diet for six weeks to these mice, which may affect the metabolism of lipids in the plasma. It is likely that *Rory* gene ablation affects the uptake or secretion of these lipids from the liver. We did not observe any changes in the expression of *Mttp*, a gene responsible for the secretion of lipid rich lipoproteins, suggesting that secretion of lipids

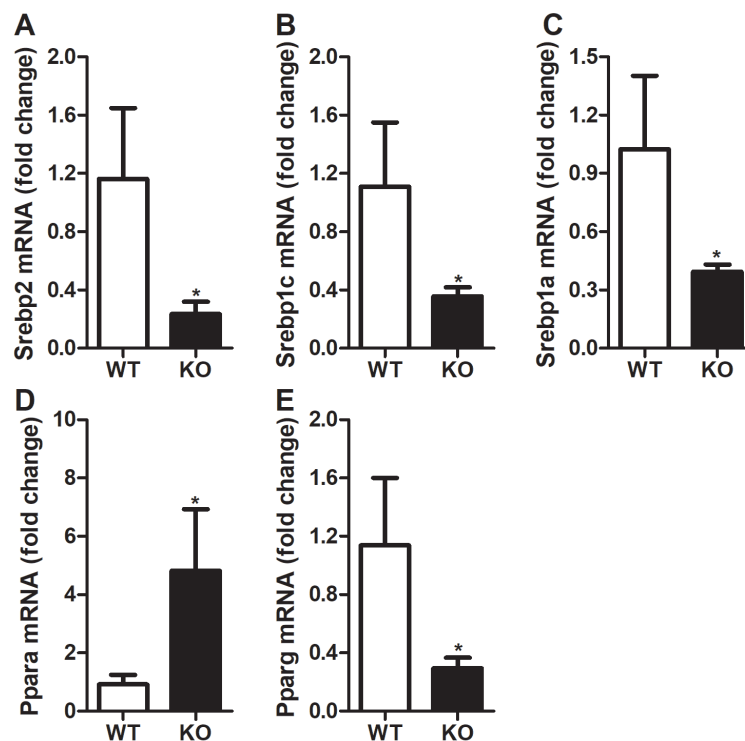


Figure 6. Changes in hepatic expression of lipid metabolism transcription factor genes in *RORγ* knockout mice. Total mRNA from the livers of WT and KO mice were used to determine the expression of transcription factor genes. Relative changes in the mRNA expression were calculated based on the expression of 18 sRNA. Values were plotted as mean \pm SD. *P* values were calculated using two-tailed Student's *t* test. **P* < 0.05. WT: wild type; KO: knockout; *RORγ*: retinoic acid-related orphan receptor γ ; *Srebp2*: sterol regulatory-element binding protein 2; *Srebp1c*: sterol regulatory-element binding protein 1c; *Srebp1a*: sterol regulatory-element binding protein 1a; *Ppara*: peroxisome proliferator-activated receptor α ; *Pparg*: peroxisome proliferator-activated receptor γ .

by the liver may not be affected by *Rory* gene deletion. However, our data indicate that *Rory* gene deletion decrease the levels of *Ldlr* gene expression implicating that uptake of lipid rich lipoproteins may be reduced in the KO mice leading to increase in the levels of plasma triglycerides. It is possible that the increase in plasma triglycerides may also be due to reduced activity of lipoprotein lipase. In fact, our data show that expression of *Lpl* and *ApoC2* genes were reduced in the livers of *Rory* KO mice. *ApoC2* acts as an activator of lipoprotein lipase^[33] and its reduction in the circulation may lead to reduced lipolysis, thereby causing increased plasma triglycerides.

RORγ functions as a transcriptional mediator that has been shown to regulate the rhythmic expression of certain metabolic genes downstream of clock machinery^[17,18]. Regulation of these genes is complex and may involve other nuclear receptors or transcriptional factors downstream of *Rory* to modulate lipid metabolism directly or indirectly^[17]. There is also a possibility that changes in these transcriptional factors may be secondary to the changes in the homeostasis of lipids in the cells^[34]. Expression of several lipid metabolism genes is regulated by various SREBPs that sense the changes in lipid levels in the cells. Stimulation of SREBP-2 regulates cholesterol homeostasis by activating the transcription of genes involved cholesterol metabolism^[35]. On the other hand, stimulation of SREBP-1c increases lipogenesis by increasing the expression of fatty acid metabolism genes^[36]. SREBP-1a has been shown to be an important regulator of both cholesterol and lipid synthesis genes^[37]. Our results suggest that deletion of *Rory* decreases the expression of *Srebp1a*, *Srebp1c*, and *Srebp2* in the liver [Figure 6]. Changes in the expression of these transcription factors decreased the overall biosynthesis of cholesterol, fatty acids, and triglycerides in the liver cells. Our data also indicate that deletion of *Rory* may increase the oxidation of fatty acids due to increased expression of *Ppara* in the liver.

We observed that expression of genes involved in biosynthesis, efflux, and esterification of cholesterol was down-regulated in *Rory* KO mice [Figure 3]. This may be the reason for less cholesterol levels in the livers of these mice [Figure 2A]. Interestingly, we noticed a significant increase in the expression of *Cyp7a* gene, which is responsible for the biosynthesis of bile acids in the liver. Although we do not know the exact mechanism of how hepatic bile acid signaling was impaired in *Rory* KO, we speculate that a larger portion of the hepatic cholesterol pool may be directed towards bile acid biosynthesis in the absence of ROR γ . Takeda *et al.*^[17] also observed an increase in the expression of *Cyp7a* gene in *Rory* KO mice fed a chow diet. However, when these mice were fed a high fat diet for eight weeks, the levels of their liver, serum, and fecal bile acid pools were reduced significantly. It is possible that the high fat diet is repressing the expression of some other genes involved in the bile acid biosynthesis pathway.

In this study, we demonstrated that ROR γ plays a significant role in the regulation of the expression of lipid metabolism genes that are involved in lipid biosynthesis, storage, and fatty acid oxidation. Our data also suggest that a cross-talk may occur between various transcription factors to maintain overall lipid homeostasis in the body. Previously, it has been shown that hepatic circadian clock plays a critical role in regulating major aspects of lipid metabolism through ROR γ activity^[17]. In a different experimental setting, we expanded the investigation to look at the changes in several lipid metabolism genes that were not studied previously. We also looked at the expression of different transcription factors to study the cross-talk between ROR γ and these transcription factors to regulate lipid homeostasis. Most of the findings in this current study are consistent and added substantive findings to the previously published reports^[17,19].

In summary, we show here that ablation of ROR γ activity reduce lipid biosynthesis but enhance fatty acid oxidation by modulating the expression of several lipid metabolism genes. However, this study has some limitations because global ROR γ -deficient mice are born healthy and fertile but die within the first four months after birth due to high incidence of T-cell lymphomas in the thymus^[15]. Therefore, further studies are warranted to show the effect of ROR γ deficiency on lipid metabolism in liver-specific KO mice to avoid any complications or confounding effects due to T-cell lymphomas. We will extend the studies of Takeda *et al.*^[17] to investigate role of ROR γ on lipid metabolism genes in chow-fed mice that were not studied previously. We will also look at the expression of different transcription factors to study the cross-talk between these factors and ROR γ to regulate lipid homeostasis. Furthermore, these mice may also be a good model to study the effect of liver-specific *Rory* gene deletion on diet-induced obesity. In conclusion, it may be beneficial to target ROR γ activity for the management of obesity and related lipid metabolic disorders.

DECLARATIONS

Authors' contributions

Conducted experiments: Jahangir Z, Veluru D, Otaibi AA, Mubarak SA, Subie BA, Alghanem AF
Contributed to the conception and design of the study: Iqbal J, Hawwari A, Bakillah A, Qarni AA
Performed data analysis and interpretation: Iqbal J, Bakillah A
Wrote the paper: Iqbal J, Bakillah A, Hawwari A

Availability of data and materials

All data and materials and mice are publicly available.

Financial support and sponsorship

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Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

All experiments were approved by Institutional Animal Care and Use Committee at King Faisal Specialist Hospital and Research Center.

Consent for publication

Not applicable.

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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.
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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;

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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);

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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.

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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.

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