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Editorial

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Acknowledgement to reviewers of *Vessel Plus* in 2019

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Original Article

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The intracoronary injection of recombinant human prourokinase in emergency interventional therapy for high thrombus load during ST-segment elevation myocardial infarction: a retrospective cohort study

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Abstract

Aim: The clinical effect of the injection of recombinant human prourokinase to infarction-related arteries during percutaneous coronary intervention (PCI) in ST-segment elevation myocardial infarction (STEMI) patients who have slow reflow/no reflow was investigated.

Methods: Data from STEMI patients who underwent emergency PCI at the Chest Pain Center of the First People's Hospital of Changde City from April 2017 to December 2018 were collected for analysis.

Results: Whether the ST segment had decreased by more than 50% at 90 min and whether the creatine kinase isoenzyme and cardiac troponin I had decreased by more than 50% were investigated. There were no significant differences in the indexes of color Doppler echocardiography (left ventricular ejection fraction, left ventricular end-diastolic diameter, ventricular aneurysm, and ventricular thrombus) within seven days after surgery and in the ventricular tachycardia/ventricular fibrillation between the two groups ($P > 0.05$). There was no significant difference in major adverse cardiovascular event between the two groups within one month after surgery ($P > 0.05$), but, there were significant differences in the Thrombolysis in Myocardial Infarction (TIMI) classification, corrected TIMI frame count, creatine kinase peak value, and the third-degree atrioventricular block ($P < 0.05$).



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Conclusion: The intracoronary injection of recombinant human prourokinase in patients with STEMI undergoing emergency PCI is safe and effective.

Keywords: ST-segment elevation myocardial infarction, primary percutaneous coronary intervention, recombinant human prourokinase, infarction-related artery

INTRODUCTION

The guidelines of the European Society of Cardiology^[1] recommend percutaneous coronary intervention (PCI) as the preferred strategy for reperfusion therapy in patients with acute ST-segment elevation myocardial infarction (STEMI). However, when the thrombus load in the coronary artery is heavy, PCI cannot effectively remove the thrombus and increases the risk of thrombus shedding, leading to the occurrence of “slow blood flow” and “no reflow” in the distal infarction-related artery (IRA). For STEMI patients with a high coronary artery thrombus load, the treatment strategy is particularly critical, which has puzzled interventional doctors. The purpose of this study was to analyze the safety and efficacy of the intraoperative injection of recombinant human prourokinase (rhPro-UK) in STEMI patients with a high thrombus load during emergency PCI with IRA.

METHODS

Patient enrollment

From April 2017 to December 2018, 312 patients from the First People's Hospital of Changde City met the diagnostic criteria of STEMI based on the 2014 guidelines for the diagnosis and treatment of acute STEMI. All patients underwent emergency PCI, and the IRA was successfully opened. These patients were divided into two groups. Patients with high thrombus loads, which had been confirmed by coronary angiography, were treated with rhPro-UK by guiding catheter infusion into the proximal end of the IRA, and were placed in the Pro-UK group ($n = 127$). There were 109 males and 18 females in this group. The age of the Pro-UK patients ranged from 20 to 86 years old, and the average age was 59.56 ± 12.58 years old. Among these patients, 7 patients underwent percutaneous transluminal coronary angioplasty (PTCA) and 120 underwent PCI. Six people were treated with an intra-aortic balloon pump and 23 patients were treated with temporary pacemakers. For the affected vessels, there were 2 cases in the left trunk, 56 cases in the anterior descending branch, 8 cases in the circumflex branch, and 61 cases in the right coronary artery.

Study protocol

Pro-UK group: according to a high thrombus load, as confirmed by coronary angiography, the patients were treated with the intracoronary infusion of urokinase to the IRA. rhPro-UK was injected into the coronary artery, into a minimum of one branch, a maximum of nine branches (5 mg/branch), and a median of two branches. Seven patients underwent PTCA and 120 underwent PCI.

Control group: in patients who did not have a high thrombus load during the operation, rhPro-UK was not injected into the coronary artery. Eight patients underwent PTCA and 177 underwent PCI.

Both groups were given aspirin 300 mg, clopidogrel 300/600 mg, or Ticagrelor 180 mg immediately before the beginning of PTCA, followed by 100 mg/qd, 75 mg/qd or 90 mg/bid, respectively.

Statistical methods

If measurement data had a normal distribution, the mean \pm standard deviation ($\bar{x} \pm s$) are shown, and the difference between groups was compared by a *t* test. If the data had a nonnormal distribution, they are represented by the median and quartile limits [*M* (*Q*₁, *Q*₃)], and the differences between these groups

were compared by the Kruskal-Wallis rank sum test. The counting data are expressed as the percentage constituent ratio or rate, and the comparisons between groups were performed by the Chi-square test or Fisher test. Univariate and multivariate logistic regression analyses were used to identify the factors related to major adverse cardiovascular events (MACEs). All analyses were performed with the statistical software packages R (<http://www.R-project.org>, The R Foundation) and EmpowerStats (<http://www.empowerstats.com>, X & Y Solutions, Inc, Boston, MA). *P* values less than 0.05 (two-sided) were considered statistically significant.

Observational indicators and methods

Angiographic analysis

Coronary angiography was performed on the target lesion in the same projection to optimize the Thrombolysis in Myocardial Infarction (TIMI) blood flow classification of the IRA. A visual evaluation was performed by two experienced interventional cardiologists. The TIMI blood flow classification criteria were as follows: Grade 0 (no perfusion), no forward blood flow at the distal end of the vascular occlusion; Grade 1 (infiltration but no perfusion), the contrast medium was partially stopped and there was a plug site, but it did not fill distal blood vessels; Grade 2 (partial perfusion), the contrast medium could completely fill the distal end of the coronary artery, but the contrast medium filling and clearance speed was slow; and Grade 3 (complete perfusion), the contrast medium could completely and rapidly fill the distal blood vessels and was removed quickly.

Biochemical assays and ST-segment resolution

The concentrations of creatine kinase (CK) and creatine kinase isoenzyme (CK-MB) were measured by immunoassays (BECKMAN COULTER Au5800 instrument). Cardiac troponin I (cTnI) was measured by a fluorescence immunoassay (Mini VIDAS instrument). The peak levels of CK-MB and cTnI were used as indexes to judge the size of the infarction. An electrocardiogram (ECG) was recorded 90 min after the intervention, and a decrease rate of the ST segment of more than 50% was regarded as the cutoff of myocardial reperfusion after PCI.

Cardiac functions

One week after emergency PCI, left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDd), ventricular aneurysm, and ventricular thrombus were measured by Vivid 7 Dimension color Doppler echocardiography.

Bleeding events and severe arrhythmias

Severe postoperative bleeding events (gastrointestinal bleeding and cerebral hemorrhage) and severe postoperative arrhythmias [ventricular tachycardia/ventricular fibrillation (VT/VF) and third-degree atrioventricular block] were recorded.

Follow-up

The main MACEs within one month after operation were recorded.

RESULTS

Baseline demographic, clinical, and angiographic characteristics

There was no significant difference ($P > 0.05$) in sex, age, risk factors, Killip classification, IRA, time from onset to balloon dilatation, time from admission to balloon dilatation, number of dilated balloons used during surgery, number of stents implanted, intraoperative aortic balloon counterpulsation operations, and temporary pacemakers between the Pro-UK group and the control group [Table 1].

Table 1. Demographic, clinical, and angiographic characteristics of the subjects

Variables	Pro-UK group (n = 127)	Control group (n = 185)	P-value
Age (years)	59.56 ± 12.58	61.34 ± 11.73	0.203
Male [n (%)]	109 (85.83%)	146 (78.92%)	0.121
Hypertension [n (%)]	70 (55.12%)	113 (61.08%)	0.293
Hyperlipemia [n (%)]	34 (26.77%)	69 (37.30%)	0.052
Diabetes [n (%)]	25 (19.69%)	39 (21.08%)	0.764
Smoking [n (%)]	51 (40.16%)	65 (35.14%)	0.367
Killip [n (%)]			0.259
I	101 (79.53%)	149 (80.54%)	
II	10 (7.87%)	23 (12.43%)	
III	12 (9.45%)	10 (5.41%)	
IV	4 (3.15%)	3 (1.62%)	
pre-PCI TIMI classification [n (%)]			< 0.001
0	107 (84.25%)	121 (65.41%)	
1	14 (11.02%)	35 (18.92%)	
2	6 (4.72%)	29 (15.68%)	
Time from symptom onset to balloon dilatation (h)	6.00 (4.50-9.00)	7.00 (5.00-10.00)	0.193
Time from admission to balloon dilatation (min)	65.00 (48.50-85.00)	64.00 (47.00-93.00)	0.202
Infarction-related artery [n (%)]			0.083
LM	2 (1.57%)	4 (2.16%)	
LAD	56 (44.09%)	93 (50.27%)	
LCX	8 (6.30%)	23 (12.43%)	
RCA	61 (48.03%)	65 (35.14%)	
Number of implanted stents [n (%)]			0.864
PTCA	7 (5.51%)	8 (4.32%)	
1 stent	91 (71.65%)	134 (72.43%)	
2 stent	26 (20.47%)	36 (19.46%)	
3 stents	3 (2.36%)	7 (3.78%)	
Suction catheter [n (%)]	15 (11.81%)	7 (3.78%)	0.007
IABP used [n (%)]	6 (4.72%)	11 (5.95%)	0.640
Temporary pacemaker [n (%)]	23 (18.11%)	27 (14.59%)	0.406

LM: left main stem; LCX: left circumflex artery; RCA: right coronary artery; IABP: intra-aortic balloon pump; PTCA: percutaneous transluminal coronary angioplasty; PCI: percutaneous coronary intervention; TIMI: thrombolysis in myocardial infarction; Pro-UK: prourokinase; LAD: left anterior descending branch

Evaluation of clinical effectiveness

The following indexes of the two groups were analyzed: the TIMI classification of the IRA immediately after surgery and the corrected TIMI frame count (CTFC) were used to assess the blood perfusion of culprit vessels; whether the ST segment had decreased by more than 50% at 90 min; and whether the peak of CK-MB and cTnI had decreased by more than 50%. There were no significant differences in the indexes of color Doppler echocardiography (LVEF, LVEDd, ventricular aneurysm, and ventricular thrombus) within seven days after surgery ($P > 0.05$), but there was a significant difference in the TIMI classification, CTFC, and CK peak value ($P < 0.05$) between the two groups [Table 2].

Clinical safety assessment

Eight patients had gastrointestinal bleeding. Two patients experienced cerebral hemorrhage in the Pro-UK group, while 12 patients experienced gastrointestinal bleeding in the control group. There was no significant difference in the severity of complications ($P > 0.05$) or the length of hospital stay ($P > 0.05$) [Table 3] between the two groups. There was no significant difference in the VT/VF between the two groups ($P > 0.05$), but there was a significant difference in the third-degree atrioventricular block (III° AVB) ($P < 0.05$) between the two groups [Table 3].

Follow-up results

Cardiovascular Major Adverse Events (MACEs) is a clinically viable technique for accurate, rapid, and safe evaluation of myocardial perfusion. In this study, MACE events included stent thrombosis, angina

Table 2. Markers of infarct size, myocardial reperfusion, and cardiac functions

Variables	Pro-UK group (n = 127)	Control group (n = 185)	P-value
Postoperative TIMI classification [n (%)]			0.041
0	1 (0.79%)	2 (1.08%)	
1	0 (0.00%)	1 (0.54%)	
2	14 (11.02%)	6 (3.24%)	
3	112 (88.19%)	176 (95.14%)	
CTFC (mm)	23.26 ± 2.68	24.16 ± 3.65	0.047
CK peak value (U/L)	2625.50 (1548.75-4582.50)	2356.50 (1203.25-3797.00)	0.032
CK-MB peak value (U/L)	229.55 (110.60-371.33)	204.30 (96.30-329.65)	0.217
cTnI peak value (U/L)	9.53 (1.70-25.00)	7.55 (2.29-20.33)	0.511
ST-segment resolution (> 50%) [n (%)]	87 (68.50%)	139 (75.14%)	0.198
LVEDd (mm)	48.08 ± 5.73	47.43 ± 5.26	0.307
LVEF (%)	58.28 ± 9.30	58.88 ± 9.03	0.569
Ventricular aneurysm [n (%)]	14 (11.02%)	10 (5.41%)	0.067
Ventricular thrombus [n (%)]	1 (0.79%)	2 (1.12%)	0.769

CK: creatine kinase; CK-MB: creatine kinase isoenzyme-MB; LVEF: left ventricular ejection fraction; LVEDd: left ventricular end-diastolic diameter; Pro-UK: prourokinase; TIMI: thrombolysis in myocardial infarction; CTFC: corrected TIMI frame count; cTnI: cardiac troponin I

Table 3. Comparison of the indexes of severe bleeding events, severe arrhythmia events, and hospitalization days

Variables	Pro-UK group (n = 127)	Control group (n = 185)	P-value
Bleeding events			0.655
No	117 (92.13%)	172 (92.97%)	
Gastrointestinal bleeding	8 (6.30%)	12 (6.49%)	
Cerebral hemorrhage	2 (1.57%)	1 (0.54%)	
VT/VF [n (%)]	14 (11.02%)	10 (5.41%)	0.067
III° AVB [n (%)]	12 (9.45%)	7 (3.78%)	0.040
Hospitalization days (days)	10.00 (8.00-12.00)	9.00 (7.00-11.00)	0.444

VT/VF: ventricular tachycardia/ventricular fibrillation; III° AVB: third-degree atrioventricular block; Pro-UK: prourokinase

Table 4. Comparison of MACEs between the two groups within one month of surgery

Variables	Pro-UK group (n = 127)	Control group (n = 185)	P-value
Stent thrombosis [n (%)]	0 (0.00%)	1 (0.54%)	0.407
Angina pectoris [n (%)]	6 (4.72%)	4 (2.16%)	0.207
Stent rethrombosis [n (%)]	2 (1.57%)	1 (0.54%)	0.358
Congestive heart failure [n (%)]	32 (25.20%)	38 (20.54%)	0.333
Cardiac death [n (%)]	7 (5.51%)	9 (4.86%)	0.799

MACEs: major adverse cardiovascular events; Pro-UK: prourokinase

pectoris, stent rethrombosis, congestive heart failure, and cardiac death. There was no significant difference in MACEs between the two groups within one month after surgery ($P > 0.05$; see [Table 4](#)).

Logistic regression analysis

The MACEs that occurred within one month after surgery in the two groups were used as study Y and whether to use rhPro-UK as X, and three logistic regression models were constructed according to the different confounding factors. The results of the rough model (without adjusting for any confounding factors), the microadjustment model (adjusting for only age and sex), and the overall adjustment model (adjusting for sex, age, hypertension, hyperlipidemia, diabetes, smoking, number of stents implanted, number of balloons used, Killip, post-PCI TIMI classification, time from symptom onset to balloon dilatation, and time from admission to balloon dilatation) show that the use of rhPro-UK was not significantly associated with the risk of MACEs ($P > 0.05$; see [Table 5](#)).

Table 5. Logistic regression analysis

Exposure	Non-Adjusted	Adjusted I	Adjusted II
Pro-UK group	1.0	1.0	1.0
Control group	0.77 (0.47, 1.27) 0.3036	0.73 (0.44, 1.22) 0.2292	0.79 (0.44, 1.44) 0.4475

Adjusted model I: adjusted for sex and age; adjusted model II: adjusted for sex, age, hypertension, hyperlipidemia, diabetes, smoking, number of stents implanted, number of balloons used, Killip, post-PCI TIMI classification, time from symptom onset to balloon dilatation, and time from admission to balloon dilatation. Pro-UK: prourokinase; PCI: percutaneous coronary intervention; TIMI: thrombolysis in myocardial infarction

DISCUSSION

Emergency PCI can open IRAs in a timely and effective manner and can improve clinical prognosis. However, studies have shown that^[2] approximately 30%-50% of STEMI patients do not achieve effective myocardial reperfusion after PCI. Kaul *et al.*^[3] pointed out that myocardial perfusion disorder is an independent predictor of poor prognosis after emergency PCI. Considering that the burden of coronary artery thrombosis in patients with STEMI is often heavy, the compression of a balloon or stent during emergency PCI may result in the fragmentation and shedding of thrombi. The microthrombi that comprise thrombus segments detach from unstable plaques and aggregate with platelets, resulting in slow blood flow/no reflow^[4,5].

As part of a new generation of thrombolytic drugs, rhPro-UK can selectively activate fibrin-binding fibronase, converting fibronase into fibrinase, which can dissolve thrombi and reduce the thrombus burden. rhPro-UK can prevent the occurrence of slow blood flow/no reflow during emergency PCI and does not affect the coagulation function of the whole body. In recent years, the treatment of intracoronary thrombolysis in PCI has represented a new era for thrombolysis/PCI combination therapy. Sezer *et al.*^[6] found that the intracoronary injection of streptokinase immediately after direct PCI can effectively increase myocardial perfusion. A domestic study by Zhao *et al.*^[7] showed that rhPro-UK had a high effectiveness for opening IRAs, and the incidence of bleeding complications was low, thus rhPro-UK is a safe treatment method. In another study, Zhao *et al.*^[8] showed that mechanical thrombectomy combined with rhPro-UK thrombolysis presented a more favorable efficiency in the treatment of moderate to severe acute cerebral infarction than single treatment, and the occurrence of adverse effects was similar between the combination and single treatments. In two other recent basic studies in animal models^[9,10], the results of the studies proved that rhPro-UK promoted thrombolysis and recanalization (patency rate) and did not increase the risk of bleeding. The abovementioned studies confirmed that the use of rhPro-UK can effectively promote thrombolysis without increasing the risk of bleeding. Our study showed that there was no difference in postoperative gastrointestinal bleeding and bleeding events between the two groups, suggesting that it is safe to administer rhPro-UK to IRAs through guiding catheters. The results are consistent with the abovementioned research results. In a recent study^[11], in patients with STEMI complicated with a long delay in PCI, emergency PCI combined with rhPro-UK thrombolysis showed significantly better myocardial perfusion of IRAs than direct PCI. Geng *et al.*^[11] showed that the intracoronary injection of rhPro-UK through a balloon catheter could effectively improve myocardial perfusion in patients with STEMI. Our results show that the postoperative blood flow TIMI classification in the Pro-UK group was significantly higher than that before operation. Although there was a significant difference in the postoperative TIMI classification between the two groups, as a result of the degree of the thrombus load in the Pro-UK group, there was no difference in the ST return rate, color sonography, and MACEs between the two groups, suggesting that the administration of rhPro-UK to IRAs through guiding catheters is effective, which is consistent with the abovementioned results.

Previous studies have shown that^[12] rhPro-UK can be injected into the coronary artery in a variety of ways, such as guiding catheters, microcatheters, suction catheters, and drug balloons. Microcatheters or suction catheters require the guide wire to be withdrawn before drug injection and for the guide wire to

be reinserted during treatment, which increases the surgery time and the risk of complications; however, the new perfusion balloon is expensive, which limits its clinical application. It has been reported^[13] that this drug could be injected into the target vessel through the side hole of the balloon by using a modified balloon catheter, and the high local concentration of this drug was used to treat slow blood flow/no reflow. However, the operation including the abovementioned methods is relatively complex, requiring a more extensive operation and prolonging the operation time, thus this procedure is not suitable for use in emergency PCI at most chest pain centers. In this study, rhPro-UK was directly injected into the coronary artery through a guiding catheter. This operation process is simple and does not affect the operation flow. We aimed to adopt a simple and low-cost method. At present, in the process of promoting the construction of a nationwide “chest pain center” in China, this method has been shown to be suitable for most chest pain centers. Although the administration of a guiding catheter leads to the outflow of part of the rhPro-UK out of the guiding catheter, most of the drug can reach the middle and distal end of the affected vessel in the direction of the blood flow, achieving effective results. In this study, there was no difference in the time from the onset to balloon dilatation and from admission to balloon dilatation between the Pro-UK group and the control group, suggesting that the administration of drugs through the guiding catheter did not affect the operation time and procedure. Additionally, with the improvement of myocardial reperfusion, there was no significant difference in the results of cardiac color Doppler ultrasounds between the two groups, including LVEF, LVEDd, ventricular aneurysm, ventricular thrombus, and segmental wall motion abnormality. Reinstadler *et al.*^[14] found that the ST-segment return rate was an important index of myocardial perfusion after emergency PCI. There was no difference in the ECG ST-segment return rate between the two groups. Previous studies have shown that^[15] a higher Killip grade is associated with higher mortality. In our study, MACEs were positively correlated with the Killip grade, as higher Killip grades were associated with a higher incidence of MACEs, which is consistent with the results described above. MACEs are a new clinically feasible measure of the accurate, rapid, and safe evaluation of myocardial perfusion^[16]. During the follow-up period of one month, there was no difference in MACEs between the two groups. In this study, the ST return rate, based on ECG and color Doppler echocardiography, and MACEs were studied. There was no difference between the Pro-UK group and the control group in regard to these parameters. Multivariate regression analysis showed that there was no significant association between the use of rhPro-UK and the risk of MACEs. In conclusion, in conditions of high thrombus load during emergency PCI, rhPro-UK administration to the IRA through a guiding catheter was not associated with an increase in the incidence of bleeding complications and MACEs, suggesting that this method is safe and effective.

This study, in addition to having an insufficient sample size and a short follow-up time, was not a randomized controlled prospective study. Additionally, there was no standard dosage for all patients in the Pro-UK group. Considering that different dosages and routes of administration may affect the efficacy and safety of the drug, further study is needed to explore the optimal dose and route of administration of rhPro-UK in IRAs.

DECLARATIONS

Authors' contributions

Conceived the study, collected the data, participated in the design and drafted the manuscript: Qin ZA, Lu XL

Participated in the design, collected the data, performed statistical analysis and helped to draft the manuscript: Qin ZA, Zhou Q, Lu XL

Helped to perform statistical analysis and to revise it critically for important intellectual content: Qin ZA, Luo L, Zhan ZX, Guo N, Ge LQ

All authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Considering the retrospective nature of this study and the widely accepted use of rhPro-UK in China, specific consent form was waived.

Consent for publication

Not applicable.

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Systematic Review

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Transcatheter aortic valve implantation in the elderly: an umbrella review

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Abstract

Aim: The management of aortic stenosis has seen momentous changes thanks to the introduction of transcatheter aortic valve implantation (TAVI, i.e., transcatheter aortic valve replacement). Indications to TAVI have expanded progressively to intermediate- and low-risk patients, but trends in life expectancy have led to an increase of elderly but fit individuals with aortic stenosis eligible for TAVI.

Methods: We reviewed the current evidence base on TAVI in the elderly by conducting an umbrella review (i.e., overview of systematic reviews), based on a formal bibliographic search for systematic reviews on TAVI in elderly patients (≥ 65 years). Key, study, patient, procedural, and outcome data were extracted, and validity formally appraised with the Oxman-Guyatt index.

Results: From 71 citations, eight reviews were included (totaling 39 studies and 8579 patients): five systematic reviews, and three meta-analyses. Topics of interest were cognitive function before and after TAVI, predictive role of muscle mass and frailty on post-TAVI outcomes, comparative safety and effectiveness of TAVI, and role of rehabilitation to improve patient outlook after TAVI. Thirty-three additional studies were retrieved by means of snowballing, emphasizing the



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role of multidimensional assessment of elderly patients scheduled for TAVI, in order to maximize its appropriateness, effectiveness, and safety.

Conclusion: It is crucial to consider frailty scores, as well as nutrition and functional status, in addition to established surgical risk scores, in elderly patients considered for TAVI to improve risk prediction, reinforcing the favorable impact of this therapy to improve cognitive function.

Keywords: Aortic stenosis, elderly, transcatheter aortic valve implantation, transcatheter aortic valve replacement

INTRODUCTION

Surgical aortic valve replacement (SAVR) has been for several decades the default management strategy for severe aortic stenosis in fit patients^[1]. However, an ever increasing elderly population, often fraught with substantial comorbidities, has challenged in many cases the risk-benefit profile of surgery^[2]. Accordingly, less invasive strategies were developed, including balloon aortic valvuloplasty^[3].

Building upon developments in materials and procedures, and inspired by breakthrough results of stenting for coronary and endovascular procedures, transcatheter aortic valve implantation (TAVI), also called transcatheter aortic valve replacement (TAVR), was introduced by Alain Cribier almost two decades ago^[4-6]. The successes of TAVI have been dramatic indeed, as poignantly summarized by the recent US Food and Drug Administration approval of new-generation devices for TAVI even in patients at low surgical risk^[7]. However, TAVI continues to be considered and used mostly for elderly patients, given the uncertainty on long-term and very long-term device durability^[8].

Despite the evidently favorable risk-benefit profile of TAVI in general, and in the elderly in particular, several areas of investigation and debate persist, typically focusing on indication, timing, procedural aspects, device choice, ancillary medical management, and post-procedural results^[6]. We aimed at exploiting the synthesizing power of umbrella review studies to reconcile conflicting sources of evidence on TAVI in the elderly, in order to inform current practice and guide future research^[9].

METHODS

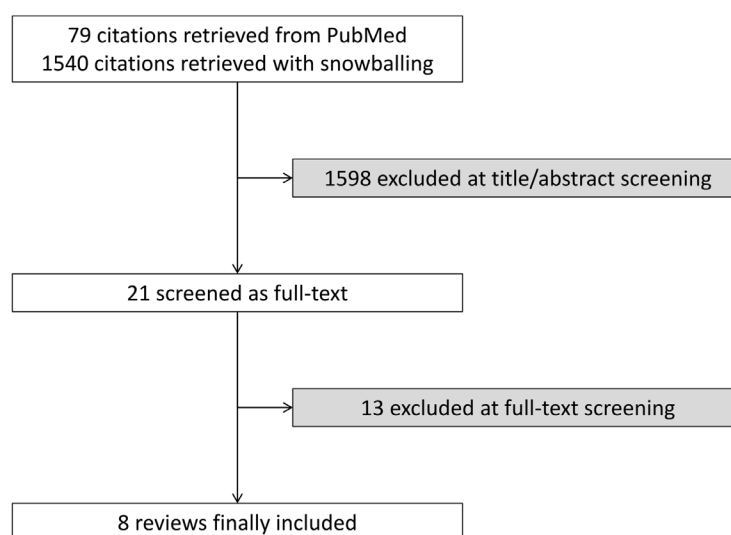
This scoping umbrella review was conducted in keeping with best practice recommendations, and reported accordingly^[9]. Specifically, we used a multifaceted approach for evidence accrual, avoiding a specific or restrictive definition of elderly. First, PubMed was searched using the following string: "{elderly OR octogenarian* OR octagenarian* OR nonagenarian* OR old OR aged OR [age AND (advanced OR old)]} AND transcatheter AND aortic AND valve AND (implantation OR replacement) AND systematic[sb]" up to 31 October 2019. Accordingly, any review detailing on, at least in part, nonagenarians, octogenarians, aged patients, or subjects with advanced or old age could be included, provided it also focused on TAVI. Thereafter, we used backward and forward snowballing to identify additional citations. Afterwards, potentially relevant citations were screened at the title/abstract level. Potentially relevant hits were then retrieved as full-texts.

We included systematic reviews (i.e., overviews of published clinical studies including two or more primary original reports) detailing TAVI in elderly patients (defined as people aged ≥ 65), irrespective of their focus on diagnosis, prognosis, device choice, procedural aspects, or outcomes, to avoid being overly restrictive. Several domains were abstracted, including review features, study aspects, and other details on included patients, procedures, and outcomes. Review validity was appraised with the Oxman and Guyatt Overview Quality Assessment Questionnaire^[10]. All reviewing activities were performed by two independent reviewers, with divergences solved after consensus.

Table 1. Included systematic reviews on TAVI in the elderly

Ref.	PubMed ID	Focus	Studies	Patients	Highlights
Anand <i>et al.</i> ^[11]	28927173	Frailty	10	4592	Frailty is a significant predictor of adverse events after TAVI
Fink <i>et al.</i> ^[12]	26192563	Cognitive function	1	64	Cognitive function may be impaired after TAVI
Furukawa <i>et al.</i> ^[13]	25916404	Frailty	6	1023	Frailty is a significant predictor of adverse events after TAVI
Lai <i>et al.</i> ^[14]	25785192	Cognitive function	6	349	Cognitive function remains stable or improves after TAVI
Mohammadi <i>et al.</i> ^[15]	26728319	Effectiveness of TAVI	NA	NA	TAVI impacts favorably on morbidity and mortality in elderly patients with AS
Ribeiro <i>et al.</i> ^[16]	28071146	Rehabilitation	5	292	Cardiac rehabilitation improves functional capacity and QoL after TAVI
Sepehri <i>et al.</i> ^[17]	25199821	Frailty	3	378	Frailty is a significant predictor of adverse events after TAVI
Soud <i>et al.</i> ^[18]	30915667	Muscle mass	8	1881	Skeletal muscle area appraised with CT is a significant predictor of adverse events after TAVI

CT: computed tomography; NA: not applicable; QoL: quality of life; TAVI: transcatheter aortic valve implantation; AS: aortic stenosis

**Figure 1.** Review profile, detailing study search and selection

RESULTS

From an initial set of 1619 citations, a subset of 21 were retrieved as full-texts, finally yielding eight reviews, totaling 39 primary studies and 8579 patients [Table 1 and Figure 1]^[11-18]. Five were systematic reviews only, and the remaining three also provided meta-analysis results^[11,16,18]. The topics of interest were cognitive function before and after TAVI^[12,14], predictive role of muscle mass and frailty on post-TAVI outcomes^[11,13,17,18], comparative safety and effectiveness of TAVI^[15], and role of rehabilitation to improve patient outlook after TAVI^[16]. Review quality ranged from high validity and low risk of bias for five reviews^[11,12,16-18], to low validity and high risk of bias in three reviews^[13-15] [Table 2], with lack of adequate reporting being the most common limitation.

In particular, Anand *et al.*^[11] performed a systematic review and meta-analysis appraising the prognostic impact of frailty in patients undergoing TAVI, including a total of 10 studies and 4592 patients. They concluded that frailty proved to be a significant predictor of adverse events after TAVI. Similar findings were reported by prior reviews such as the systematic review conducted by Furukawa *et al.*^[13], encompassing six primary studies and 1023 patients, and the one authored by Sepehri and colleagues, totaling three studies and 378 subjects^[17]. A relatively similar focus was chosen by Soud *et al.*^[18], who pooled eight studies including 1881 to appraise the predictive usefulness of appraising skeletal muscle mass by means of computed tomography (CT). CT-derived muscle area showed a significant prognostic

Table 2. Validity appraisal of included systematic reviews on TAVI in the elderly

Ref.	Search methods stated	Search for evidence comprehensive	Inclusion criteria reported	Selection bias avoided	Validity criteria reported	Validity criteria appropriate	Combination methods appropriate	Combination methods coherent	Conclusions supported by results	Overall rating
Anand <i>et al.</i> ^[11]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High validity
Fink <i>et al.</i> ^[12]	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	NA	High validity
Furukawa <i>et al.</i> ^[13]	No	NA	No	NA	No	NA	NA	NA	NA	Low validity
Lai <i>et al.</i> ^[14]	Yes	Yes	No	NA	No	NA	NA	NA	NA	Low validity
Mohammadi <i>et al.</i> ^[15]	No	Yes	No	Unclear	No	NA	NA	NA	NA	Low validity
Ribeiro <i>et al.</i> ^[16]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High validity
Sepehri <i>et al.</i> ^[17]	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	NA	High validity
Soud <i>et al.</i> ^[18]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High validity

NA: not applicable; TAVI: transcatheter aortic valve implantation

role in patients undergoing TAVI. Cognitive function before and after TAVI was the topic of interest of Fink *et al.*^[12] (who included only one study and 64 patients undergoing TAVI) and Lai *et al.*^[14] (who overreviewed six studies and 349 subjects). Notably, they found that cognitive decline is common among elderly patients with severe aortic stenosis awaiting TAVI, whereas this procedure is not associated with significant worsening in cognitive function (which can actually improve after TAVI). Finally, Mohammadi *et al.*^[15] reviewed several studies on TAVI in elderly patients to gauge the effectiveness and safety of this procedure, whereas Ribeiro *et al.*^[16] reported the results of a meta-analysis spanning five studies and 292 patients on the use of cardiac rehabilitation following TAVI, concluding that this protected discharge approach may improve functional capacity and quality of life.

Given the limited scope of the systematic reviews retrieved with a focused umbrella review approach, we also explored by means of snowballing other bibliographic sources, highlighting several important primary studies on the indications, subtleties, and outlook of TAVI in elderly patients [Table 3]. In total, 33 reports were shortlisted, including as many as 30,657 subjects. Specifically, three were reviews, one was a qualitative study, 26 were observational studies, and three were diagnostic studies. The focus of reports varied, ranging from frailty appraisal tools to the electrical risk score, N-terminal pro-brain natriuretic peptide levels, oxygen consumption formulas, diagnosis of bicuspidy, nutritional status, grip strength, cognitive function, balloon aortic valvuloplasty, postoperative delirium, and prehabilitation/rehabilitation. Overall, these reports highlight the importance of multidimensionally considering every elderly patient with aortic stenosis considered for TAVI, in order to maximize appropriateness, maximize effectiveness, and minimize risk.

DISCUSSION

The present umbrella review, aiming at summarizing the evidence base for TAVI in elderly patients, has the following implications: (1) While TAVI has been offered mostly to patients at high surgical risk with advanced age, the evidence thus far accrued on TAVI in elderly subjects is relatively limited; (2) Frailty and cognitive function were the most commonly covered topics, with reports highlighting the importance of considering frailty scores on top of standard surgical risk scores to improve the accuracy of risk prediction and ensuring decision-making, and promising data in favor of TAVI as a means to improve cognitive function; and (3) Other studies, elicited from a scoping appraisal of the scholarly literature on TAVI in elderly patients, highlighted the importance

Table 3. Selected studies on TAVI in the elderly

Ref.	PubMed ID	Design	Patients	Highlights
Amofah (2016)	26635329	Observational study	143	Sleep is disturbed in patients with AS, and may improve after SAVR and TAVI, albeit less with the latter
Bogdan (2016)	27159658	Observational study	150	Albumin predicts long-term outcomes after TAVI
Bordon (2015)	26378413	Observational study	224	Repeat BAV is a reasonable management strategy in elderly patients who are not candidate for TAVI
Boreskie (2019)	31543187	Review	NA	Prehabilitation may be beneficial in patients with AS awaiting TAVI
Cavalcante (2017)	29212513	Observational study	113	Cardiac amyloidosis is common in elderly patients with AS and predicts adverse outcomes after TAVI
Ciua (2017)	28585899	Observational study	62	Cognitive impairment is common in elderly patients with AS but is not significantly impacted by TAVI
de Thézy (2017)	29187325	Diagnostic study	49	The G8 tool is a useful screening scale for frailty in elderly patients with AS
Drudi (2018)	29344620	Observational study	1035	Depression is common in patients awaiting TAVI, and it predicts adverse outcomes, especially if persisting after the procedure
Eide (2015)	25644851	Observational study	143	Postoperative delirium is less common with TAVI than with SAVR in octogenarians
Elgendy (2019)	30569661	Observational study	6680	TAVI is associated with similar mortality but less morbidity than SAVR in nonagenarians with AS
Gertz (2014)	23704061	Diagnostic study	51	Oxygen consumption is best estimated with a modified mathematical formula
Goldfarb (2018)	29976568	Observational study	1158	Preprocedural nutritional status is associated with mortality in older adults undergoing TAVI or SAVR
Green (2012)	22331630	Observational study	102	Gait speed is associated with ADL in elderly patients with AS
Instenes (2018)	28396186	Qualitative study	10	Postoperative delirium is common after TAVI and SAVR, and its memories persist long-term
Kagase (2018)	29301641	Observational study	927	Grip strength predicts long-term outcomes after TAVI
Kamga (2013)	24579438	Observational study	30	The SHERPA frailty score in an independent predictor of post-TAVI outcome
Kim (2019)	31587128	Diagnostic study	2583	CT can reliably recognize bicuspid AS in the elderly
Lindman (2016)	27113148	Review	NA	Multimorbidity is common in elderly patients with AS
Mentias (2019)	31668118	Observational study	13,544	Outcomes of TAVI in nonagenarians have improved by considering the impact of early complications on long-term events
Murata (2019)	31462606	Observational study	58	Ventilatory efficacy predicts long-term outcomes after TAVI
Nagura (2019)	30599060	Observational study	1004	Post-procedural valvuloarterial impedance is not associated with increased mortality after TAVI
Oh (2019)	31514956	Observational study	261	Long-term outcomes are similar with TAVI and SAVR in low-risk elderly patients
Okoh (2019)	30618060	Observational study	1160	Discharge disposition impacts on post-TAVI outcomes
Olsen (2017)	27036955	Observational study	65	TAVI improves self-reported global health and generic physical health and quality of life
Orvin (2014)	24481462	Observational study	36	TAVI impacts favorably on functional performance and cognitive function
Piccirillo (2018)	30237702	Observational study	40	The 12-lead-ECG-derived electrical risk score predicts long-term outcomes after TAVI
Rabinovitz (2016)	26936468	Observational study	302	Admission Norton scale score independently predicts post-TAVI mortality
Raposeiras-Roubin (2016)	27573609	Observational study	54	NT-proBNP predicts long-term outcomes after TAVI
Russo (2014)	23757283	Observational study	78	Early cardiac rehabilitation enhances independence, mobility, and functional capacity after TAVI
Schoenenberger (2013)	23008508	Observational study	106	Post-TAVI functional decline is predicted by frailty scores
Urena (2015)	25466975	Observational study	435	Arrhythmias are common in elderly patients with AS and predict post-TAVI adverse events
Zalenska-Kocicka (2019)	30718946	Review	NA	AKI is common in patients undergoing TAVI and can be predicted by means of multidimensional risk appraisal
Zenedkun (2015)	25982494	Observational study	54	A low-dose contrast protocol for CT is associated with reduced contrast volume in patients with AS

ADL: activities of daily living; AKI: acute kidney injury; AS: aortic stenosis; BAV: balloon aortic valvuloplasty; CT: computed tomography; NT-proBNP: N-terminal pro-brain natriuretic peptide; SAVR: surgical aortic valve replacement; SHERPA: Score Hospitalier d'Evaluation du Risque de Perte d'Autonomie; TAVI: transcatheter aortic valve implantation; NA: not applicable

of multidimensional appraisal and management of these subjects, while confirming the promising role of TAVI in comparison to medical therapy, balloon aortic valvuloplasty, and SAVR in elderly patients.

The evolution of TAVI has been momentous, and, since the first pioneering cases, TAVI is challenging the role of SAVR even in low-risk patients^[6]. These successes depend on major refinements in diagnostic tools (e.g., CT angiography for precise sizing), patient preparation, device improvements, ancillary management approaches, and post-procedural management^[19-26]. These refinements and the fact that TAVI was initially validated in trials enrolling mostly high-risk patients with advanced age would suggest that all major issues concerning TAVI in the elderly have been solved^[6]. This is of course false, and substantial research is still ongoing on several related topics. For instance, the aspects of cost utility and futility remain actively debated, as well as all issues pertinent to patient preparation, device selection, predilation vs. postdilation, embolic protection, and post-procedural antithrombotic therapy^[6,11-15,27-30].

The present umbrella review, albeit limited in comparison to other umbrella reviews authored by our research group given the limited scope of the available evidence base, highlights the importance of frailty assessment to predict short-term complications and long-term results of TAVI in the elderly, the emerging role of cognitive assessment before TAVI and prevention of cognitive decline due to TAVI complications, and the usefulness of cardiac rehabilitation in all old patients with severe aortic stenosis undergoing TAVI. Further evidence highlights the importance of assessing in a multidimensional fashion the presence of comorbidities, nutritional status, grip strength, gait speed, and overall functional status, while confirming the favorable clinical performance at short- and mid-term follow-up of TAVI, without discounting the niche role of balloon aortic valvuloplasty in patients at prohibitive risk, and the pivotal function of SAVR in fit patients.

Limitations of this umbrella review are of course those typical of overviews of reviews, including the risk of ecological fallacy^[9]. In addition, while studies on TAVI usually enroll mostly patients with advanced age, only a limited set of systematic reviews explicitly aimed at the topic of TAVI in the elderly. Accordingly, further reviews are eagerly awaited to more poignantly summarize the evidence base for this important topic in structural heart disease. Focusing on the definition of elderly, our definition of elderly as aged ≥ 65 years is quite arbitrary, especially in the context of TAVI, which is often performed in much older subjects^[31,32]. However, this remains a common pragmatic definition for many patients, non-specialists, and decision-makers^[32]. In addition, by default, umbrella reviews have limited room to select primary studies from included reviews. Similarly, having an unrestrictive approach at TAVI indication (e.g., stenosis, regurgitation, and valve-in-valve) risks mixing “apples with oranges” and providing overly heterogeneous results. Most importantly, the TAVI landscape continues to change, shifting from prohibitive and high-risk patients, to subjects at intermediate or low risk. Another crucial evolution has centered on devices, which evolved from the crude Cribier-Edwards device to current-generation, low-profile and fully repositionable/retrievable ones^[21]. However, as stated above, by definition, umbrella reviews cannot limit inclusion to a given group of primary studies. Accordingly, we can only let readers subset the included systematic reviews/studies according to the specific features they are most interested in, when wishing to apply to specific patient subgroups the findings of our umbrella review.

In conclusion, the scholarly literature on TAVI continues to accrue, reaffirming the favorable risk-benefit balance of this breakthrough technology in patients with severe aortic stenosis, including selected low-risk subjects. Our umbrella review, including eight systematic reviews, 39 primary studies, and 8579 patients, highlights the importance of considering frailty scores, as well as nutrition and functional status, in addition to established surgical risk scores in elderly patients considered for TAVI to improve risk prediction, reinforcing the favorable impact of this therapy to improve cognitive function.

DECLARATIONS

Authors' contributions

Designed the review, performed all reviewing activities and drafted the manuscript: Antonazzo B, Biondi-Zoccai G

Participated in review design, supervised all reviewing activities, and provided critical contributions to the manuscript: Marullo AGM, Frati G, Ronzoni S, Chiariello GA, Versaci F, Giordano A

All authors eventually approved it in its final version.

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All authors declared that there are no conflicts of interest.

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Not applicable.

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Review

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Therapeutic properties of the new phytochemical osmotin for preventing atherosclerosis

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Abstract

Osmotin, a natural plant protein found in tomato, potato, pepper, and tobacco, is a homolog of human adiponectin. It exerts multiple biological activities through adiponectin receptors in a variety of mammalian cells. The therapeutic properties of osmotin have recently been shown in the pathogenesis of atherosclerosis by *in vitro* and *in vivo* experiments. Osmotin suppresses the adhesion of monocytes to endothelial cells by downregulating inflammatory cytokines and adhesion molecules in both cells. It suppresses oxidized low-density lipoprotein-induced foam cell formation by downregulating cluster of differentiation 36 and acyl-coenzyme A:cholesterol acyltransferase 1 as well as upregulating ATP-binding cassette transporter A1 in monocyte-derived macrophages. In vascular smooth muscle cells, osmotin suppresses the migration, proliferation, and production of collagen 1, fibronectin, and matrix metalloproteinase 2 by decreasing the phosphorylation of extracellular signal-regulated protein kinase 1/2 and nuclear factor- κ B as well as increasing AMP-activated protein kinase (AMPK) expression. Treatment with osmotin suppresses abdominal fat accumulation in C57BL/6 mice and prevents the development of aortic atherosclerotic lesions, improving vascular inflammation and plaque instability in apolipoprotein E-deficient (*ApoE*^{-/-}) mice. Osmotin protects against obesity- and diabetes-induced nonalcoholic fatty liver disease in leptin-deficient obese (*ob/ob*) and leptin receptor-deficient diabetic (*db/db*) mice. These effects are attributed to the stimulatory actions of osmotin on peroxisome proliferator-activated receptor- α and AMPK. Moreover, osmotin lowers serum levels of total cholesterol and triglyceride in non-diabetic and diabetic rats. These findings suggest that osmotin contributes to improving the extracellular risk factors for atherosclerosis and vascular intracellular and molecular responses. Therefore, the novel phytochemical osmotin may serve as a novel therapeutic target for atherosclerosis and related diseases.



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Keywords: Osmotin, atherosclerosis, inflammation, diabetes, obesity, phytochemical, vascular cells, animal models

INTRODUCTION

Atherosclerosis is clinically manifested as coronary artery disease, stroke, and peripheral arterial disease, which are major causes of mortality and morbidity worldwide^[1]. Atherosclerosis is a chronic inflammatory vascular disease induced by endothelial injury followed by atheromatous plaque formation, leading to thickening and loss of elasticity in the arterial wall of medium- and large-sized arteries, including the coronary, cerebral, and carotid arteries and the aorta^[2]. Risk factors of atherosclerosis include dyslipidemia, diabetes, obesity, and hypertension. The pathophysiology of atheromatous plaque development involves endothelial cell (EC) inflammation and proliferation, monocyte adhesion to ECs and infiltration into the under-endothelial space, inflammatory cytokine release from monocyte-derived macrophages, oxidized low-density lipoprotein (Ox-LDL)-induced macrophage foam cell formation, vascular smooth muscle cell (VSMC) migration and proliferation, and extracellular matrix (ECM) production by VSMCs^[3]. The progression and rupture of atheromatous plaques in coronary arteries lead to myocardial ischemia and infarction. Timely reperfusion is critical for the salvage of ischemic myocardium. After coronary angioplasty, restoration of blood flow to the damaged myocardium triggers further ischemic myocardial damage. This paradoxical phenomenon is known as ischemia-reperfusion injury, which is a serious clinical problem in the treatment of patients with coronary artery disease. Several studies have investigated preventive effects of plants on atherogenesis^[4-9]. Osmotin, a plant-derived natural protein, is receiving the most attention as a therapeutic target for atherosclerosis and myocardial ischemia-reperfusion injury^[10,11].

Osmotin was first isolated from tobacco (*Nicotiana tabacum*) cells by Singh *et al.*^[12]. Later, osmotin was also found in other plant species, including tomato, potato, oat, pepper, and grape^[13]. Osmotin is a plant peptide hormone, also called phytochemical, which belongs to the fifth class of the group of pathogenesis-related proteins^[14]. The osmotin gene *AP24* is known to be activated by environmental and phytohormone signals^[15]. Osmotin plays an important role in the protection against osmotic and oxidative stresses caused by higher salt concentration, cold, and drought^[16], and has anti-fungal activity in plants^[17]. In plants and yeasts, osmotin exhibits anti-fungal, anti-oxidant, and anti-apoptotic effects through PHO36, which is an adiponectin receptor (AdipoR) homolog^[13,18].

Osmotin has recently attracted attention as a homolog of mammalian adiponectin^[13,18], which is the most famous adipocytokine (adipokine) with anti-inflammatory, anti-diabetic, and anti-atherogenic properties^[19-21]. Osmotin is composed of 246 amino acids including a 21-amino acid signal peptide, which do not share remarkable similarity to human adiponectin (AdipoQ, 244 amino acids) in the amino acid sequence alignment^[13]. However, the domain I of osmotin is demonstrated to overlap with the β -barrel domain of AdipoQ by three-dimensional structure analyses^[13,18]; thereby, osmotin binds to AdipoR1 and then leads to intracellular signaling^[18,22,23]. Therefore, osmotin is regarded as a natural agonist for human AdipoR1^[22]. Osmotin exhibits anti-inflammatory and anti-apoptotic effects in mammalian cells through AdipoR1^[24,25]. Takahashi *et al.*^[10] recently showed that osmotin exerts atheroprotective effects through human AdipoR1 in human vascular cells. In intracellular signaling pathways, both nuclear factor- κ B (NF- κ B) and extracellular signal-regulated protein kinase 1/2 (ERK1/2) suppression and AMP-activated protein kinase (AMPK) activation play a pivotal role in the preventive effects of osmotin on inflammation and atherosclerosis^[10].

Osmotin as well as adiponectin binds to AdipoR1, leading to the activation of adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1) followed by phosphoinositide 3-kinase (PI3K)/Akt, AMPK, peroxisome proliferator-activated receptor- α (PPAR- α), and protein-tyrosine

phosphatase 1B (PTP1B)^[10]. The PTP1B activation suppresses RAF1 and ERK1/2 phosphorylation^[10]. The activation of PI3K/Akt and/or AMPK suppresses apoptosis of vascular cells (ECs, VSMCs, and macrophages) and cardiomyocytes. NF- κ B and ERK1/2 suppression and AMPK activation inhibit inflammation, differentiation, migration, and proliferation of the vascular cells; thus, these signalings are therapeutic targets for atherosclerosis^[26-28]. In adipocytes, hepatocytes, and skeletal muscle cells, AMPK and PPAR- α activation contributes to improving insulin resistance and lipid metabolism, respectively. AdipoR1 is expressed at high levels in monocytes, macrophages, ECs, VSMCs, cardiomyocytes, adipocytes, hepatocytes, and skeletal muscle cells^[29]. The AdipoR agonist AdipoRon, an adiponectin-like synthetic small molecule, is in the spotlight as an oral anti-atherosclerotic drug^[30].

Several lines of evidence have recently shown the protective effects of osmotin against atherosclerosis, hyperlipidemia, diabetes, and obesity^[10,31-33]. This review article summarizes the therapeutic properties of osmotin for preventing atherosclerosis and myocardial ischemia-reperfusion injury as well as inflammation and neurodegeneration. In addition, the article describes the comparisons of the atheroprotective effects between osmotin and adiponectin or AdipoRon. However, there are no data comparing the exact difference in the potency of these effects among the three agents.

ATHEROPROTECTIVE EFFECT

In vitro anti-atherosclerotic effects of osmotin

Osmotin has been shown to exert the multiple effects in different cell types including ECs, monocytes/macrophages, VSMCs, adipocytes, and cardiomyocytes^[10,11,33]. The anti-atherosclerotic effects of osmotin have been investigated using cultured human vascular cells *in vitro*. In this section, the evidence regarding the anti-atherosclerotic effects of osmotin is introduced, as summarized in Figure 1.

Preventive effects of osmotin on vascular inflammation

Osmotin suppresses lipopolysaccharide (LPS)-induced upregulation of monocyte chemoattractant protein 1 (MCP1), tumor necrosis factor- α (TNF- α), intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), and E-selectin in human umbilical vein endothelial cells (HUVECs)^[10]. It suppresses the TNF- α -induced adhesion of human THP1 monocytes to HUVECs^[10]. Osmotin shifts toward an anti-inflammatory phenotype (M2) rather than pro-inflammatory phenotype (M1), associated with ERK1/2 and NF- κ B downregulation and PPAR- γ upregulation in human THP1 monocyte-derived macrophages^[10]. It also suppresses the LPS-induced secretion of interleukin 6 (IL6), pentraxin 3 (PTX3), and TNF- α from human THP1 monocyte-derived macrophages^[10]. These findings indicate that osmotin suppresses vascular inflammation and endothelial dysfunction. In addition, it suppresses the proliferation of human EA.hy926 ECs^[10], thus preventing intimal medial thickness. Osmotin mimics the suppressive effects of adiponectin on the expression of ICAM1, VCAM1, and E-selectin in ECs, monocyte-EC adhesion, and EC proliferation as well as inflammatory phenotype (M1) and TNF- α expression in macrophages^[34-38].

Preventive effects of osmotin on macrophage foam cell formation

Osmotin suppresses Ox-LDL-induced accumulation of cholesterol ester (foam cell formation) by downregulating cluster of differentiation 36 (CD36) and acyl-coenzyme A:cholesterol acyltransferase 1 (ACAT1) as well as upregulating ATP-binding cassette transporter A1 (ABCA1) in human THP1 monocyte-derived macrophages^[10]. These effects are consistent with the effects of adiponectin in suppressing Ox-LDL-induced foam cell formation and ACAT1 expression and enhancing ABCA1 expression in human monocyte-derived macrophages^[39-41].

Preventive effects of osmotin on the migration and proliferation of VSMCs

Osmotin suppresses angiotensin II-induced migration of human aortic smooth muscle cells (HASMCs)^[10]. It also suppresses the proliferation of HASMCs by decreasing the phosphorylation of RAF1, ERK1/2,

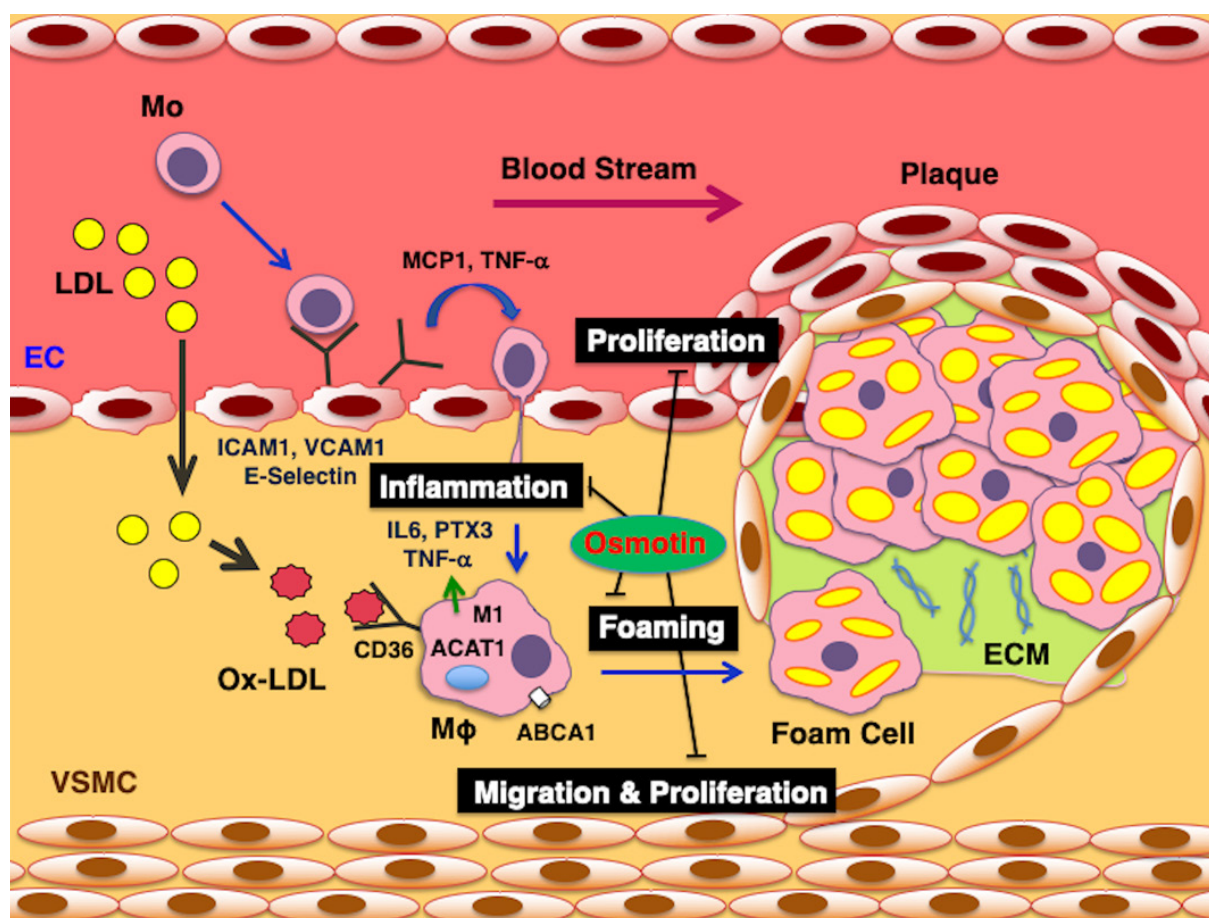


Figure 1. Cellular and molecular mechanisms mediating the preventive effects of osmotin on atherosclerosis. Osmotin suppresses the proliferation of vascular ECs. It suppresses vascular inflammation, characterized as monocyte-EC adhesion, by downregulating MCP1, TNF- α , ICAM1, VCAM1, and E-selectin in ECs, and suppresses inflammatory phenotype (M1) and secretion of IL6, PTX3, and TNF- α in monocyte (Mo)-derived macrophages (M ϕ). Osmotin suppresses Ox-LDL-induced foam cell formation by downregulating CD36 and ACAT1 as well as upregulating ABCA1 in Mo-derived macrophages. In VSMCs, osmotin suppresses the migration, proliferation, and production of ECM proteins, such as collagen 1, fibronectin, and matrix metalloproteinase 2. Therefore, osmotin prevents the development and instability of atheromatous plaques. EC: endothelial cells; MCP1: monocyte chemoattractant protein 1; TNF- α : tumor necrosis factor- α ; ICAM1: intercellular adhesion molecule 1; VCAM1: vascular cell adhesion molecule 1; IL6: interleukin 6; PTX3: pentraxin 3; Ox-LDL: oxidized low-density lipoprotein; CD36: cluster of differentiation 36; ACAT1: acyl-coenzyme A:cholesterol acyltransferase 1; ABCA1: ATP-binding cassette transporter A1; VSMCs: vascular smooth muscle cells; ECM: extracellular matrix

and NF- κ B as well as increasing AMPK expression^[10]. Osmotin exerts the same suppressive effects of adiponectin and AdipoRon on the migration and proliferation of VSMCs^[42-44].

Modulatory effects of osmotin on ECM production in VSMCs

The intercellular networking that occurs among ECs, VSMCs, and macrophages leads to a fibroproliferative response, in which ECM plays an important role in atheromatous plaques. The ECM is composed of a mixture of vastly different macromolecules including collagens, fibronectin, elastin, and matrix metalloproteinases (MMPs), which are produced by VSMCs in the arterial wall. Osmotin suppresses the production of collagen 1, fibronectin, and MMP2, and increases that of elastin and MMP9 in the HASMCs^[10]. The former contributes to preventing the development of atheromatous plaques, while the latter contributes to vascular elasticity and remodeling. However, osmotin has no effect on collagen 3 production in HASMCs^[10]. Osmotin mimics the suppressive effects of adiponectin on collagen 1 expression in VSMCs^[45].

Suppressive effects of osmotin on the glucose uptake and differentiation of adipocytes

Visceral adipose tissue promotes insulin resistance and metabolic disorders, resulting in the development of atherosclerosis. In particular, perivascular adipose tissue has been recently shown to have a close linkage with atherosclerosis^[46]. Perivascular adipocytes residing in the vascular adventitia are recognized as endocrine cells^[46]. Cross-talk between perivascular adipocytes and vascular cells in blood vessel wall modulates the formation of atherosclerosis by releasing adipocytokines^[47]. Similar to adiponectin^[48], osmotin suppresses the differentiation and proliferation of adipocytes by regulating the expression of p21, p27, and cyclin-dependent kinase 2, as well as improves glucose uptake in 3T3-L1 adipocytes^[33].

Protective effects of osmotin against ischemia-reperfusion injury in cardiomyocytes

A recent study has shown the protective effects of osmotin against myocardial ischemia-reperfusion injury^[11]. Osmotin protects rat cardiac myoblast H9c2 cells against ischemia-reperfusion injury through AdipoR1 by increasing phosphorylation of PI3K/Akt and decreasing that of NF- κ B^[11]. Osmotin exhibits the same cardioprotective effects of adiponectin and AdipoRon against ischemia-reperfusion injury^[49,50]. These findings indicate that osmotin as well as adiponectin and AdipoRon could prevent myocardial damage following coronary events and ischemia-reperfusion injury.

***In vivo* anti-atherosclerotic effects of osmotin**

Several studies have shown that adiponectin and AdipoRon suppress the development of atherosclerotic lesions in apolipoprotein E-deficient (*Apoe*^{-/-}) mice, an atherogenic mouse model, on a normal or high-fat diet^[51-53]. Recently, the anti-atherosclerotic effects of osmotin have also been investigated using a variety of animal models *in vivo*. Treatment with osmotin suppresses abdominal fat accumulation in C57BL/6 mice fed with a high-fat diet^[33]. In *Apoe*^{-/-} mice on a high-cholesterol diet, chronic infusion of osmotin prevents the development of aortic atherosclerotic lesions accompanied by an improved vascular inflammation and plaque instability^[10]. In this model, osmotin also improves fasting plasma glucose level, free fatty acid level, and insulin resistance^[10]. Similarly, injection of osmotin lowers serum levels of total cholesterol and triglyceride and prevents atherosclerosis in Wistar rats fed with a high-cholesterol diet^[31]. Osmotin injection decreases serum levels of glucose, insulin, total cholesterol, and triglyceride in streptozotocin-induced diabetic rats fed with a high-fat diet^[32]. In addition, it protects against obesity and diabetes-induced nonalcoholic fatty liver disease in leptin-deficient obese (*ob/ob*) mice and leptin receptor-deficient diabetic (*db/db*) mice^[54]. These effects are attributed to the stimulatory actions of osmotin on AMPK and PPAR- α pathways. Therefore, osmotin is also expected to be useful in the preventive health care in diabetics in future^[55].

ANTI-INFLAMMATORY EFFECT

Atherosclerosis is an inflammatory vascular disease. The Canakinumab Anti-inflammatory Thrombosis Outcome Study trial provided direct evidence that inflammation accelerates cardiovascular disease in humans, by showing that a therapeutic antibody targeting IL1 β decreased recurrent cardiovascular events^[56]. This section introduces the beneficial effects of osmotin on inflammatory diseases other than atherosclerosis. Osmotin suppresses LPS-induced neuroinflammation through AdipoR1 followed by toll-like receptor 4 and NF- κ B pathways in BV2 microglial cells^[57]. In addition, infusion of osmotin using osmotic pumps attenuates dextran sodium sulfate-induced colitis in mice^[58]. These effects are consistent with anti-inflammatory effects of adiponectin and AdipoRon^[58-60]. The results from *in vitro* and *in vivo* experiments indicate that osmotin as well as adiponectin and AdipoRon could prevent inflammatory diseases.

NEUROPROTECTIVE EFFECT

Alzheimer's disease is the most common form of dementia. The pathogenesis of Alzheimer's disease involves characteristics such as amyloid- β deposition, tau phosphorylation, and apoptotic neurode-

generation^[61]. The risk factors of Alzheimer's disease are known to be inflammation, lipid metabolism (*Apoe*), and atherosclerosis^[61-63]. A clinical prospective study has shown that atherosclerosis in carotid arteries leads to the progression of Alzheimer's disease^[64]. Osmotin protects against amyloid- β deposition, tau phosphorylation, and apoptotic neurodegeneration through AdipoR1 followed by the AMPK/sirtuin 1/sterol regulatory element-binding protein 2 pathway in neuronal cells^[25,65-67]. It also enhances neurite outgrowth and synaptic complexity via AdipoR1/nogo-66 receptor NgR1 signaling^[68]. These findings indicate that osmotin is a potential candidate for the treatment of neurological disorders such as Alzheimer's disease. A preclinical trial study recently reported the usefulness of intravenous administration of osmotin-loaded magnetic nanoparticles in combination with electromagnetic guidance in the treatment of Alzheimer's disease^[69]. These effects of osmotin are compatible with neuroprotective effects of adiponectin and AdipoRon in Alzheimer's disease^[70-73].

ANTI-TUMORIGENESIS EFFECT

Cancer and atherosclerosis have been classified as non-communicable diseases by differing in target cells^[74]. However, both diseases have principally identical mechanisms such as cell proliferation induced by inflammation and reactive oxygen species^[75]. Adiponectin induces anti-angiogenesis and anti-tumor activity through AdipoR1 via caspase-mediated EC apoptosis^[13]. Actually, adiponectin and AdipoRon suppress the proliferation of a variety of human cancer cells^[76-78]. Similarly, osmotin induces cell cycle arrest in the G0/G1 phase^[33] and suppresses cell proliferation^[10]. These findings suggest the possibility that osmotin may suppress tumor growth. However, further studies are needed to clarify this hypothesis.

THERAPEUTIC STRATEGY

Osmotin is a natural plant protein that is ubiquitous in fruits and vegetables^[13]. Osmotin is also known to be a phytochemical that is resistant to heat treatment (cooking)^[13]. Furthermore, the osmotin protein is relatively stable and may maintain activity even through contact with the human digestive system^[13]. Therefore, this phytochemical could be administered by ingestion of fruits and vegetables containing a great amount of osmotin or by oral administration of osmotin and/or its analogs that consist of the amino acid residue including an active center^[10]. However, the best drug delivery system (DDS) for osmotin administration is considered to be enveloped in nanocapsules in order to avoid digestive degradation^[10,69]. Moreover, continuing preclinical and clinical investigations with osmotin, in particular combined with a nanocapsule system as carrier vehicles into atherosclerotic lesions, are essential for future studies^[10]. It is very important for DDS establishment to design the size of the nanocapsule so that it can go through a relatively wide gap between damaged vascular ECs, but not a normal gap between intact ECs in humans. The magnetic nanocapsule-based DDS with external electromagnetic guidance may be more useful to make osmotin cross endothelial gap junction^[69]. Future studies are needed to clarify whether oral administration of osmotin-loaded nanocapsules can treat vascular lesions in patients with atherosclerosis. Similarly, osmotin-like proteins derived from many vegetables and fruits are also regarded as adiponectin peptidomimetics and gather attention as novel therapeutic drug candidates for atherosclerosis and related diseases^[79].

CONCLUSION

These findings indicate that the novel phytochemical osmotin might be an effective therapeutic agent for atherosclerosis, inflammation, neurodegeneration, and their related diseases and that AdipoR1 might be a crucial therapeutic target for these diseases. Osmotin may open up a new therapeutic window in the treatment of atherosclerosis in cases with hypoadiponectinemia and/or resistance against adiponectin and AdipoRon^[10,30,80]. Osmotin also provides benefits to maintain vascular health and prevent vascular disease in healthy individuals.

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Authors' contributions

The author contributed solely to the article.

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The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

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Not applicable.

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Commentary

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Why and how to achieve total arterial revascularisation in coronary surgery

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Abstract

Single internal mammary artery and supplementary saphenous vein grafts (SVG) continues to be used in approximately 95% of coronary surgery as of 2019. The late failure of SVG is very well documented yet remains the predominant conduit used - why? The left internal mammary artery almost never fails, and late angiography of patent radial artery grafts also appear entirely normal. Logic would suggest that avoiding the conduit known to progressively fail would lead to improved late outcome. Our studies have demonstrated such findings in large single centre and national registry datasets. We describe strategies to achievement of total arterial coronary revascularisation.

Keywords: Total arterial revascularisation, radial artery, total arterial revascularisation, radial artery, Y graft

INTRODUCTION

This paper is intended to be a pragmatic guide including diverse considerations related to the attainment of total arterial revascularisation. It is not a comprehensive review and limited discussion of some topics is intended to provide a summary of a topic rather than an exhaustive review of the topic.



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THE WHY: ACHIEVING TOTAL ARTERIAL REVASCULARISATION

As of 2019, almost all cardiac surgeons perform 90%-95% of coronary bypass surgery (CABG) with the use of saphenous vein graft (SVG) worldwide^[1,2]. Of the patients receiving total arterial revascularisation (TAR), a proportion will have been performed without specific strategic intent to achieve TAR [e.g., a single or double CABG using the internal mammary artery (IMA)]. Consequently, a deliberate strategy to achieve TAR in every patient is uncommon.

Consider the following proposition: if SVG develops atherosclerosis and fails - and that failure, then leads to the failure of the treatment of CABG - why is it that we count the number of arterial grafts?

The argument in favour of counting veins rather than arteries

“The LIMA-LAD is the only important graft in CABG”

The seminal publications of Lytle and Loop in the 1980s^[3-5] reported data acquired during the 1970s, the period of IMA introduction. They found that use of left IMA (LIMA) significantly improved survival compared to exclusive use of SVG. The popular interpretation of these data over the following decades established the LIMA-LAD as somehow “sacrosanct”. It should be borne in mind that LIMA was almost always the only arterial graft used, and the left anterior descending (LAD) almost always the only coronary target. All other targets including branches of the LAD were grafted with SVG. As of 2019, it is almost universally accepted that CABG is not of adequate quality if the LIMA-LAD is not used.

There are two apparent interpretations to the original papers by Lytle and Loop:

- A. The IMA has “magical” properties which are impossible to define (not real, not scientific).
- B. The observation arises from the difference between the late patency of an arterial graft compared to the late patency of a venous graft^[3,6-14].

Interpretation B is not popular with surgeons or cardiologists^[3,15,16], whilst Interpretation A is inconsistent with science or logic.

Modes of conduit failure

Progressive atherosclerosis of SVG is very widely documented with up to 50% of grafts occluding by about 10 years postoperative^[8,17-19]. An angiographic classification was developed to categorise diseased SVG (see the work by Fitzgibbon^[20]). At durations longer than 10 years, the progressive decline in SVG patency continues, although may be ameliorated with the widespread use of optimal medical therapies including statins^[21].

It is widely accepted that the LIMA has a small early graft failure rate, considered to reflect flow competition from the native coronary circulation, and thereafter there is no clear evidence of any progressive conduit atherosclerosis as is the case for SVG. A LIMA which survives the early postoperative period is considered by most to be a “permanent graft” without prospects of progressive failure irrespective of the duration postoperative. The angiographic evidence in the late period supports this contention^[22]. Indirectly, the greater survival of patients who have received LIMA grafts also supports this contention^[23,24].

For the LIMA, and most likely any IMA, the late angiographic findings are of: (1) patency (Patent); and (2) normal conduit lumen (Perfect Patency); or (3) Occluded/String sign. A string sign has not been well documented in the literature as being capable of reversal and is considered occluded (permanently). Thus, the angiographic findings are “binary” - Patent + Normal or occluded. Therefore, the Fitzgibbon classification is not suited to the use for this arterial conduit. A subtlety of complex arterial reconstructions is the variable diameter of an arterial conduit related to “autoregulation” of flow via changes to the diameter of the conduit. For example, the proximal segment of the LIMA in a composite graft such as LIMA-RA-Y

Table 1. Patency and perfect patency for asymptomatic patients receiving conventional angiography 13 ± 3 years postoperative with at least one internal mammary artery, radial artery and saphenous vein graft

Comparison	Perfect patency <i>n</i> (%)	<i>P</i>	Patency <i>n</i> (%)	<i>P</i>
IMA, RA, SVG		< 0.001		0.015
IMA <i>vs.</i> RA	60/62 (96.8) 71/77 (92.2)	0.461	60/62 (96.8) 72/77 (93.5)	0.298
IMA <i>vs.</i> SVG	60/62 (96.8) 10/57 (17.5)	< 0.001	60/62 (96.8) 47/57 (82.5)	0.013
RA <i>vs.</i> SVG	71/77 (92.2) 10/57 (17.5)	< 0.001	72/77 (93.5) 47/57 (82.5)	0.055
Arterial, SVG		< 0.001		0.009
Arterial <i>vs.</i> SVG	131/139 (94.2) 10/57 (17.5)	< 0.001	132/139 (95.0) 47/57 (82.5)	0.009

IMA: internal mammary artery; RA: radial artery; SVG: saphenous vein graft.

(RAY) or BIMA-Y (BIMAY) is usually larger in diameter proximal to the Y graft than distal to the Y graft, as it needs to be to provide additional flow to the second conduit as well as the continuation of the LIMA. These findings are normal and expected and do not represent a pathological state.

The most common alternative arterial conduit is the radial artery (RA) and, infrequently, the gastroepiploic artery (GEA). Controversy continues to surround the use of RA in CABG, its perioperative and intraoperative management and long-term outcomes. Data outcomes using statistics that estimate future events (Kaplan-Meier actuarial survival) suggest a progressive decline in patency of RA. This is discordant with the directly measured angiographic findings of RA, which are also “binary” - being either patent and normal or occluded/string sign. There are no serial angiographic reports in the literature of normal RA grafts in the early postoperative period, and then later developing progressive atherosclerosis and eventual occlusion. In our institutional experience of more than 20,000 RA use, we also have not documented progressive atherosclerosis leading to RA occlusion in serial angiograms. The longest duration postoperative for RA angiography in our experience is 22 years, whereby the RA appeared patent and normal.

In a research angiographic series we conducted with patients selected to have received all three IMA, RA and SVG conduits (*n* = 50) at 13 ± 3 years postoperative, we found that the Patency and Perfect Patency was the same for IMA (97%, 97%) and almost the same for RA (94%, 92%), respectively (one RA was diseased at the time of implantation, and remained patent and diseased). The patency of SVG was significantly lower (83%) and the Perfect Patency of SVG was only 18% [Table 1]. There were no significant differences in perfect patency or patency between IMA and RA, despite IMA being almost exclusively grafted to the LAD and RA being grafted to the other two territories.

The angiographic appearance of RA and IMA appeared to be the same in the late period and different to SVG indicating a different mode of graft failure.

Predicting future failure of grafts

It is understandable that a heavily diseased SVG may occlude in the foreseeable future - from logical first principles. Equally, a normal appearing arterial graft at angiography would suggest that this conduit is not likely to fail in the foreseeable future - from logical first principles. A further extension to the logical argument is that SVG is first exposed to the arterial blood pressure and flow rates on the day of surgery. These newly introduced factors may then lead to the development of accelerated atherosclerosis, which can be observed in the cardiac surgery, vascular surgery and renal access surgery domains. Arterial conduits have been exposed to the arterial blood pressure and flow since birth and should therefore not be expected to develop sudden or rapidly progressive atherosclerosis after being transplanted to the coronary circulation and being exposed to the same or similar blood pressure and high flow state.

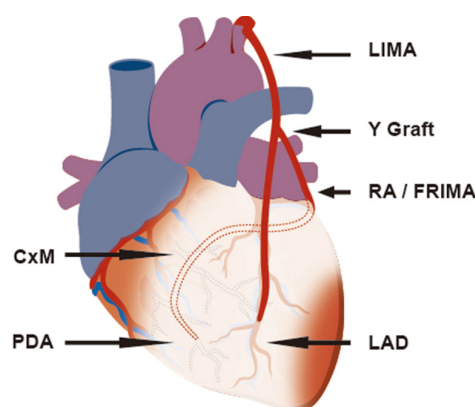


Figure 1. Schematic diagram of radial artery Y graft operation. LIMA: left internal mammary artery; RA: radial artery; LAD: left anterior descending artery; OM: obtuse marginal artery; PDA: posterior descending artery

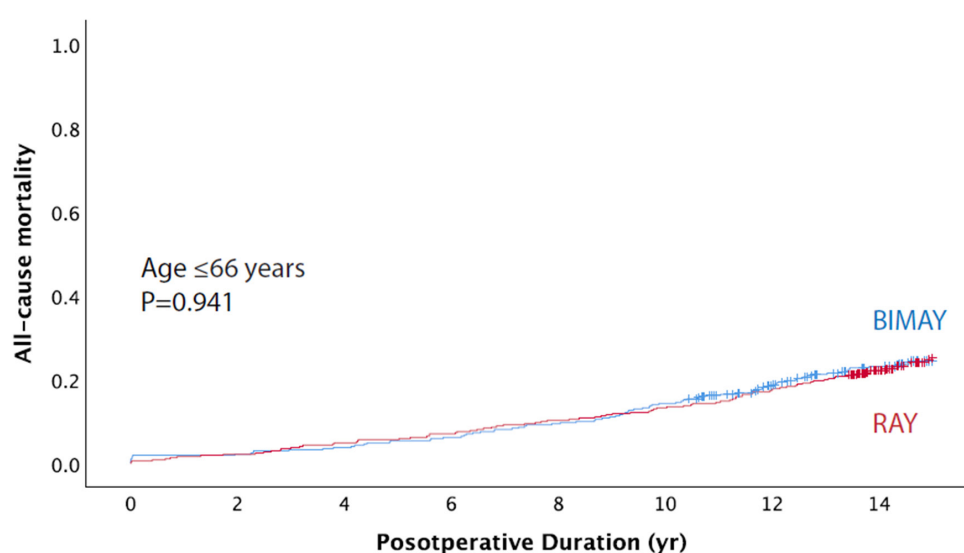


Figure 2. Mortality of propensity score matched cohorts of radial artery Y graft versus bilateral internal mammary artery Y graft operations, $n = 371$ pairs. RAY: radial artery Y graft; BIMAY: bilateral internal mammary artery Y graft

All-cause mortality as a surrogate for coronary graft failure

In large-scale randomised or very well-matched series, it is expected that the non-cardiac cause of death will be equally distributed between groups. For a revascularisation procedure such as CABG, it would be expected that non-coronary causes of cardiac death such as progressive native valve disease would also be equally distributed between groups. Hence, excess mortality is considered to be caused by graft failure, which leads to ischaemic complications that may lead to premature death^[7,25].

Could IMA and RA be similar?

In a series of composite arterial grafted patient (Y graft) [Figure 1] to all three coronary territories and closely propensity score matched, we compared bilateral internal mammary artery Y graft (BIMAY) and radial artery Y graft (RAY) in the Australian population [Figure 2]. All-cause mortality was measured by the national death register and is considered robust. Comparing single surgeon experience from Sydney and an institutional experience from Melbourne, we found no difference in mortality between the two techniques for age at surgery (≤ 66 years, $P = 0.941$, Figure 2) or when all ages were included (due to a severe age distribution imbalance, $P = 0.239$).

Equivalence of arterial conduits?

There is considerable theoretical or *in vitro* evidence to suggest that the LIMA has properties that renders it “superior”. These are claimed to include increased synthesis of nitric oxide, resistance of smooth muscle cells to proliferation, tight endothelial junctions, decreased expression of adhesion molecules and other theoretical correlations between histological structure and long-term function^[26-29]. The discussion, however, has mainly been confined to the comparison with SVG, where the RA is considered to have a thicker muscular layer and *in vitro* and *in vivo* experimentation suggests that it is prone to spasm and theoretically more likely to develop atherosclerosis^[30-36].

However, direct observation suggests otherwise. Consider the following propositions:

1. If a 75-year-old patient had a LIMA, RA and GEA harvested, and all were normal - why should they be so (given that the popular belief that only the LIMA is especially resistant to the development of atherosclerosis)? Specifically, all conduits have been exposed to the arterial blood pressure and flow for 75 years prior to harvest - and are normal - suggesting that all three behave similarly.
2. Angiograms of patent arterial conduits more than 10 or 20 years postoperative reveal a “binary” outcome. Either the conduit is Patent + Normal or Occluded/String sign. These findings are the same for all arterial conduits. Specifically, all conduits are exposed to the arterial blood pressure and flow postoperatively - and remain normal - suggesting that all three behave similarly.
3. If a length of artery remains normal whilst in the arterial circulation for decades prior to surgery - why should it suddenly develop atherosclerosis within a few years of being transplanted into the arterial circulation of the heart (when it is exposed to the same blood pressure and flow as pre-harvest)? Specifically, the haemodynamics of the coronary circulation are essentially the same as for the chest wall, forearm or stomach and there is no credible reason to expect that an arterial conduit will alter its behaviour merely upon use as a coronary graft.

How should the many theoretically- or laboratory experiment-based predictions as to the short- and long-term behaviour of various arterial conduits which conflict with direct observations be interpreted? In the view of the authors, direct observation overrides theoretical- or laboratory-based conclusions, and suggest that further refinement of both theory and interpretation of experimentation is required.

It is suggested that there is a striking similarity for all arterial conduits used in coronary surgery, and, if they do not fail early due to competitive flow, they may be expected to remain patent. All behave differently to SVG, giving rise to the saying, “arteries are arteries - and veins are not”. Should all arterial conduits be considered as interchangeable - would it be acceptable to use RA or GEA to graft the LAD? This is the position that the authors now accept as valid. Please refer to Part 2 for why widespread adoption of this concept is not likely to be rapid.

Complete revascularisation

The consideration of incomplete revascularisation relates primarily to a difference in strategy between PCI, where treatment of the “culprit lesion” is preferred (at least in the acute setting), and the usual surgical strategy of full revascularisation with CABG. Within CABG, off pump CABG has been associated with few grafts, suggesting that incomplete revascularisation is more common, which may impact on outcome. This is beyond the scope of this paper and for the remainder of this paper it is assumed that all patients received full revascularisation.

Could RAY and TAR achieved by any other reconstruction technique be similar?

In a large institutional cohort, we demonstrated that the survival of RAY and TAR by other techniques was not different ($P = 1.000$ ^[37], [Figure 3](#)).

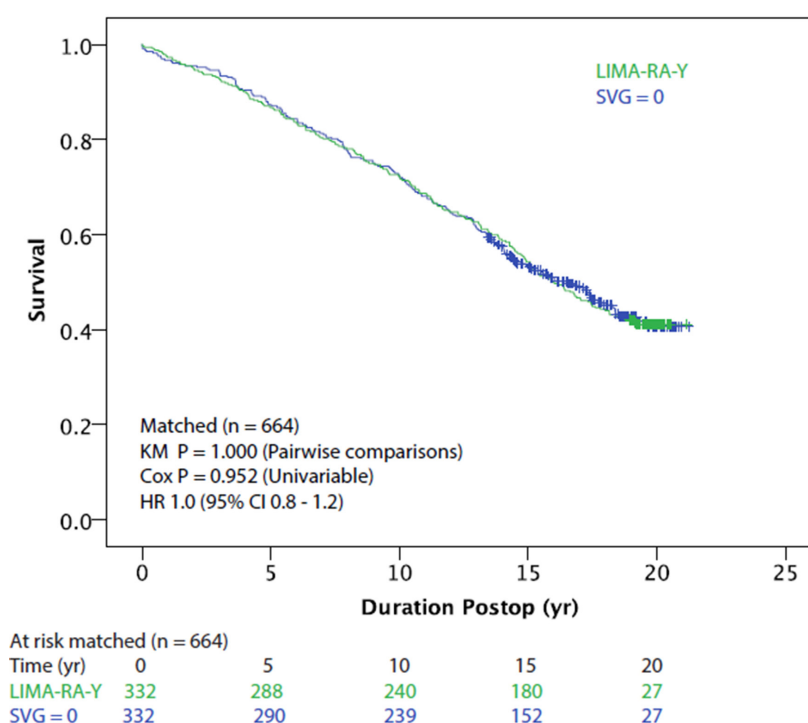


Figure 3. Survival of propensity score matched radial artery Y graft versus total arterial revascularisation patients by any other reconstruction technique, $n = 332$ pairs. Reproduced with permission, JACC 2018^[37]. LIMA-RA-Y: left internal mammary artery radial artery Y graft; TAR: total arterial revascularisation

Is RAY or TAR survival significantly better than single internal mammary artery and saphenous vein graft?

We demonstrated that the survival of the “conventional CABG” of left internal mammary artery and supplementary saphenous vein graft (LIMA + SVG) was significantly lower (HR 1.3 95%CI: 1.0-1.6, $P = 0.043$ ^[37], Figure 4).

Is multi-arterial grafting significantly better than single IMA + SVG?

The theoretical framework is that, if one arterial graft is better than SVG, then two or even three arterial grafts should have incrementally greater survival, and this contention is supported by multiple publications^[38-40]. In our own national database analysis, we conducted a series of large-scale propensity score matches using 17 variables. One arterial graft with supplementary SVG (1A + SVG) was compared to 2A + SVG ($n = 7895$ pairs) with a mortality hazard in favour of more arterial conduits of 1.21 (95%CI: 1.12-1.30, $P < 0.001$). For the comparison of 3A + SVG ($n = 3017$ pairs), the mortality hazard in favour of more arteries was 1.41 (95%CI: 1.24-1.60, $P < 0.001$). Our findings are consistent with the literature and with greater sample size.

The 10-year Arterial Revascularisation Trial outcome remains highly controversial and variously claimed to support the lack of evidence for two IMA leading to improved late survival as would otherwise be predicted by the evidence presented earlier^[41]. Clarity of interpretation is gained by understanding the key confounders and thereby discounting the “as randomised” (intention-to-treat) analysis in favour of the “as treated” analysis. The all-cause mortality was not different for those randomised to one or two IMA ($P = 0.62$).

One key weakness of the study design was to allow use of RA without consequence - this was consistent with the popular belief at the time of study design - that RA was perhaps “no better” than SVG. Although this view remains commonplace to this day, the evidence is that RA has superior angiographic, clinical and

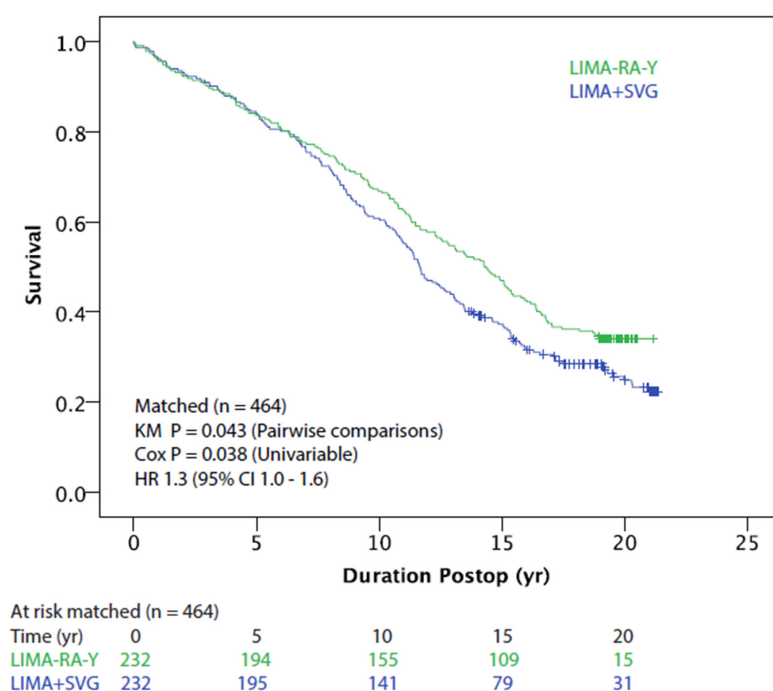


Figure 4. Survival of propensity score matched radial artery Y graft versus left internal mammary artery and supplementary saphenous vein graft patients, $n = 332$ pairs. Reproduced with permission, JACC 2018^[37]. LIMA-RA-Y: left internal mammary artery radial artery Y graft; LIMA + SVG: left internal mammary artery and supplementary saphenous vein graft

survival outcomes compared to SVG^[37,40,42-44]. Therefore, the study design of one versus two IMA, rather than one versus two arterial grafts was confounded by RA being used. Additionally, there was a high cross-over from the randomised allocation. For patients randomised to one IMA, RA was used in 22% and BIMA used in 4% resulting in 26% receiving more than one arterial graft. Additionally, of patients randomised to two IMA, 14% crossed over to a single IMA, resulting in a 40% crossover rate of one versus two arterial grafts. It was not reported how many patients randomised to BIMA received additional RA grafts, which could potentially further magnify the groups discrepancy. By contrast, the *post-hoc* (non-randomised) dataset analysed all-cause mortality according to the use of 1 or ≥ 2 arterial grafts and found a significant survival advantage for more arterial grafts (HR 0.81, 95%CI: 0.68-0.95). Criticism of *post-hoc* non-randomised data is reasonable and valid.

However, this argument misses an even more important consideration of this dataset, which is that both groups in either of these analyses include supplementary SVG. If SVG is the conduit known to progressively fail and leads to ischemic events, of which some lead to death (see below), then the two groups remain relatively similar because both groups still use SVG. Specifically, SVG is more important in the causation of failure of CABG than the arterial grafts, in the long term. A further analysis of this dataset according to TAR vs. ≥ 1 SVG has not been published.

“The chicken and the egg” dilemma

The obvious consequence of increasing use of arterial grafts is that there is a compensatory reduction in the number of SVG being used. We conducted an analysis whereby the number of grafts was restricted and then performed separate matching for all grafting combinations within each stratum^[42] [Figure 5]. For each stratum, the increasing use of SVG (and reduction in the use of arterial grafts) resulted in an increasing mortality hazard.

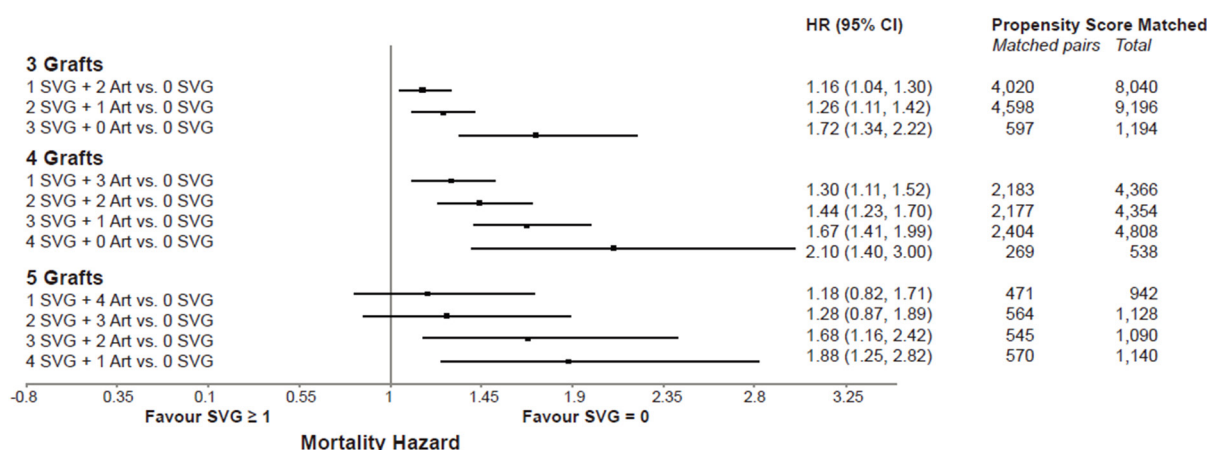


Figure 5. Multiple propensity score matched analysis according to increasing saphenous vein graft use within restricted number of grafts. Reproduced with permission EJCTS 2018^[42]. SVG: saphenous vein graft; Art: arterial grafts; 0 SVG or SVG = 0, total arterial revascularisation

Count the veins, not the arteries

We know that SVG will develop progressive atherosclerosis and progressively fail in the long term. We know that, except for a small percentage of arterial grafts failing in the short term due to flow competition from the native coronary circulation, there is a paucity of evidence to suggest that arterial grafts will ever progressively fail in the long term. Survival with one or more SVG was lower than for TAR [Figure 6]. The survival advantage of TAR remained even when there was a majority of grafts that were arterial (multi-arterial) and with one SVG^[42] [Figure 7].

The relationship between graft failure and reduced survival has a plausible mechanism and therefore it is suggested that the conduit of most interest is the SVG rather than the arterial conduits^[37,38,40,45,46]. It would be logical, therefore, that the number of SVG grafts has greater effect on survival than the number of arterial grafts. The strategy of TAR is predicated on the principle of reducing or eliminating SVG grafts, rather than the principle of greater number of arterial grafts.

Early mortality of TAR

Whilst it is a common belief that TAR is more complex and therefore results in a higher perioperative mortality, there appears to be very little evidence to support this view. Indeed, the evidence points to a lower perioperative mortality with TAR^[47-49]. There is also a paucity of evidence that “low volume” centres exhibit a disproportionally higher perioperative mortality with TAR; indeed, the same argument was present in the 1980s and 1990s when there was resistance to the increased use of IMA^[46,50-52].

Potential risks of multi-arterial grafting

The principle risk of BIMA is a higher rate of deep sternal wound infection. The literature is vast with considerable debate; however, large-scale or meta-analyses indicate a consistent finding that the rate is approximately double that of single IMA, higher in diabetics and obese females and possibly lower with skeletonised IMA^[53-56]. Logically, harvesting the IMA by any method substantially reduces the blood supply to one side of the sternum, and any difference between harvest techniques is not likely to be substantial.

The risks to the forearm following RA harvest are surprisingly low^[57-61]. Ischemic complications affecting the hand are exceptionally rare. Wound infections are infrequent due to the superior blood supply of the forearm relative to the leg, and nerve damage is confined to local nerves that are entirely cutaneous and which do not supply the palm or fingertips. There is no evidence that the forearm has reduced blood flow following RA harvest^[62].

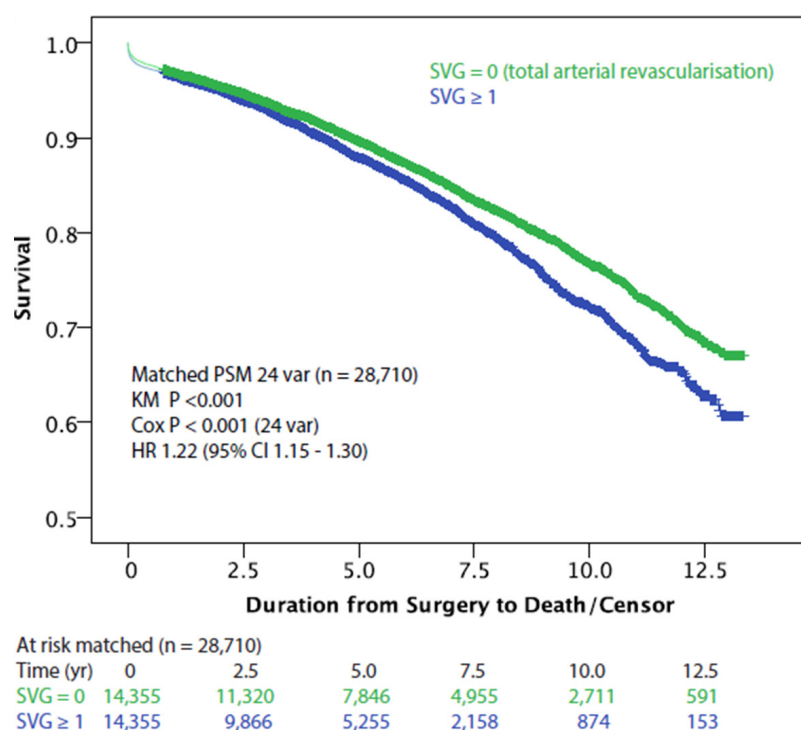


Figure 6. Propensity score matched comparison of total arterial revascularisation and the use of one or more saphenous vein grafts, $n = 14,355$ pairs. Reproduced with permission EJCTS 2018^[42]. SVG = 0, total arterial revascularisation; SVG ≥ 1, use of one or more saphenous vein grafts irrespective of the number of arterial grafts; KM: Kaplan-Meier; Cox: Cox proportional hazards method; HR: hazard ratio; 95%CI: 95% confidence interval; SVG: saphenous vein graft

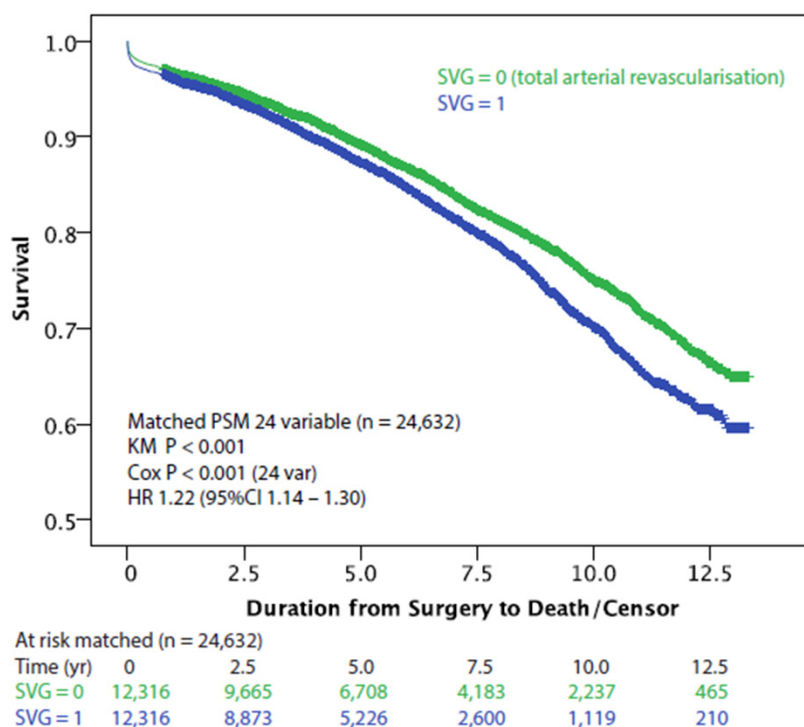


Figure 7. Propensity score matched comparison of total arterial revascularisation and the use of one or more saphenous vein grafts, $n = 12,316$ pairs. Reproduced with permission EJCTS 2018^[42]. SVG = 0, total arterial revascularisation; SVG = 1, use of one single saphenous vein graft irrespective of the number of arterial grafts (number of arterial grafts 1.7 ± 1.1); KM: Kaplan-Meier; Cox: Cox proportional hazards method; HR: hazard ratio; 95%CI: 95% confidence interval; SVG: saphenous vein graft

The key perioperative risks relate to end-organ dysfunction due to iatrogenic systemic hypotension leading to end organ hypoperfusion. Acar first advanced the hypothesis that RA was prone to spasm that could be managed by intravenous vasodilators^[63]. The media of RA is thicker than for IMA, and the logic that RA appeared likely to be prone to spasm gained momentum, resulting in a widespread use of vasodilators only when RA was used. This continues in most centres today, resulting in early abandonment of RA use. Remarkably, there has been no large-scale study undertaken to date. Two small randomised controlled trials (RCT) failed to show benefit for vasodilators, but they were criticised for lack of power^[64,65]. In a meta-analysis of six RA RCTs, benefit for vasodilators was found^[66], but caution in interpretation was advised^[67]. This author has used vasoconstrictors in the majority of patients since 1996, and 100% of patients since 2008 including all patients where RA was used. The author observes that RA spasm is less severe and less frequent than for IMA.

A theoretical risk for composite grafting is a poorly constructed Y anastomosis, which could compromise one or both of the conduit limbs distal to the Y graft. However, there are no reports that relate specifically to this risk. It is assumed that the patency of both limbs is primarily dependent on the integrity of the conduits and flow dynamics affecting the distal anastomoses. The influence of competitive flow has been suggested to influence composite grafts to a greater extent than aorta-coronary grafts^[68-70]; however, the bulk of the literature would suggest that the outcomes are similar^[71-76].

The relationship between surgeon or institutional operative volume and mortality or other outcomes is generally strongly held. There is some evidence from the surgical literature (often not relating to CABG) that lower volume equates with higher mortality or adverse outcome^[77-80], whereas other studies found no such relationship^[81,82]. For the Royal Melbourne experience, the mortality of CABG when routine TAR was adopted was lower^[83], and mortality is lower or similar in many reports^[52,84-89].

However, this argument misses the point of change in CABG practice. In the examples above, mostly the analysis relates to performance of a procedure in sufficient volume (or not performing the procedure - insufficient volume). In the case of CABG using more arterial conduits, there is usually no change to the number of cases performed or the reconstruction methods used. Thus, the central consideration relates to a minor change in practice rather than a consideration of the volume of cases. Specifically, the number of CABG cases for each surgeon or institution, the number and distribution of grafts, the management of cardiopulmonary bypass, and for the most part the postoperative management of the patient will all remain the same. The differences in operative technique for the harvest of arterial rather than venous conduit, the anastomosis suture technique and any other aspect of the surgical procedure are minor. It is a common view, therefore, that that differences between non-complex arterial reconstructions and conventional surgery are greatly exaggerated. The adoption of TAR represents a relatively small and easily managed alteration to the operative technique.

PSYCHOLOGICAL BARRIERS TO ACHIEVING TOTAL ARTERIAL REVASCULARISATION - THE KEY BARRIERS

The conformist pressure

A remarkably powerful force in medicine is the desire of a group to be homogenous or at least in conformity with the leader of the group. In addition, since the cardiac surgeon is dependent on the cardiologist for patient referrals (but the cardiologist is not dependent on the surgeon for referrals), many surgeons are conscious of the need to ensure that their referring cardiologists prefers greater use of arterial grafts - before the surgeon changes their technique. In practice, there is often a misalignment between cardiologist and surgeon which results in maintenance of the status quo.

Complications occur in all medical treatments, but, when new techniques are introduced, there is often a new benchmark established by those resisting change - that of a zero tolerance of complications, different from normal practice of an acceptable rate of complications as dictated by local guidelines, societies or the literature. This practice leads to excessive psychological stress on the surgeon attempting the change, often leading to return to the status quo (the intent of the criticism).

Guidelines are the result of a consultative and consensus opinion of experts reviewing the existing literature. Level A evidence always relates to techniques that have been in use for a long time, usually decades. The key intended effect of guidelines is to enforce conformity: for those whose practice lags behind the mainstream to update to the mainstream practice as well as for pioneers and early adopters of new techniques to return to the mainstream. "Evidence-based medicine" usually refers to conformity to guidelines, increasing the risk to the individual practitioner if they practice outside of the guidelines; hence, risk aversion is a powerful force acting against new change.

Technical difficulty of grafting

The technical difficulty is usually exaggerated. Coronary surgery is fairly basic conceptually and most surgeons are competent at suturing a conduit to a target coronary artery.

The simple path to evolve practice is to substitute an arterial conduit for a venous conduit - and not to change any other aspect of the surgical technique. By the use of both IMAs and both RAs, four grafts can be easily achieved without alteration of the surgeon's usual operative routine. In this way, the majority of CABG could achieve TAR and the remainder of the patients could receive multi-arterial grafting. This change in practice is simple and easily achievable, with minimal risk to the surgeon's reputation and results but with long-term survival benefit to the patient.

To achieve routine TAR or at least high rates of TAR does require the use of more complex reconstructive techniques, which are discussed below, and it is recommended that surgeons who rarely perform TAR commence with substituting arterial conduits in the first instance before attempting more complex methods.

The "R" word

Surgeons who infrequently use a RA will usually articulate that the late outcomes of RA are:

- (1) "no good";
- (2) RA is difficult to harvest and their assistants are untrained;
- (3) RA takes too much time to harvest and they cannot afford the time; or
- (4) they have to introduce the use of vasodilators to their practice due to the reports of the high tendency of RA to spasm, which may then introduce iatrogenic complications related to low blood pressure and reduced end organ perfusion.

The use of RA is integral to the achievement of TAR for most surgeons (although a BIMAY technique could be an alternative). In our Australian series, RA represented 47% of all grafts in the TAR group and 25% in the Multi-Arterial Grafting (MAG) group prior to propensity score matching. Our group has a large experience of RA use and considers:

- (1) RA exhibits late angiographic patency very similar to IMA - a patent graft in the short term appears to remain patent and normal in the long term. This contributes to the TAR group maintaining the highest late survival, as well as to survival in the MAG group exceeding 1A + SVG. The in-hospital mortality is not increased in our experience.
- (2) RA is the simplest conduit to harvest, and substantially easier than IMA. It is also the easiest harvest technique to learn. We harvest RA with similar technique methods as for IMA: enter the correct anatomical

plane (within the fascia surrounding the neurovascular bundle), dissect the tissues with low power electro-cautery, divide the branches between two small metal clips with electro-cautery and retain the satellite veins. Alternatively, use of scissors or an ultrasonic scalpel is also very safe. Topical and intraluminal 1% papaverine is used.

(3) Harvest of the RA is faster than for IMA and similar to or faster than SVG.

(4) RA is less prone to intraoperative spasm requiring topical therapy than IMA.

(5) For more than two decades, our institution has liberally used intermittent and/or continuous intravenous vasoconstrictors to maintain physiological blood pressure (usually > 100 mmHg systolic) in order to maintain end organ perfusion (including cardiac graft perfusion). In the case of these authors, since 2008, 100% of all cardiac operations have had a low dose of norepinephrine commence at the start of the anaesthetic and continued throughout the operation and into the ICU period - sometimes for days. In the case of a severe systemic inflammatory response syndrome, the infusion may be substantially increased even to 50 µg/min. We have not observed RA spasm clinically, and, rarely when a patient undergoes perioperative angiography, we have not documented RA spasm. We do not use intraoperative calcium channel blocking agents and there is a highly variable use of these agents postoperatively with poor compliance on follow up. We therefore advocate that surgeons using RA for the first time or infrequent users of RA should not vary their intraoperative or postoperative management from their usual practice and not make any adjustments to their usual blood pressure guidelines or to the drugs that they normally use.

(6) RA is longer than IMA.

(7) RA is larger and more robust, making anastomosis construction easier than for IMA.

(8) RA is less prone to kinking than SVG.

Cognitive dissonance

Leon Festinger was a New York born social psychologist, who published the Theory of Cognitive Dissonance^[90,91]. This wide-ranging theory has some applicability to cardiac surgery.

Consider: Example from the theory: a smoker, who knows that smoking is harmful and that he should stop, continues to smoke. Why?

The theory explains that the desire to smoke conflicts with the desire to be healthy, resulting in internal psychological tension (dissonance) and they will take steps to try and reduce this tension. For example, they may rationalise their behaviour, excuse it, blame others, deny it, etc. However, a further facet of the theory is that, if another person were to try and persuade them to stop smoking, or even blame them for smoking, then they will vigorously defend their right/desire/intent to keep smoking. That is, the help offered is frequently seen as more threatening to their sense of self than is their own internal conflict on the same subject.

Example from cardiac surgery: a cardiac surgeon, who believes that SVG will ultimately fail and would prefer to use more arterial grafts but continues to use SVG.

THE HOW: PRACTICAL STEPS TO BECOME A ROUTINE TAR SURGEON

Keys to success

1. It is relatively easy and safe to become a “mostly” TAR surgeon.
2. Stage the introduction of more complex techniques and become comfortable with them before proceeding.

Radial artery harvest

The key to simple RA harvest is to enter the correct anatomical plane, which is within the fascia immediately surrounding the superficial radial nerve, radial artery and veins. Here, the RA lies in loose areolar tissue and can be harvested with its two satellite veins with the minimum of dissection.

Tips: Please do not include surrounding tissues such as additional deep fascia, muscle or tendon fragments, as this increases the trauma to the forearm without benefit. Specifically, such a harvest has no advantage for influencing spasm.

An alternative is endoscopic harvest of RA and it is not recommended that this technique is used in the initial experience. The open technique will provide for a clear understanding of the anatomy and minimise any risk to the RA or forearm structures [Video 1].

Tips: Division of the proximal end of RA can be undertaken with metal clips or suture ligation (the latter recommended in the initial experience). No separation of the satellite veins from the RA should be performed prior to proximal division, as this often causes spasm of the proximal 1 cm of RA. However, separation of the veins after division does not cause spasm, and the reason for this is not apparent. Generally, spasm is less common or severe than that seen in the distal segment of the IMA and treatment is the same, with use of papaverine and rarely use of a metal probe.

RA may be stored with the usual preparation used by the surgeon for IMA. The author uses 1% papaverine with heparin and no blood, whereas others may dilute the solution with some blood of similar volume. There is no convincing evidence of efficacy superiority for any method.

Grafting aorta-coronary with radial artery (keep it simple)

Using the same suture technique and suture material, as is the normal practice of the surgeon when grafting with SVG, should result in the same technical result as is their usual practice.

Tips: The RA is easier to use than SVG as it has a thicker wall and is more elastic and so less prone to kinking if it is of redundant length. A minor technique difference of the distal anastomosis is that the RA does not require the construction of a “hood”. For SVG to adopt a satisfactory lie without flattening over the toe (distal) region of the anastomosis, it is common for surgeons to leave some of the SVG redundant so that a rounded “hood” is created. This occurs when the conduit anastomosis area is larger than the coronary anastomosis area. This is not necessary for all arterial conduits, and exact size matching is preferred and does not ever lead to restriction at the anastomosis.

The proximal anastomosis to the aorta is constructed as for an SVG anastomosis with the same suture materials (e.g., 6/0 Prolene). No SVG intermediary hood between the aorta and the RA, or any alternative anastomosis method, is required.

Sequential grafting

The rationale for single SVG conduits per distal coronary anastomosis arose from the experience gained during the 1970s. It was common to use a single length of SVG to commence at the aorta and then graft all distal targets with sequential anastomoses - the so-called “round the world” graft. Since the most common site of SVG graft failure was near to the aortic anastomosis, the danger was that all grafts would fail simultaneously, leading to large ischemic implications. The strategy of a separate conduit for each anastomosis was therefore a strategy of limiting the impact of SVG graft failure to just one target anastomosis. Sequential grafting therefore fell out of favour.

In the era of arterial grafting, sequential grafting is essential to maximise the efficient use of conduit. The underlying premise is that arterial conduits will remain patent in the long term, distinct from the SVG experience.

A wide variety of sequential anastomosis techniques has been described^[92-95], but we describe a simple and pragmatic approach in this section. The conduit is then draped over the coronary artery at whatever angle appears to offer the best “lie” without tension. This may vary from directly overlying (“parallel”), to crossing at right angles (“diamond”) or somewhere in between (“oblique”). An incision is made longitudinally in both coronary artery and conduit of equal size. If the first suture placed passes through the native coronary artery at a point where the heel of the conduit would lie, and the second needle is then used to pass through the conduit at the heel, this angle is preserved. The anastomosis may then be completed with whatever usual suture method is preferred by the surgeon.

Tips: Arterial conduits may be constructed with parallel or oblique anastomoses due to the ability of the arterial conduit to adopt curves with redundant length of conduit as they are not prone to kinking. However, the SVG is very prone to kinking and it is essential for most sequential anastomoses that it be constructed to prevent any curvature to SVG, which usually results in the lie of the SVG and the coronary target being at right angles to each other - the diamond anastomosis.

Tips: It is generally easier to construct sequential anastomoses along the length of the conduit from its proximal to distal end, as this facilitates the movement of the free distal end of the conduit during suturing. Consideration should be made to creating the aortic anastomosis first, followed by sequential grafting along the length of the conduit as required.

Y (composite) grafting

The key elements of Y grafting are established by the techniques already described^[95-99]. Use of RAY where the LIMA and left RA are harvested simultaneously results in no prolongation of the operation over usual practice and additionally may be useful if the right RA has been used to perform diagnostic coronary angiography.

To perform the Y anastomosis, place two folded gauze over the distal ascending aorta after division of the thymus. This stabilises the movement from the heart facilitating suturing. At the level of the inferior aspect of the brachiocephalic vein, make a longitudinal incision on the chest wall aspect of the LIMA approximately 50% longer than for the LAD anastomosis. The proximal end of the RA [or free right internal mammary artery (RIMA)] is then sutured using the usual method of the surgeon. The two conduits should be tacked together to prevent inadvertent torsion occurring.

The LAD territory should be grafted with the LIMA first, and sequential grafts performed to diagonal arteries as appear comfortable by draping the LIMA over the branches. This sequence is necessary to position the Y graft correctly, which should lie near to the lateral border of the pulmonary artery. If a hole was made in the pericardium to pass the conduits, the Y should be positioned near to this hole. The RA (or second IMA) should then be used to revascularize the circumflex and/or the right coronary territory by performing sequential anastomoses along its length from proximal to distal. The final anastomosis for each conduit is an end-to-side anastomosis.

If cardioplegic arrest is used, release of the conduit clamps may allow early reperfusion as well as optimal conditions for inspection of the anastomoses for leaks and for the lie of conduits.

Tips: The complexity of this operation is usually exaggerated; however, caution is advised during the early experience when using it for unstable patients, rescue situations or other factors that lead to additional

independent stress for the surgeon. It is far better to become familiar with the technique under ideal or optimal circumstances, so that under urgent or non-optimal circumstances the methods are well practiced.

Alternative combinations of graft reconstructions

Many variants are described but these may be summarised as “mini-Y” grafts. Specifically, short lengths of conduits that may have been redundant from a previous aorta-coronary graft can be effectively utilised by creating a Y graft and using the conduit to graft a coronary artery with a more ideal lie. For example, a short length of IMA or RA could be sutured to the LIMA as a Y graft and used to graft a diagonal artery that was adopting a course widely divergent from the LAD, making a conventional sequential anastomosis with the main LIMA more difficult. Generally, more complex iterations of this technique do not facilitate ease of grafting.

Relatively common grafting configurations are depicted in [Figure 8](#).

Alternative conduits

Second internal mammary artery

Use of a second internal mammary artery in composite grafting is discussed above. The alternative is the use of an *in situ* or RIMA. The key limitation of the *in situ* graft is the frequent inability to reach distal coronary targets. Devotees of the use of *in situ* graft usually claim that the PDA and sometimes the left ventricular branch, distal LAD or more distal marginal arteries can be reached; however, this is often not the case in the hands of many surgeons. The trajectory to the right coronary territory may be at risk of tension with excessive inflation of the lungs. Additionally, for circumflex territory targets, the trajectory of the RIMA may limit grafting by being constrained to passage through the transverse sinus or anterior to the ascending aorta to reach the LAD. For these left coronary vessel targets, the presence of a patent IMA graft in close proximity to the ascending aorta results poses a significant risk of inadvertent injury during redo surgery. Additionally, the meta-analysis data would suggest that, on balance, the risk of deep sternal wound infection with bilateral compared to unilateral IMA harvest roughly doubles^[53-56]. The free graft is commonly believed to confer a lower patency compared to the *in situ* graft. However, this may be an anomaly of grafting strategy, as it is most commonly grafted to the RCA targets, or to circumflex targets of secondary importance. Therefore, when compared to LIMA-LAD grafts, the patency is lower, yet similar to other arterial grafts^[100,101]. However, when a free IMA is grafted to the LAD, the survival is similar^[102]. It is likely therefore that the higher patency of the LAD graft principally reflects the larger “run-off” of this vascular bed, compared to the conduit.

Gastroepiploic artery

The proponents are predominantly Japanese surgeons and advocate a pedicled over a free graft configuration^[87,103-107]. The reported complication rate of harvesting this conduit is low, but it is rarely used outside of Japan. The popular view internationally is that the harvest of this conduit is a major additional step to surgery and that the complication rate would be higher than for either SVG or RA harvest. Logically, this appears likely, especially with the higher obesity rates in Western countries, but there is very little direct evidence. The artery itself would be expected to behave in a similar manner to IMA or RA, by being resistant to the development of conduit atherosclerosis over the long term^[103,108].

Degree of coronary stenosis and graft selection

This topic is beyond the scope of this article. Competitive flow may affect all conduits and is present in all coronary territories. General observations are that the effects are greatest in the RCA territory and least in the LAD territory. For the surgeon early in their experience with multiple arterial grafting, it is wise to be conservative and graft lesions of high severity (e.g., $\geq 80\%$ stenosis). The alternative to grafting should be not to graft, rather than grafting with SVG.

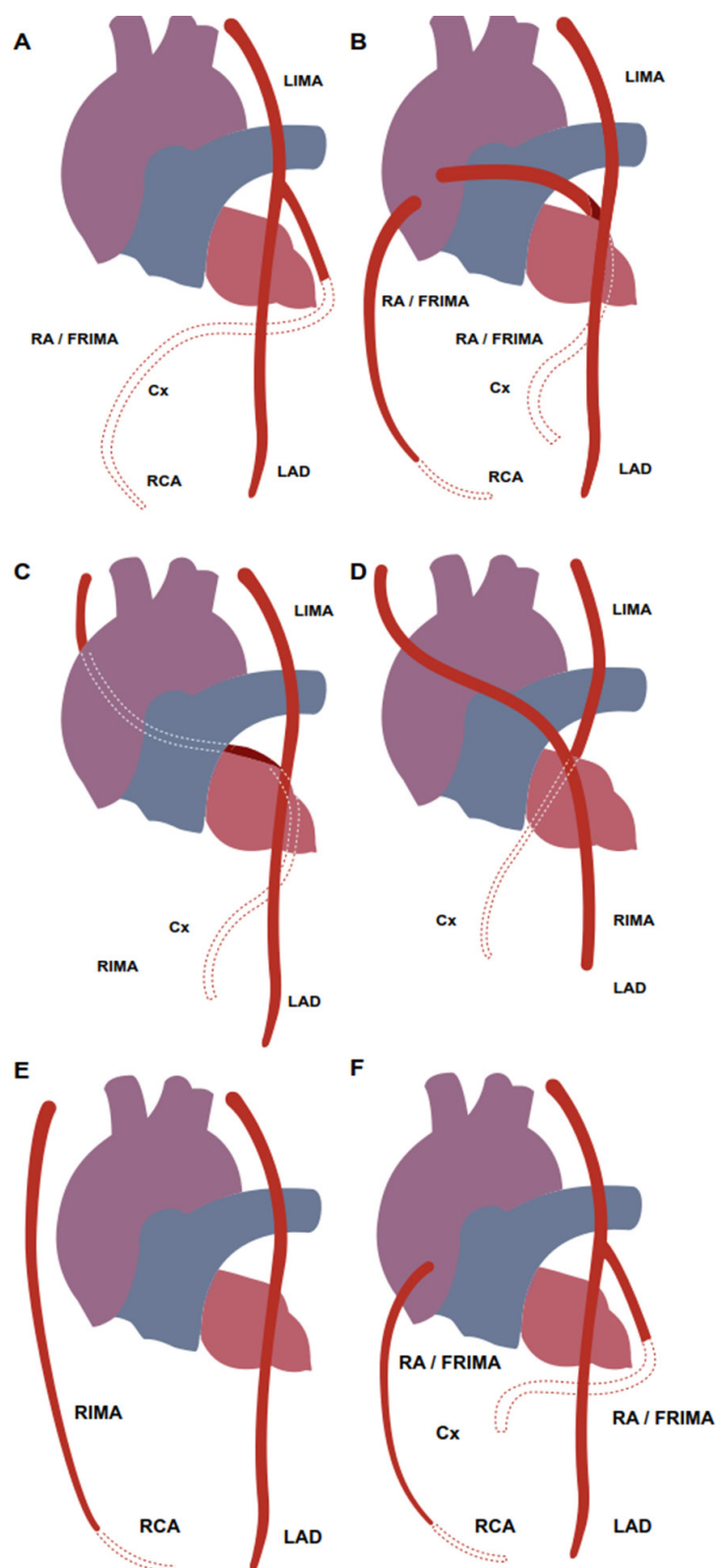


Figure 8. Commonly performed alternative grafting configurations achieving total arterial revascularisation: A: classical Y graft; B: conventional grafting strategy (sequential grafting as required for each conduit within each coronary territory); C: *in-situ* RIMA to circumflex via transverse sinus; D: *in-situ* RIMA to LAD and LIMA to circumflex; E: *in-situ* RIMA to RCA territory; and F: "mini" Y grafts from LIMA to left sided coronary targets, with additional aorta-coronary conduit for RCA territory. LIMA: left internal mammary artery; RIMA: right internal mammary artery; RA/FRIMA: radial artery or free right internal mammary artery; LAD: left anterior descending artery; Cx: circumflex; RCA: right coronary artery

The effects of competitive flow are subject to some popular misconceptions:

1. Reduced arterial graft patency with reduced degree of coronary stenosis is not a “binary” phenomenon. Surgeons often assume that patency will be 100% when grafted to coronary lesions of more than a particular value (e.g., 80% or 90% stenosis) - and, conversely, they assume the patency will be 0% when grafted to lesions of less than this value. This is incorrect and exaggerated. The patency progressively diminishes as the degree of coronary stenosis decreases, and there is no specific cut-off value whereby patency falls to zero^[71,109-112]. In our as yet unpublished experience, the patency of all conduits exceeds 50% at angiography when grafted to coronary lesions of $\geq 50\%$.
2. Use of SVG is resistant to the effects of competitive flow and can be used without adverse consequence for mild or moderate lesions. Our data indicate that even single SVG is associated with long-term adverse survival implications and we would advise avoidance of grafting with any conduit under these circumstances.
3. Early arterial graft failure from competitive flow is still a failure. From the clinical perspective, this is not true. The lack of a flow limiting coronary lesion (at rest) results in a minimal blood pressure drop distal to the lesion, and therefore a minimal pressure gradient driving flow across the conduit. The lack of conduit flow presumably leads to conduit ischemia, although the precise mechanism of arterial graft failure is unknown. Late histology of a string sign reveals a viable media and adventitia; however, the lumen is mostly obliterated with intimal hyperplasia. Because the target myocardium remains well supplied by blood at rest, there is no ischemic consequence to this graft failure.
4. A “string sign” (an angiographic appearance of severe and diffuse narrowing of an arterial conduit lumen, which does not fill the native coronary artery via the graft) is thought to be a reversible state. Many would believe that this is caused by spasm of the conduit (rather than the intimal hyperplasia seen at histology) and that relaxation of the conduit wall will reverse patency. We have never documented a case of reversal of string sign and consider it permanently occluded.

FINAL WORD: BUYING INTO THE LEFT MAIN CORONARY STENOSIS PCI VS. CABG DEBATE

Head *et al.*^[113] performed a patient level pooled analysis of 11 RCT examining PCI vs. CABG. The findings were of five-year all-cause mortality benefit for CABG (HR = 1.20, 95%CI: 1.06-1.37, $P = 0.004$). However, the benefit was confined entirely to multivessel and complex coronary disease and in diabetic patients (HR = 1.28, 95%CI: 1.09-1.49, $P = 0.002$). Non-diabetics and those with left main stenosis had no significant difference.

In late 2019, the five-year data for the EXCEL^[114] and NOBLE^[115] trials examining left main coronary stenosis of low and intermediate complexity were released. Great controversy surrounding the definition used for myocardial infarction (alleged to favour PCI) was ongoing at the time of this manuscript preparation. Nevertheless, these two trials reported apposing analyses using composite endpoints. The EXCEL trial reported no significant difference (HR = 1.19, 95%CI: 0.95-1.50, $P = 0.13$). The NOBLE trial reported superiority for CABG (HR = 1.58, 95%CI: 1.24-2.01, $P < 0.001$).

The key relevance to this manuscript is that *none* of the CABG vs. PCI trials accurately describe the revascularisation techniques of the CABG arm. Since the vast majority of patients are from North American or European centres, it is left to the reader to assume that the technique of CABG would closely reflect “routine” clinical practice in these regions. Specifically, it is therefore likely that the vast majority of patients undergoing CABG in these trials would have received a single arterial graft and supplementary SVG. What this suggests, is that the superiority of CABG over PCI in these trials - may be further magnified by a factor of at least 1.22 - if all of the CABG arm were to have received total arterial revascularisation.

It is therefore a matter of speculation as to whether PCI vs. CABG that is comprised entirely of total arterial

revascularisation would demonstrate magnified significant differences, or converted non-significant to significant differences. There is a strong logical basis to propose new PCI vs. CABG (TAR) trials.

CONCLUSION

To increase the use of arterial grafts by initially substituting arterial conduits for venous conduits - but not altering the techniques of the surgeon - is a simple and pragmatic approach toward routine total arterial revascularisation. To achieve universal arterial graft use, a stepwise approach, practising the various sequential and Y graft methods, should be initially undertaken in elective and low risk surgery to minimise stress on the operating team.

DECLARATIONS

Authors' contributions

Authored this manuscript based on the work of many other studies and publications including manuscript design, writing, incorporation of prior analyses and of clinical opinion offered: Royse A

Co-authored all of the referenced studies and was involved in study design, execution and analysis in all: Royse C

Contributed to data collection and analysis in all of the studies referenced: Boggett S

Involved in the analysis of all of the studies referenced and co-written all: Clarke-Errey S

Involved in writing, data collection, data analysis and study design of the original study for the comparison of total arterial revascularisation and use of saphenous vein graft published in the European Journal of Cardiothoracic Surgery: Pawanis Z

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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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Imaging and computational modeling of tricuspid regurgitation and repair

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Abstract

The tricuspid valve (TV) and right ventricle (RV) are a complex mechanical system. Tricuspid regurgitation impacts a growing and heterogeneous population, leading to right-sided volume overload and right heart failure if left untreated. In part because isolated surgical tricuspid valve repair (TVR) is performed infrequently and has a high mortality, several percutaneous TVR options are being developed. However, the mechanical effects of different types of percutaneous or surgical TVR are unclear. Both the quality of RV imaging and the power of cardiac computational modeling have increased, and accurate computational models of the TV + RV with simulated TVR are now possible. Computational models of TV + RV may aid in patient selection and procedural planning prior to surgical and percutaneous TVR.

Keywords: Adult cardiac surgery, tricuspid regurgitation, bioengineering, computational modeling, finite element modeling

INTRODUCTION

Tricuspid regurgitation (TR) affects an estimated 0.5% of the US adult population, equivalent to the prevalence of aortic stenosis^[1]. The population of patients with moderate to severe TR is increasing with



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the increasing incidence of atrial fibrillation and with use of mechanical devices such as pacemakers, and it is currently estimated at around 1.6 million Americans^[2,3]. Population-based studies have determined that isolated TR in the absence of other cardiac disease is a risk factor for death even when incidentally identified on echocardiography. Further work has shown that, when controlling for other measures of cardiac disease such as depressed left ventricular (LV) ejection fraction and elevated pulmonary artery pressure, moderate to severe TR remains an independent risk factor for both morbidity and mortality^[4,5].

Links between TR and increased mortality risk may stem from adverse right ventricle (RV) remodeling. TR provides a nidus for progressive right ventricular dilation, volume overload, and ultimate right heart contractile dysfunction^[6]. Supporting this is the finding that increasing severity of TR on imaging is associated with markers of progressive RV dysfunction, such as RV dilation and increased right atrial pressure^[4]. The greater the initial burden of TR in untreated patients, the more likely they are to progress over time, and the greater the associated progression of RV dysfunction^[7]. Correspondingly, isolated severe TR is associated with increased incidence of heart failure despite maximal medical therapy, a 3-4 × risk of major adverse cardiac events, and a 2-3 × risk of death, after controlling for age and presence of other comorbid conditions^[8]. Despite these sobering statistics, ideal management of moderate to severe TR remains unclear.

While surgical valve repair is the standard of care for patients with severe and symptomatic TR, it carries with it a 2-5 × higher risk of mortality than surgery on other cardiac valves, and consequently repair of isolated severe TR is rare^[3,9-13]. Furthermore, because of the complex relationship between TR and RV dysfunction, for a subset of patients with a clear indication for surgery, correction of TR results in florid right heart failure, and can be fatal^[6]. At present, no predictive index exists to identify such patients preoperatively^[14]. Because of the high morbidity and mortality rate of open tricuspid surgery, there is a growing interest in minimally invasive therapies for the tricuspid valve (TV). However, while transcatheter therapies for TR are evolving, they have lagged behind similar interventions for the aortic and mitral valves, and have yet to be incorporated into routine clinical practice^[15].

Advances in cardiac imaging have enabled high-resolution assessments of both the RV and TV, providing improved assessment of both structure and function^[9,14,16]. Computational models of the RV and TV are underway to accurately represent the mechanical behavior of the right heart under varied conditions^[17,18]. The utility of this type of cardiac modeling has been previously demonstrated in the LV and mitral valve, illuminating the effects of surgical procedures such as ring annuloplasty, MitraClip placement, and surgical ventricular restoration^[19-22]. Using these techniques to examine the TV will provide insight into the mechanical effects of surgical repair and of novel transcatheter devices, leading to improved care of this large group of patients with diseases of the “forgotten” valve.

RIGHT HEART ANATOMY

The right heart includes the right atrium (RA) and RV, separated by the TV. Deoxygenated venous blood drains from the body into the RA, and is propelled forward into the RV during atrial systole; the TV then acts as a one-way valve preventing regurgitant flow of blood back to the RA during ventricular systole, as blood is ejected from the RV out to the pulmonary circulation to participate in oxygen and carbon dioxide exchange.

TV ANATOMY

The TV is a unique and complex anatomic structure, in a dynamic relationship with the RA and ventricle. The TV is the largest and most apically positioned of the four cardiac valves with a normal orifice area of 7-9 cm²^[23]. The TV complex encompasses valve leaflet, papillary muscles, chordae, and annular components. These components work in a well-coordinated symphony in order for the TV to

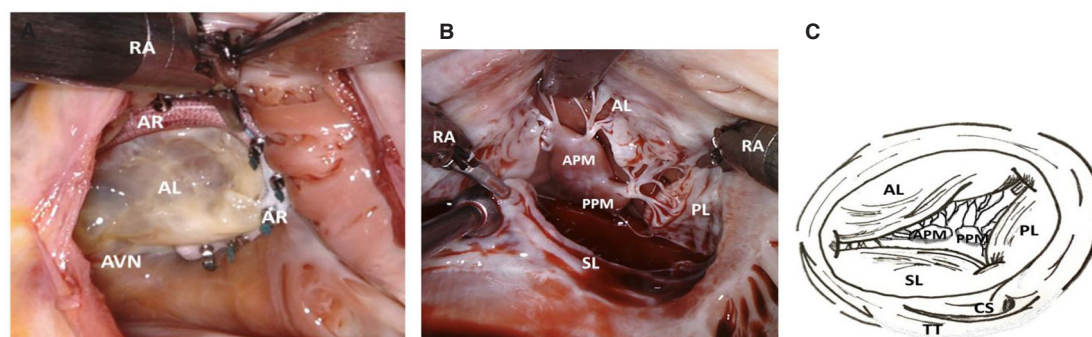


Figure 1. A: Intra-operative photograph of the tricuspid valve with closed leaflets; B: Intra-operative photograph of the tricuspid valve with visible papillary muscles. AL: Anterior leaflet; PL: Posterior leaflet; SL: Septal leaflet; CS: Coronary sinus; TT: Tendon of Todaro; APM: Anterior papillary muscle; PPM: Posterior papillary muscle; RA: Robot arm

physiologically function as a one-way valve. Disorders of the RV or TV disrupt the unidirectional flow of blood, leading to TR.

The TV has three leaflets: anterior, posterior, and septal [Figure 1]. The anterior leaflet is the largest, most mobile, and longest in the radial direction of the three leaflets. The posterior leaflet is the shortest circumferentially and is the least consistently present; in approximately 10% of people, the posterior leaflet and anterior leaflet are fused^[24]. The septal leaflet is the least mobile of the three leaflets and is attached to the tricuspid annulus directly above the interventricular septum. These leaflets are suspended in a flexible D-shaped annulus, and tethered to the RV by the sub-valvular apparatus, which is composed of the papillary muscles and their associated chordae tendinae [Figure 1B and C]. The flexible nature of the TV annulus allows it to adapt and change shape during the cardiac cycle, such that it can increase up to 30% in area during end systole/early diastole^[23]. There are classically three papillary muscles: the anterior and posterior, which are most reliably present, and the septal papillary muscle, which is absent in approximately 20% of patients^[25]. However, the number of papillary muscles is highly variable and can range from two to nine distinct entities. The anterior papillary muscle is the largest, and, in some patients, the moderator band, which carries part of the right bundle branch of the atrioventricular (AV) conduction system, can be found to join the anterior papillary muscle.

The anatomy of adjacent structures is equally important in the operative management of TV disease. There are three important structures to consider: (1) the noncoronary sinus of Valsalva; (2) the conduction system; and (3) the right coronary artery^[24]. The noncoronary sinus is located near the commissure between the anterior and septal leaflets of the TV, increasing the risk of aortic perforation with transcatheter devices, which require anchoring in this area. The AV node lies in the apex of the triangle of Koch, and the bundle of His crosses the attachment to the septal leaflet approximately 3-5 mm posterior to the antero-septal commissure, which can result in heart block if there is excess pressure on the AV node^[26]. The right coronary artery originates from the right coronary sinus of Valsalva and courses adjacent to the tricuspid annulus, and it can result in cardiogenic shock secondary to direct injury to the right coronary artery in TV repair^[27].

ETIOLOGIES OF TR

TR can be divided into two overarching categories: primary and secondary. Primary TR indicates dysfunction of the valve leaflets or chordae and makes up about 10% of TR in adults^[3]. Causes of primary TR include: leaflet damage due to implanted devices, such as pacemakers and catheters; myxomatous valve disease; carcinoid heart syndrome; congenital anomalies of cardiac development; endocarditis; and rheumatic valve disease. In the absence of other cardiac disease, isolated moderate to severe primary TR carries with it an estimated $1.6 \times$ risk of mortality over the general population^[1,3].

Secondary TR makes up the remaining 90% of TR in adults and results from right ventricular remodeling in the presence of an otherwise normal TV. Etiologies are diverse and include chronic atrial fibrillation, pulmonary hypertension, left ventricular failure, left to right shunts, right ventricular infarcts, and cardiomyopathies^[28]. Secondary TR is associated with papillary muscle displacement, and dilatation and remodeling of the TV annulus, leading to tethering or tenting of the TV leaflets and progressive RV dysfunction.

CLINICAL IMPACT OF TR

It is estimated that about 1.6 million Americans have moderate to severe TR, while only about 8000 will undergo TV surgery annually^[3,28]. TV surgery carries a high risk of mortality compared to other cardiac operations: overall in-hospital mortality from isolated TV repair has been reported at around 8.5%, remaining stable over the past decade; this is compared to the 1%-5% mortality expected with isolated repair of any of the other three main cardiac valves^[11,12]. This is likely reflective of the late referral of these patients, such that, by the time of surgical evaluation, they often have systemic manifestations of right heart failure (i.e., coagulopathy, hepatic dysfunction, and renal failure). This is in contrast to the paradigm for intervention on left-sided valves, which is to repair or replace before the onset of structural changes to the heart^[9].

TIMING AND METHODS OF TRICUSPID INTERVENTION

The 2014 American College of Cardiology/American Heart Association valvular heart disease guidelines strongly recommend isolated TV surgery in severe symptomatic tricuspid stenosis, recommend isolated TV surgery in patients with severe TR who do not respond to medical therapy, and recommend isolated TV surgery in asymptomatic patients with severe TR and at least moderate RV dilation or dysfunction^[9]. Despite these recommendations, there remains a large disparity between the number of affected patients and the number surgically repaired^[11,12]. It has been suggested that early surgery should be considered in severe TR with RV dilation before the onset of symptoms^[6,14]. There are currently no significant published data on an accurate way to assess the TV and RV to determine the potential clinical evolution or recommend the timing of intervention for isolated TR.

Various surgical methods have been applied to repair of the TV, including leaflet augmentation, suture annuloplasty, and the “clover” technique of suturing the center-point of each of the tricuspid leaflets together^[29]. The most common method of open surgical repair is ring annuloplasty, whereby a prosthetic, incomplete ring is sutured to the tricuspid annulus to decrease annular size, restore leaflet coaptation, and prevent further annular enlargement [Figure 2A]^[29]. Because of the high-risk nature of open tricuspid surgery, and the general presence of comorbid conditions among patients with TR, significant interest exists in percutaneous options for tricuspid repair. Methods to deploy the MitraClip (a percutaneous clip designed for mitral valve repair) in the tricuspid position have yielded positive initial results, and work is ongoing to design and test dedicated tricuspid clips and deployment devices [Figure 2B]^[30].

INCIDENCE OF RV FAILURE AFTER REPAIR AND NEED FOR MECHANICAL RV SUPPORT

The RV is more sensitive to changes in preload and afterload than is the LV^[31,32]. RV function can deteriorate in context of alterations in preload or afterload, or direct alterations of RV contractility due to infarction or ischemia. Importantly, acute increases in afterload are especially poorly tolerated by the thin-walled RV. Regardless of surgical or percutaneous repair type, correction of moderate to severe TR results in sudden exposure of the RV to increased afterload when the valve becomes competent. In patients with limited RV reserve, this can result in acute postoperative RV failure, a unique management challenge associated with increased complications and death after tricuspid surgery^[33]. The risk of RV dysfunction is higher in the setting of structural remodeling (RV dilation) and/or high pulmonary vascular resistance,

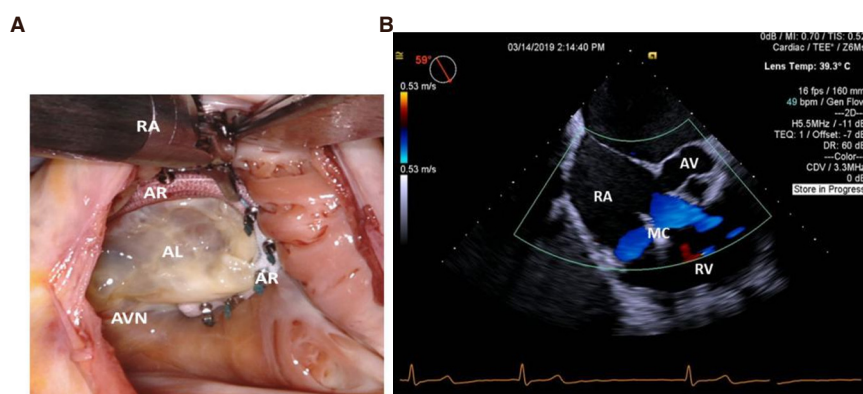


Figure 2. A: Intra-operative photograph of tricuspid annuloplasty ring; B: TEE of MitraClip repair of the tricuspid. Doppler blood flow on either side of the MitraClip during RV filling is seen in blue. AR: Annuloplasty ring; AL: Anterior leaflet; AVN: AV node; RA: Robot arm; RV: Right ventricle; AV: Aortic Valve; MC: MitraClip

and retrospective studies demonstrate significantly increased mortality and morbidity after TV repair in patients with preexisting reduced right ventricular function^[10]. However, there is a paucity of clinical data on the incidence of postoperative RV failure after isolated TV surgery.

Perioperative RV failure can be managed by manipulating preload, afterload, and contractility; however, these efforts may ultimately fail to provide adequate forward flow, leading to a vicious cycle wherein low cardiac output decreases coronary perfusion and further worsens RV function, necessitating initiation of mechanical support for the failing RV^[34]. Right ventricular mechanical support methods include percutaneous axial flow pumps such as the Impella RP (Abiomed, Danvers, MA), extracorporeal centrifugal pumps such as the ProtekDuo (LivaNova, London, England), or venoarterial extracorporeal membrane oxygenation (VA-ECMO). However, no predictive model yet exists to identify patients preoperatively who are at high risk of RV failure or who may benefit from preemptive initiation of RV mechanical support.

IMAGING OF THE RV

RV imaging has been traditionally deemed challenging due to its irregular shape (prohibiting geometric assumptions) and thin-walled structure with extensive trabeculations^[26,35]. Advances in imaging technology have facilitated higher resolution cardiac assessment, enabling reliable assessment of RV structure, function, and tissue characterizations.

Magnetic resonance imaging

Cardiac magnetic resonance imaging (MRI) is the gold standard for RV imaging, allowing quantification of ventricular volume throughout the cardiac cycle, identification of areas of scar using late gadolinium enhancement, and measurement of global and local RV strain [Figure 3A-C]^[36]. MRI-based population database studies of thousands of patients have defined normal values for RV mass, volume, and ejection fraction, indexed by age, race, and gender^[37]. Cardiac MRI also identifies subtle changes in dysfunctional myocardium, such as non-ischemic fibrosis, which correlate with decreased RV function but are not visible by other imaging modalities^[38]. High resolution MRI of *ex-vivo* hearts reveals the complex underlying structure of the RV myocardial fibers, which change angle from the base to the apex of the RV as well as transmurally through the RV wall [Figure 4]^[39]. Key drawbacks to conventional MRI include significant time for image acquisition, need for operator expertise, cost, closed space imaging environment (in which a patient is supine), and clinical patient stability to tolerate repeated respiratory breath-holds. Furthermore, many implantable cardiac devices are incompatible with MRI scanners, limiting imaging of patient

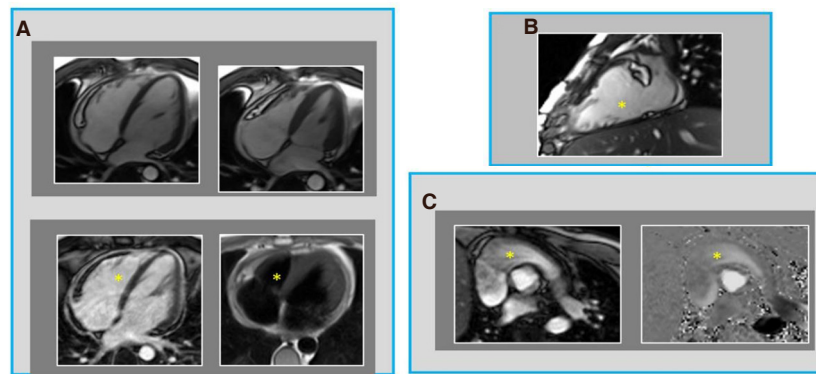


Figure 3. Representative examples of right ventricular assessment via cardiac magnetic resonance (CMR). A: Top: Functional assessment via steady state free precession cine-CMR (left = end-diastole, right = end-systole). Bottom: Myocardial tissue characterization via late gadolinium enhancement CMR (for infarction/fibrosis) and T1-weighted spin echo-CMR (for fat infiltration); B: Dedicated right ventricular functional assessment via cine-CMR; C: Flow assessment via phase-velocity encoded CMR (left = magnitude data, right = phase-encoded data). Asterisk corresponds to the right ventricle in each image

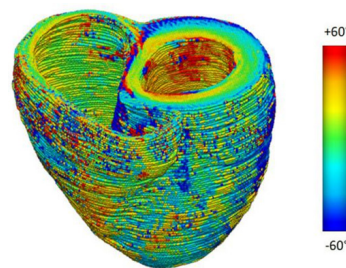


Figure 4. A color map of myofiber helix angles (angles defined with respect to the local circumferential direction) of the LV and RV, derived from an *ex vivo* diffusion tensor MRI of a human heart

populations with pacemakers and implantable defibrillators - patients specifically at high risk for TR and of interest to the current review. While use of MRI conditional devices and free breathing MRI techniques as well as growing expertise in cardiac MRI have bypassed some of these challenges, substantial impediments to widespread utilization persist, highlighting the importance of alternative imaging modalities for RV and TV assessment.

Echocardiography

Transthoracic echocardiography (TTE) is the least invasive and most readily available of all cardiac imaging modalities, and is safe for all patients regardless of clinical condition or presence of implantable devices. To maximize utility of this imaging modality, recent guidelines have been published to standardize RV echocardiography, establishing normal reference values and systematizing the approach to two-dimensional RV imaging^[40]. Unfortunately, the RV is retrosternal and visualization may be limited with TTE, depending on patient body habitus. However, general estimates of RV size and function are possible, and correlate well with RV dysfunction identified on MRI^[35]. Further information is gained with three-dimensional TTE, allowing quantification of RV ejection fraction (RVEF); depressed RVEF (< 35%-40%) measured by echo has been clinically linked to risk of major adverse cardiac events and cardiac death^[33,41]. Structural data from 3D TTE have also been used for RV shape analysis, which is discussed in further detail below. Trans-esophageal echo (TEE) is more invasive and is limited by the anterior position of the TV as compared to the MV, but should be employed when trans-thoracic windows are inadequate for tricuspid visualization^[40]. 3D TEE and TTE measures of RV dysfunction provide the closest correlate to RVEF as measured by MRI^[35,38].

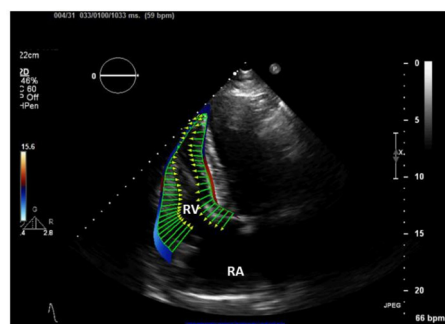


Figure 5. Transthoracic echo with RV speckle tracking based calculation of RV endocardial deformation (green vectors). RA: right atrium; RV: right ventricle

Strain

Myocardial strain is a measurement of relative deformation of a region of myocardium, from a reference state (e.g., end-diastole) to a deformed state (e.g., end-systole). In the context of continuum mechanics, myocardial strain is quantified using a second-order strain tensor given its complex 3D motion^[42]. Existing biomechanics and cardiac image analysis often report strains along local wall coordinate system, namely circumferential, longitudinal, and radial directions, because the local wall coordinate system is better aligned with the major mode of deformation of the heart. Global longitudinal strain, especially end-systolic longitudinal strain, is the most commonly used metric in cardiac imaging^[43]. For clinical purpose, negative end-systolic longitudinal strain indicates shortening of the LV with respect to end-diastole, with more negative values indicating greater shortening and therefore greater contractility of a region of the heart. Regional Strain can be measured by echocardiography, using techniques such as speckle tracking and tissue Doppler; RV strain measured by 2D echo has been shown to be a stronger predictor of outcomes in patients with heart failure than more conventional measures of cardiac function such as LV ejection fraction and B-type natriuretic peptide [Figure 5]^[44]. Echo-based RV strain also predicts mortality in heart failure patients listed for cardiac transplantation^[45]. As compared to other echocardiographic measures of RV function such as tricuspid annular plane systolic excursion, longitudinal strain measured by echo correlates most strongly with decreased RVEF measured by MRI^[38].

Multiple techniques have been developed for MRI-based measures of myocardial strain. Conventional MRI strain measures rely on “tagging”, i.e., the encoding of magnetic lines orthogonal to myocardial tissue^[46]. The tags are then tracked with post-processing software, yielding fine-detail information on the circumferential, longitudinal, and radial motion of the myocardium. One such MRI technique, known as Displacement ENcoding with Stimulated Echoes (DENSE) MRI, shows promising capability in imaging myocardial displacement on a voxel-by-voxel basis. This technique has been successfully applied to examine LV motion for both human and animals under physiological and pathological conditions^[47]. Effort has also recently been made to extend the analysis to the RV in healthy rat hearts^[48].

All tag-based techniques require high-resolution MRI and can be limited by the thin wall and trabeculations of the RV, which is often only one to two voxels thick, especially on the free wall. A further limitation is the need for additional time in the MRI scanner for each specialized sequence obtained, which can be a significant factor for patients with TR and heart failure symptoms, with limited ability to lay flat. Feature-tracking is a post-processing solution using standard cine MRI sequences (obtained during every cardiac MRI), applying software that identifies features in an image and tracks them in successive time points, to quantify motion of various regions of the RV^[46]. This method has its own limitations, including inability to track motion of less than one-pixel magnitude, and difficulty with out-of-plane motion in 2D images. Both DENSE and feature tracking are evolving tools for detailed analysis of RV function.

IMAGING OF THE TV

Imaging of the right heart, specifically the TV, is imperative to not only understanding TV anatomy and function in each individual to optimize future management, but also to identify the underlying etiology of TR. Thus, evaluation of the structural integrity of the TV leaflets in multiple views with cardiac imaging is critical, as this is the distinguishing feature between primary and secondary TR. With respect to assessing severity of functional TR, special attention should be paid to the tricuspid annulus (TA) size. This can be a challenge, as the tricuspid annulus is a dynamic structure with changing shape and size during respiratory and cardiac cycles, due to the contraction of the surrounding myocardium^[49]. Echocardiography is the primary modality used for evaluating the TV^[50,51]. The application of 2D TTE to image the TV has several limitations. First, 2D TTE does not provide a complete visualization of the TV; only two TV leaflets can be seen at the same time, while 3D TTE or TEE provides a view of all leaflets simultaneously. Second, 2D TTE underestimates the maximal dimension of the tricuspid annulus compared to 3D TTE or cardiac MRI^[52]. This is important to emphasize because there is a growing body of evidence demonstrating the importance of TA diameter as a marker for TV dysfunction, even in the absence of clinically significant TR^[53]: normal TA diameter in an adult is 28 ± 5 mm; concomitant TV surgical intervention at the time of left-sided valve surgery is recommended when the TA diameter is ≥ 40 mm^[9,54]. Third, 3D TTE provides a more reliable means to identify the TV leaflets and commissures compared to 2D TTE, which is important in evaluating the effect of damage secondary to pacemaker leads or other implanted devices in patients with primary, catheter-related TR^[26]. Lastly, in addition to TA diameter, decreased TV leaflet coaptation and degree of leaflet tethering are important prognostic factors predicting outcomes in patients undergoing tricuspid repair^[55]. Both of these anatomic pathologies are better visualized using 3D (rather than 2D) TTE.

MODELING TR

Large animal models

Foundational *in-vivo* experiments with sonomicrometry crystals placed in ovine RVs demonstrate that acute RV failure increases RV size, RV free wall strain, and size of the TA with corresponding increase in grade of TR^[56,57]. In these large-animal models, tricuspid repair with annuloplasty led to decreased RV size, normalized RV strain, and resolution of TR, supporting tricuspid annuloplasty as a therapy of choice for TR, with potential to improve RV function^[56]. Such animal models provide valuable insights into the origins of RV failure and choice of intervention; however, they are invasive, cumbersome, and by nature cannot be replicated in humans. Development of non-invasive techniques to reproduce these results is consequently an important clinical target.

Computational models of the RV, LV, and TV

With the above advances in cardiac imaging, it is possible to accurately define RV structure, including fiber angles, shape, and areas of ischemia and fibrosis; RV function, including strain in varying regions; TV structure, function, and pathology; and the geometric and functional relationships among the RV, LV, and TV. Using these imaging modalities, an important clinical target is development of accurate computational models of the LV, RV, and TV. Such models will help predict the mechanical effects of tricuspid repair and identify patients at risk of RV dysfunction after such repair. The following efforts are underway in model development.

Shape analysis

Cardiac shape is intimately related to function and provides visual evidence of the pathological changes of cardiac disease. This has been demonstrated most extensively in the LV, where remodeling towards a spherical shape correlates with decreased exercise tolerance and increased mortality^[58]. However, as discussed above, the shape of the RV is not simple to describe, and more nuanced methods are required. One such method is statistical shape analysis, a mathematical tool allowing non-invasive identification of

patterns of cardiac structure, which can then be related to function and correlated with clinical outcomes. This has been previously used on the RV to identify differences in patients with pulmonary hypertension (PH) compared to normal controls, demonstrating that patients with PH have increased RV eccentricity (a rounder shape), with bulging of the apex and the tricuspid annulus^[59]. Increased RV size and sphericity have also been associated with the known cardiovascular risk factors of hypertension, diabetes, obesity, and smoking^[60]. In congenital heart disease, for patients with single-ventricle pathology, changes in ventricular shape have been clinically correlated to symptom severity^[58]. As data accrue for different RV and TV pathologies, shape analysis will allow creation of non-invasive predictive tools of disease severity and outcomes.

Finite element modeling

Finite element (FE) modeling is a tool that allows the calculation of the stress-strain behavior of complex materials by breaking them down into small pieces, or elements. This tool has been applied for the last three decades to increase understanding of cardiac mechanics in diverse ways, including modeling the LV and mitral valve and simulating mitral ring annuloplasty, mitral leaflet resection, and percutaneous mitral clip application^[19-22,61]. Modeling of the TV has lagged behind models of the left heart; the RV and TV are more complex in shape and behavior than the LV and mitral valve; their anatomy is more variable between patients and between time points in the same patient; the right and left heart are interdependent, with RV behavior altered by LV pressure and by the function of the interventricular septum; and, to model the right heart, some measures of right-sided cavity pressures are required, which typically involve invasive procedures such as cardiac catheterization. Furthermore, extant computational models ignore the complex interaction between the TV and RV, and as yet, no patient-specific models of the RV + TV, or the LV + RV + TV, have been created. Development of such models will allow direct comparison of different repairs for a specific patient to determine the ideal repair type, accounting for the effect of such repairs on the ventricles and valve as a unit.

Modeling the RV + LV

To create an FE model of the myocardium, the 3D geometry of the ventricles is first captured with cardiac imaging, using echo, computed tomography (CT), or cardiac MRI. These images are “contoured”, outlining the endocardium and epicardium, to create a virtual mesh of the LV, RV, or both. The stress-strain relationships, or material property laws, of regions of the myocardium are then specified. Multiple formulations of these material property laws exist; our lab employs the version developed by Drs. McCulloch and Guccione (CMISS/Continuity)^[62]. These laws are based directly on the 3D fiber angle distributions of the myocardium [Figure 4], are commonly used, and have been validated experimentally under multiple conditions^[63,64]. Biventricular models of rat, swine, and canine hearts have been created with this technique to demonstrate increased myocardial stress in heart failure and pressure overload^[65-67]. In addition, of more direct clinical relevance, human, patient-specific biventricular models have been created to accurately predict the effect of cardiac resynchronization therapy^[68]. To date, these models have not examined the ventricles in patients with TR, and they have not incorporated the TV.

Modeling the isolated TV

To create patient-specific models of the TV, high-resolution images are required to describe its complex valvular geometry. Initial studies focused on excised cadaver valves, which are stationary and easy to structurally define^[69]. In living patients, 3D echocardiography images of the TV can be contoured in individual slices at a particular point in the cardiac cycle, to obtain a mesh of the overall valvular structure. Material properties are then applied as described above for the myocardium; these properties have been obtained experimentally from excised leaflet tissue, using predominantly biaxial stretching to determine stress-strain relationships^[70]. Using these techniques, the first computational model of the TV, based on excised human cadaver tissue, was created in 2010^[69]. Initial patient-specific models of the

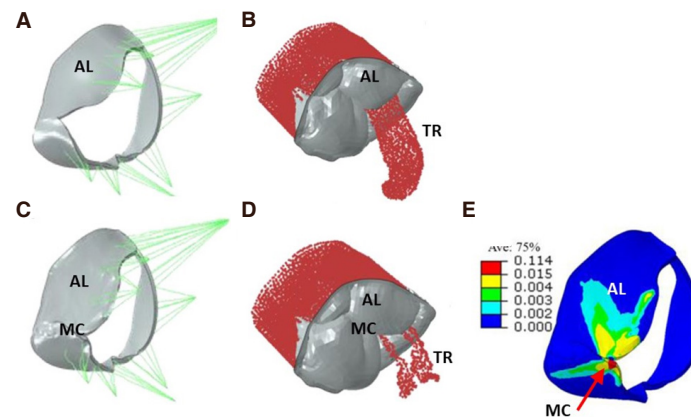


Figure 6. FE model with simulated tricuspid regurgitation (TR) before and after virtual MitraClip (MC). A: baseline mode; B: with TR jet; C: model after virtual MC; D: showing a reduced amount of TR and E) leaflet stress after virtual MC. AL: Anterior leaflet

isolated TV in healthy subjects have established normal values for stress and strain in the valve leaflets and chordae^[18,49]; the first foray into modeling TV pathology modeled the geometry of intact and prolapsing valve leaflets based on multi-slice CT images^[71]; and the first simulated tricuspid repair incorporated fluid-structure interaction models of MitraClip application to a regurgitant TV, demonstrating the utility of percutaneous therapy as an addition to the limited armamentarium of TR treatments [Figure 6]^[17]. The details of fluid-structure interaction are beyond the scope of this review; however, incorporating the specifics of regurgitant volumes, as well as the number and direction of regurgitant jets, does allow further accuracy in modeling, providing finer details of the effect of specific valvular repair techniques. Currently, no computational model of the TV includes the RV or LV, limiting investigations into functional TR.

Future directions: modeling the TV + RV + LV

Creating a patient-specific model of the TV + RV + LV is within the current bounds of computational and imaging possibility. This model will allow description of such pathologies as TR secondary to LV failure and prediction of RV dysfunction after tricuspid repair. The addition of the ventricles into models of TR will also delineate the ventricular effects of percutaneous tricuspid interventions, as well as tricuspid repair; because abnormal ventricular strain predicts adverse ventricular remodeling and repair failure over time, including the ventricles into mechanical models of tricuspid disease is a key component of predicting the long-term effects of tricuspid interventions. As statistical shape analysis techniques identify key changes in RV and LV geometry by varying etiologies of tricuspid disease, patients may be classified by non-invasive imaging into groups according to disease severity and type of ventricular derangement; with FE modeling techniques, the understanding of tricuspid interventions can move beyond patient specific modeling to generalized applications based on these imaging characteristics^[58].

CONCLUSION

With advances in cardiac imaging come new insights into the importance of the RV and TV in cardiovascular disease. TR remains an under-treated entity, and surgical tricuspid repair carries a high mortality rate. Computational modeling of the TV, RV, and LV based on high-resolution cardiac MRI and echocardiography allow creation of non-invasive patient-specific models of tricuspid repair, helping to solve several vexing clinical problems. First, right heart failure after cardiac surgery requires complex supportive care and carries with it a high mortality rate; no models yet exist to accurately predict or deter this outcome. A combination of statistical shape methods and FE models of the right heart may help identify at-risk RVs, allowing for better preoperative patient selection, and identification of patients who may need prophylactic right-sided mechanical circulatory support at the time of valve repair. Second, open

cardiac surgery is not a practical option for many patients with TR, due to the prevalence of comorbid conditions; computational modeling allows development and testing of novel devices for percutaneous repair, potentially reaching a greater population of patients with TR. Third, and beyond the scope of this review, the realm of congenital cardiac surgery involves treatment of patients with a broad spectrum of valvular and ventricular disease; the opportunity to model such repairs in-silico prior to operating on an individual patient provides for customization of patient-specific repair types with deeper understanding of their immediate and long-term mechanical effects.

DECLARATIONS

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Review

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Mitral valve repair in infective endocarditis: which evidence?

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Abstract

Infective endocarditis is still a challenging clinical condition undergoing continuous epidemiologic changes, involving both the population at risk and the microbiological etiology. Antibiotic treatment alone is not effective in presence of structural abnormalities of native valves, leading to heart failure and/or to high embolic risk. Moreover, some patients despite being treated with antibiotics, their valve leaflets may undergo profound degenerative changes responsible for significant hemodynamic abnormalities. The resulting valve disease may lead to a decreased life expectancy. In these patients, surgery was the only independent factor associated with long-term survival. Valve repair in the last two decades has demonstrated to be a valuable alternative to valve replacement in mitral valve endocarditis. Mitral valve repair was associated with decreased hospital and long-term mortality, recurrent endocarditis and overall need for reoperation in comparison to valve replacement. Furthermore, repair limits the risks related to prolonged anticoagulation. However, these results suffer from several limitations: results of repair are dependent on the experience of surgical team, valve damage is usually less extended in patients undergoing repair as well clinical and hemodynamic impairment are more severe in patients undergoing replacement. Therefore, although repair should be preferred when technically feasible caution must be paid to assess its absolute superiority in comparison to valve replacement.

Keywords: Infective endocarditis, valve repair, valve replacement, survival

INTRODUCTION

Epidemiology of native valve endocarditis

The epidemiology of native valve endocarditis has undergone significant changes over the past few decades. The incidence is reported between 1.5 and 5 per 100,000 persons per year, although large epidemiological



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studies have shown a continuous increasing trend^[1-3]. This is associated with a significant increase in economic costs, above \$120,000 per patient^[4]. Both native and prosthetic valve endocarditis epidemiology are affected by the increasing number of health care-associated infections, contributing for not less than 25% of overall cases. Hemodialysis, implantable cardiac devices, venous catheters, immunosuppression, and intravenous drug use are main risk factors for infective endocarditis (IE)^[5,6]. Patients are older and frail, often affected by serious comorbidities. Infections due to staphylococci are continuously increasing in comparison to oral streptococci. Staphylococci at present are the most frequent etiologic organism. A Danish study showed that between 1957 and 1990, *Staphylococcus aureus* bacteremia increased from 3 to 20 per 100,000 person-years, paralleling the increase of hospital admissions and invasive medical procedures^[7]. Hand-hygiene, barrier precautions, and antisepsis are effective measures in reducing the rate of bacteremia. Microbial eradication is the goal of treatment in IE. Broad spectrum empiric antibiotic treatment should be started as soon as possible, immediately after collection of samples for blood-culture. Identification of etiologic microorganism allows tailored therapy to be administered, even if antibiotic resistance is a growing worrying phenomenon^[2,8]. Overview of medical management of IE is beyond the aim of present review. It must be emphasized that in patients in medical treatment, a close reassessment of clinical, laboratory and echocardiographic findings is mandatory since infective endocarditis may rapidly progress even under antibiotic treatment. Moreover, in patients without indication to urgent surgery, hemodynamic changes related to residual valve damage are associated with a decreased life expectancy. A large study conducted in France showed that surgical treatment was the only independent predictor of long-term survival in patients admitted to hospital for infective endocarditis^[9].

Indications to surgery

The indications for surgery in patients with IE have been defined by American Association for Thoracic Surgery, American Heart/American College of Cardiology and European Heart Society (ESC)^[10-12]. According to guidelines, surgery is defined as urgent, usually within index hospitalization before completion of a full course of antibiotics. The ESC guidelines distinguish emergency surgery (within 24 h) from urgent surgery (within a few days), or elective surgery (after 1 to 2 weeks) of antibiotic therapy. More than 50% of patients with native valve endocarditis needs surgery, more frequently on an urgent basis.

Hemodynamic impairment due to severe valve regurgitation, characterized by severe left ventricular dysfunction, refractory pulmonary edema and/or cardiogenic shock, is the more frequent indication for urgent surgery (class I level of evidence B). Early surgery is also indicated in the case of extension of the infection beyond the valve annulus, with perivalvular abscess, fistula, or pseudoaneurysm formation or with involvement of heart conduction system (AV block).

In native valve endocarditis, extra valvular spreading occurs in about 30% of cases. Urgent surgery may be needed to prevent potential catastrophic embolism in the presence of large (> 10 mm) and/or mobile vegetations. A randomized study showed that in native valve endocarditis characterized by large vegetations (> 10 mm) and/or severe valve regurgitation, surgery was associated with a significant decreased risk of death and embolic events in comparison with medical care^[13].

Neurologic involvement, not rarely asymptomatic, may be demonstrated in about 50% of patients with infective endocarditis. *Staphylococcus aureus* is the more frequent etiologic agent. The timing of surgery in patients after embolic stroke is challenging and controlled studies are not available. Delay in surgery may be associated with risk of recurrent embolism, however patients undergoing early surgery are at the risk of hemorrhagic transformation of the stroke since full anticoagulation is needed for cardiopulmonary by-pass. Moreover, hypoperfusion during surgery may be associated to an extension of ischemic area. A careful multidisciplinary evaluation weighing the relative role of severity of hemodynamic impairment against that of neurologic damage may help in scheduling surgery. Results from observational studies suggest that the

Table 1. The endocarditis team - role

Should have meetings on a regular basis in order to discuss cases, take surgical decisions, and define the type of follow-up
Chooses the type, duration, and mode of follow up of antibiotic therapy following the current guidelines
Should participate in national or international registries, publicly report the mortality and morbidity of their center, and be involved in a quality improvement and patient education programs
The follow-up should be organized on an outpatient visit basis at a frequency depending on the patient's clinical status (ideally at 1, 3, 6, and 12 months after hospital discharge, since the majority of events occur during this period)

Modified from ref.^[12]

Table 2. Factors related to choice of surgical strategy

Favor mitral valve repair	Favor mitral valve replacement
Single scallop or leaflet valve involvement	Extensive damage of anterior leaflet
Isolated vegetation	Large lesion of posterior leaflet and/or commissures
Valve perforation	Annular abscesses
Less extensive valve damage with enough tissue after debridement	Low volume repair centers in patients with severe valve damage
allowing repair with patches, neo-chordae, annular ring	Cardiogenic shock

risk of further neurologic impairment is related more to severity of baseline neurologic damage than to surgery timing. In the case of hemorrhagic stroke surgery should be delayed at least 30 days.

ESC guidelines suggest that patients with IE should be referred to specialist centers and managed with a multidisciplinary specialized team (the “Endocarditis Team”) including “at least cardiac surgeons, cardiologists, anesthesiologists, infectious disease specialists, microbiologists and, when available, specialists in valve diseases, CHD, pacemaker extraction, echocardiography and other cardiac imaging techniques, neurologists, and facilities for neurosurgery and interventional neuroradiology” [Table 1]^[11]. An approach by a formalized multidisciplinary team led to a reduction in in-hospital and long-term mortality. This decrease in mortality was even more impressive since patients were old and suffered from several comorbidities^[14,15].

Despite a clear indication, about 25% of all patients with IE still do not undergo surgery. Independent factors associated with a decision not to proceed with surgery include liver disease [odds ratio (OR) for surgery: 0.16; 95%CI: 0.04-0.64], stroke before surgical decision (OR = 0.54 ; 95%CI: 0.32-0.90), and *Staphylococcus aureus* infection (OR = 0.50; 95%CI: 0.30-0.85)^[16].

A comprehensive multidisciplinary evaluation in IE may be extremely useful for the individualisation of proper surgical strategy, whose objectives are total removal of infected tissues and reconstruction of cardiac morphology.

With mitral and tricuspid involvement, the extent of valvular destruction and of extra valvular extension are the main determinant in the choice between valve repair and replacement. Involvement of valve leaflets, including perforation, favors mitral valve (MV) repair. Neo-chordae may be used in the case of isolated or multiple ruptured chordae. Extensive damage of a single leaflet or abscess formation are not necessarily a contraindication for valve repair. Extensive damage of the anterior leaflet, large lesions involving the posterior leaflet or the MV commissures and perivalvular extension with annular abscesses are considered the main technical difficulties for mitral repair. Intraoperative assessment of the valve after initial debridement allows to evaluate whether the remaining tissue is of sufficient quality to achieve a durable result. Intraoperative transesophageal echocardiography should guide surgeons in assessing residual valve regurgitation after valve repair [Table 2].

MV repair vs. valve replacement

No randomized trial has been conducted comparing MV repair and replacement in patients with infective endocarditis. In light of present evidence, it may be ethically unfeasible in the future to conduct such

Table 3. Results of study comparing MV repair and replacement in infective endocarditis

Retrospective studies	Repair <i>n</i> (%) <i>vs.</i> replacement	Hospital mortality repair	Hospital mortality replacement	Follow-up mortality repair	Follow-up mortality replacement	Microbiology repair-replacement	IE recurrence
El Gabry <i>et al.</i> ^[19]	35 (NA)	11%	-	23% 10 m	-	Strept 28% Staph 29% Other 11% Culture 31% Negative	5%
Alkhouli <i>et al.</i> ^[18]	NA (25%)	8.1%	11.3%	-	-	NA	0 %
Tepsuwan <i>et al.</i> ^[22]	114 (52%)	-	-	9% 1 y	30% 1 y	Strept 51%-50% Staph 11%-2% Other 6%-16% Culture 32%-32% Negative	-
Lee <i>et al.</i> ^[23]	454 (21.2%)	6.3%	10.8%	19% 4 y	31% 4 y	Strept 60%-48% Staph 30%-40% Other 10%-12%	-
Rostagno <i>et al.</i> ^[20]	34 (68%)	11%	15%	14.7% 1 y	22% 1 y	Strept 52%-36% Staph 31%-34% Other 10%-12% Culture 7%-18% Negative	2.9%
Solari <i>et al.</i> ^[21]	155 (81%)	11.6%	29.7%	43% 15 y	64% 15 y	Strept 36%-19% Staph 43%-54% Other 11%-12% Culture 10%-15% Negative	2.4%
Cuerpo <i>et al.</i> ^[24]	68 (18.4%)	16%	27%	20% 1 y	30.7% 1 y	Strept 38%-38% Staph 26%-32% Other 20%-24% Culture 6%-6% Negative	0.1%
Review/meta-analysis Feringa <i>et al.</i> ^[17]	490 (39%)	2.3%	14.2%	7.8% 3 y	40.5% 3 y	Strept 43%-42% Staph 24%-31% Other 13%-7% Culture 20%-20% Negative	1.8%
Harky <i>et al.</i> ^[25]	2906 (32%)	5%	10%	8.3% 1 y	17.3% 1 y	Strept 43%-33% Staph 33%-35% Other 13%-14% Culture 11%-18% Negative	2.9%

y: year; m: months; Strept: Streptococcus species; Staph: Staphylococcus species; NA: not available; IE: infective endocarditis; MV: mitral valve

randomized studies. Available information relies on observational studies often reporting a small number of patients. Valve repair has decreased risk of prolonged anticoagulation and to left ventricular geometric changes, which are associated with valve replacement. In a pivotal review by Feringa *et al.*^[17], 470/1194 (39%) patients with MV endocarditis underwent valve repair. In-hospital mortality (2.3% *vs.* 14.4%) and long-term mortality (7.8% *vs.* 40.5%) were significantly lower after valve repair in comparison with replacement. Moreover, after MV repair, the authors reported a significant decrease in the rates of early and late reoperation, early and late cerebrovascular events and late recurrent endocarditis.

In the last decade, the number of patients with IE undergoing MV surgery has increased significantly as well as the number of mitral valve repair^[18]. However, the percentage of repair *vs.* replacement showed high variability in different centers. Overall MV repair is associated with a better outcome in comparison to valve replacement both in term of in-hospital and long-term mortality^[19-25]. Furthermore, the risk of recurrence of endocarditis is significantly lower after MV repair. Finally MV repair was associated with shorter length of hospitalization and reduced cost. Results were not significant influenced by the need

for concomitant surgical procedures (CABG, aortic replacement, tricuspid repair/replacement), although MV replacement was sometimes preferred in these patients^[25]. It must be emphasized that patients who underwent MV replacement were on average older with more preoperative comorbidities and severe clinical conditions^[22].

CONCLUSION

Results from previous investigations [Table 3] suggest that MV repair in native MV endocarditis is associated with a significant decrease in early and long-mortality, endocarditis recurrence and need for re-intervention. In particular, the risk of reinfection is significantly higher (from 8% to 27% vs. less than 3%) in patients with MV replacement. The number of patients undergoing repair is still highly variable in different centers (from 18% to 80%). Experience of surgical team in repair techniques is essential particularly when facing extensive damage of MV components. Low-volume centers show significantly lower repair rates with suboptimal results and higher re-intervention rate. Several limitations should be considered in the evaluation of studies reporting favorable results of MV repair in infective endocarditis.

Firstly, all published investigations are retrospective observational studies and no randomized control study has ever done comparing the two techniques. Considering present evidence, it appears difficult that it will ever be proposed in the future. Nevertheless, a selection bias may affect most of reported experience since valve replacement was mainly performed in patients with more severe clinical conditions and extensive valve damage. This may have contributed both to higher hospital and long-term mortality reported in valve replacement group. Concomitant surgical procedures were performed more frequently in patients undergoing valve replacement than in patients with MV repair, carrying a higher surgical risk.

Thirdly in the everyday life, management of MV endocarditis depends on experience of surgical teams. This appears the main determinant in the strategy adopted for surgery and its outcomes. A lower repair rates and less optimal outcomes, with residual valve regurgitation and need for re-intervention, has been reported in low-volume centers.

Finally, microbiological etiology may play a relevant role in establish surgical strategy. *Staphylococcus aureus* related IE are associated with more extensive valve lesions and significantly higher mortality. A lower repair rate, although not uniformly reported in different centers, is often observed in patients with *staphylococcus* infection.

In conclusion, MV repair may be considered the first choice treatment of MV endocarditis “with favorable anatomy” in experienced centers. However, multidisciplinary evaluation should direct definite choice in the individual patient.

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Perspective

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Role of exosome-associated adenosine in promoting angiogenesis

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Abstract

The role of exosomes in different physiological and pathological settings is an emerging field of great current interest. One hallmark of exosomes is the promotion of blood vessel formation. Exosomes of different cellular origin have been shown to be enriched in angiogenic proteins which directly promote angiogenesis. In addition, exosomes are also efficacious producers of adenosine and potentially encapsulate adenosine in their lumen. The adenosine content of exosomes has been linked to their immunosuppressive effects. In this communication, we consider the possibility that adenosine production by tumor cell-derived exosomes may represent a novel pathway for stimulation of angiogenesis in the tumor microenvironment.

Keywords: Exosomes, extracellular vesicles, angiogenesis, adenosine, adenosine receptors, endothelial cells

INTRODUCTION

Extracellular vesicles (EVs) and their functional role in health and disease are of great current interest. Especially exosomes, a virus-size subset of EVs (~30-100 nm), show great potential as disease biomarkers, drug carriers, or therapeutics. They are actively produced by parent cells and carry a complex cargo, which includes proteins, nucleic acids, and lipids^[1].



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ROLES OF EXOSOMES IN ANGIOGENESIS

One hallmark of exosomes is the promotion of angiogenesis. Skog *et al.*^[2] reported in 2008 that glioblastoma-derived exosomes contain mRNA, microRNA (miRNA), and angiogenic proteins and that these exosomes reprogram endothelial cells (ECs) to an angiogenic phenotype. A variety of research groups extended the work of Skog *et al.*^[2] addressing the pro-angiogenic functions of exosomes in different health and disease settings. Multiple pathways which are used by exosomes to stimulate blood vessel formation were uncovered in recent years and most research focuses on the direct interaction of exosomes with ECs^[3]. It was shown that exosomes can deliver signals to receptors on ECs, which activate the relevant molecular pathways and contribute to altered cellular responses^[3]. The reported ligands on the surface of exosomes which can induce an angiogenic response include vascular endothelial growth factor (VEGF), interleukin 6 (IL-6), IL-8, fibroblast growth factors (FGF), and urokinase-type plasminogen activator (uPA)^[2,4]. Besides surface-mediated receptor-ligand interactions, ECs internalize exosomes within 2-4 h^[4]. Numerous pathways are utilized by ECs for the internalization of exosomes, such as phagocytosis, micropinocytosis, or lipid raft-mediated internalization^[5]. However, the main uptake mechanism is endocytosis, as recently reported^[4]. The internalization of exosomes allows for the delivery of messages that are then translated by ECs^[2]. miRNAs are frequently described components of the exosome cargo which can induce pro-angiogenic responses in ECs^[6].

ADENOSINE PATHWAY AND ANGIOGENESIS

In addition to these well-described pathways, signaling via purines may also contribute to exosome-mediated effects on angiogenesis. One pathway that has not been studied thus far and that is particularly interesting in the context of exosomes and angiogenesis is the adenosine pathway. Extracellular adenosine exhibits a broad range of effects on cell cycle control, immunoregulation, and cytokine regulation through both direct and indirect mechanisms and ultimately leads to the progression of malignant diseases^[7]. Additionally, adenosine has been recognized as a potent stimulator of angiogenesis and Adair estimated that adenosine can contribute up to 50%-70% of the angiogenic response in some situations^[8,9]. Adair *et al.*^[10] also described that intravenous infusion of adenosine can increase plasma levels of VEGF in humans. In cultured cells, it was shown that adenosine induces EC proliferation and migration by increasing levels of VEGF and other angiogenic growth factors^[9]. Additionally, adenosine can stimulate EC proliferation independently of VEGF, which probably involves modulation of other pro-angiogenic and anti-angiogenic growth factors and perhaps an intracellular mechanism^[9].

Initiation of the adenosine signaling cascade requires binding of adenosine to the specific adenosine receptors (ADORs), which are divided into four subtypes: A₁R, A_{2A}R, A_{2B}R and A₃R. The main difference between these receptors is the affinity for adenosine, since adenosine binds to A₁R, A_{2A}R and A₃R in the nanomolar range, whereas adenosine binds to A_{2B}R in the micromolar range^[11]. This indicates that physiologic concentrations are sufficient to induce A₁R-, A_{2A}R- and A₃R-mediated signaling, and elevated adenosine concentrations, which are usually found in inflammatory or tumor microenvironments, can activate an A_{2B}R-signaling cascade^[11]. It was shown that adenosine induces EC growth by activating A_{2B}R and that A_{2B}R plays a critical role in regulating vascular remodeling associated with EC proliferation in angiogenesis, collateral vessel development, and recovery after vascular injury^[12,13]. However, the other receptor subtypes have also been reported to be involved in angiogenesis. Although there is no direct stimulation of ECs, ADORs act in a functional cooperative fashion to promote angiogenesis by a paracrine mechanism involving the differential expression and secretion of angiogenic factors from other cell types^[14,15]. Ernens *et al.*^[16] reported that adenosine upregulates thrombospondin-1 production by macrophages via A_{2A}R and A_{2B}R, resulting in stimulation of angiogenesis. Adenosine also stimulates the production of VEGF, IL-8, and angiopoietin-1 from mast cells via A_{2B}R and A₃R, as reported by Feoktistov *et al.*^[15]. Clark *et al.*^[17] demonstrated that A₁R activation elicits an angiogenic response and promotes VEGF-release from cultured monocytes. Thus, the literature suggests that all ADORs are involved in regulating blood vessel development, and that the underlying pathway for stimulating angiogenesis is highly context/microenvironment-dependent.

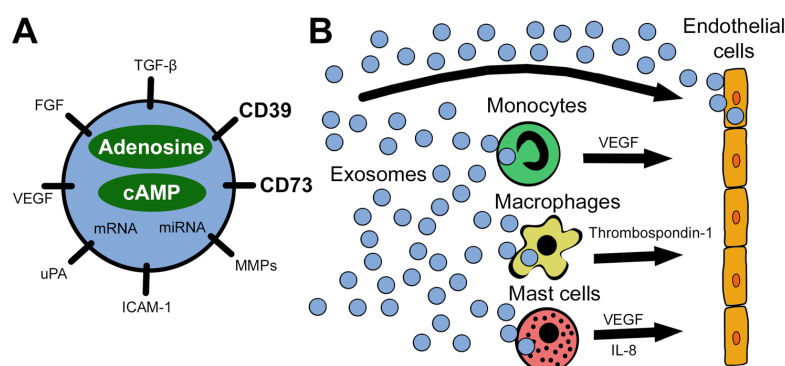


Figure 1. A schematic visualizing the reprogramming of endothelial cells by exosomes. A: exosomes carry a variety of pro-angiogenic factors including the ectonucleotidases CD39 and CD73. Besides surface-bound molecules, exosomes encapsulate pro-angiogenic factors, nucleic acids, adenosine, and cAMP, as well as other purine metabolites; B: tumor-derived exosomes interact directly with endothelial cells or reprogram other cells in the tumor microenvironment to release pro-angiogenic factors. All these interactions involve signaling via adenosine receptors expressed on responder cells: specifically, $A_{2b}R$ on endothelial cells, A_1R on monocytes, $A_{2A}R$ and $A_{2b}R$ on macrophages, and $A_{2b}R$ and A_3R on mast cells. miRNA: microRNA; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; TGF-β: transforming growth factor beta; MMPs: matrix metalloproteinases; ICAM-1: intercellular adhesion molecule 1; uPA: urokinase-type plasminogen activator; IL-8: interleukin 8; cAMP: cyclic adenosine monophosphate

ADENOSINE-MEDIATED STIMULATION OF ANGIOGENESIS BY EXOSOMES

Taken together, the above findings suggest the presence of a possible link between angiogenesis and exosome-associated adenosine, as presented in Figure 1. Specifically, it was shown that exosomes contribute to extracellular adenosine production and hence might modulate ECs indirectly^[18]. Exosomes from diverse cancer cell types exhibit potent ATP- and 5'-AMP-phosphohydrolytic activity, partly attributed to activity of CD39 and CD73, respectively, on the surface of exosomes^[19]. This exosome-generated adenosine is functionally active and can trigger a cyclic adenosine monophosphate (cAMP) response in $A_{2A}R$ -positive but not $A_{2A}R$ -negative cells^[19].

While it is well recognized that exosomes encapsulate functional proteins and nucleic acids, it is currently unclear whether purine metabolites are encapsulated within exosomes. Sayner *et al.*^[20] recently reported that EVs encapsulate cAMP to provide an additional second messenger compartment. Our preliminary data show that exosomes not only encapsulate cAMP but also adenosine and adenosine metabolites (inosine, hypoxanthine, and xanthine). This may indicate that exosomes can induce ADOR signaling independently of the production of adenosine by exosome-associated enzymes. Adenosine in the lumen of exosomes is protected against uptake and metabolism by other cells, such as red blood cells. Thus, exosomal adenosine may represent a mechanism for adenosine to serve as a circulating, rather than strictly local, factor.

Exosome-associated adenosine emerges as a potential stimulator of angiogenesis in different settings. Studying this pathway promises to uncover important aspects of exosome functions, which are ultimately leading to the stimulation of blood vessel formation. Understanding this pathway might also help to find targets for the stimulation or inhibition of exosome-induced angiogenesis.

DECLARATIONS

Authors' contributions

Writing of the manuscript: Ludwig N

Supervision and editing of the manuscript: Jackson EK, Whiteside TL

Availability of data and materials

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Editorial

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Advancing the care of patients with cerebrovascular disease: editorial

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Contemporary understanding of cerebrovascular disease has seen rapid development coupled with translational clinical advancements. Increased knowledge of the basic processes of the disease has provided the basis for significant developments in the detection, prevention, and treatment of focal and systemic conditions whose combined effects present a disproportionately high burden on the health care system, associate with multiple significant co-morbidities, and often carry high and protracted morbidity and mortality^[1]. Such trends have been the result of an increased progressive and coordinated effort in various related basic science and clinical fields. In this editorial, we review some important examples of the work providing multidisciplinary contributions to the understanding and treatment of Cerebrovascular Disease, as featured in this special issue.

Critical to the development of effective prevention and treatment strategies is an advanced understanding of underlying disease pathophysiology and molecular biology. Accordingly, Padarti *et al.*^[2] provided a comprehensive and well written review on the epidemiology, cellular biology, and phenotypic expression of cerebral cavernous malformations (CCM) in the context of known CCM proteins. The article effectively describes disease biology in a stepwise fashion following a brief summary on present epidemiology and clinical presentation^[2]. Current understanding of the structural biology and genotype of each individual implicated protein complex is provided prior to a discussion on consequent epigenetic processes and inter- and intracellular interactions^[2]. A thoughtful review of possible mechanisms of disease is then presented, supported by current *in vitro* and *in vivo* data and anecdotal evidence^[2]. Together, the authors provided a succinct and comprehensive report on our modern understanding of CCM pathobiology and suggested potential routes of future investigation. Such reviews are a vital substrate in the development of



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translational investigations and clinical advancement.

Along with an understanding of disease basic science, modern clinical practice mandates introspective evaluation of current practice. Such methods allow for the identification of areas of improvement in treatment and prevention strategies. Im *et al.*^[3] investigated the compliance and appropriate prescription of aspirin in the primary prevention of cerebrovascular and cardiovascular disease. The authors prospectively analyzed the baseline demographics of 1125 patients without prior vascular disease undergoing physicals with primary care physicians at their center^[3]. Using patient demographics, the authors evaluated discrepancies in aspirin use at the time of their routine medical checkup^[3]. The authors found that, while aspirin administration was indicated^[4] in 23.6% of patients, only 3.9% of patients were actively taking the drug^[3]. Investigation of this discrepancy illustrated that only 10% of patients were appropriately prescribed aspirin based on current international guidelines, and thus through secondary calculation reasoned that 6% in total were taking the medicine appropriately^[3]. The authors suggested that improvement in primary prevention may be made through increased education on aspirin use, at the level of both the prescriber and the patient^[3]. Such studies provide opportunities in the prevention of complex cerebrovascular disease by highlighting areas where active guidelines are not being employed efficiently.

Similarly, effective selection of patients to undergo primary prevention of treatment measures allows for efficient resource management and decreases risks of adverse events. As such, growing interest in radiographic and molecular biomarkers has sparked interest in multiple aspects of cerebrovascular disease. In their prospective analysis, Mueller *et al.*^[5] sought to characterize radiographic features and various potential serum biomarkers and their association with clinical presentation of atherosclerotic carotid disease. Transcranial ultrasound bubble studies and radiotracer computer tomography angiography were utilized to evaluate the stability of plaque formation in symptomatic and asymptomatic patients with clinically significant carotid stenosis^[5]. Given the uncertain nature regarding the stability of atherosclerotic plaques and their tendency to rupture and produce atheroembolic events, the subject has seen significant interest in active studies involving the evaluation of prognostic factors and underlying biology in plaque formation^[6]. The authors chose to compare important clinical indicators, such as propensity of microemboli formation, with biomarkers under investigation, concluding that ICAM-1 was associated with thrombotic plaque formation^[5].

As an adjunct to active research, case reports provide valuable anecdotal evidence of the variable presentation and management techniques of disease. Candelaresi *et al.*^[7] presented a well written report of a patient who developed posterior reversible encephalopathy syndrome attributable to the administration of the myeloma protease inhibitor bortezomib. The case details the clinical course and association with drug and discusses potential mechanisms^[5]. The patient in the case was ultimately successfully treated with systemic glucocorticoids^[5], a convenient option as such drugs are often a component of anti-myeloma drug regimens.

Cerebrovascular disease has undergone a recent and synergistic increase in disease understanding and treatment options. Such phenomena are only possible with continued and persistent multidisciplinary academic interest. Critical literature takes a variety of forms. Literature reviews provide a concise and relevant presentation of basic science knowledge of disease processes and may provide functional benefit to other basic scientists, clinicians, and translational scientists. Introspective evaluations of current disease practices allow quality improvement and an effective utilization of active management strategies. Studies identifying and validating disease biomarkers are increasingly necessary given the heterogeneous nature of multiple disease processes to assure effective patient selection and resource allocation. Finally, case reports allow investigators to be aware of variations of disease presentations and effects of seemingly unrelated events to provide anecdotal evidence that may affect clinical decision making or inspire connections in

scientific hypotheses. This special issue of *Vessel Plus* provides valuable contributions for those investigating cerebrovascular disease and treating patients harboring disease of the cerebral circulation.

DECLARATIONS

Authors' contributions

Drafting the work: Scullen T

Substantial contributions to the conception or design of the work, revised the work critically for important intellectual content, and made final approval of the version to be published: Dumont A

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Scullen T, Dumont A

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Both 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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Atherosclerosis microbiome: upcoming target for vaccine and drug development

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Abstract

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in adults and is one critical area of the medical sciences. Atherosclerosis is the main underlying pathology and is characterized by chronic inflammation of the arterial walls. The current treatment modalities for CVD target hypertension, hyperlipidemia and hemostasis, and suppress inflammation without directly addressing the origin of inflammation. Thus, many individuals with multiple classic risk factors for CVD do not experience acute ischemic events. Moreover, myocardial infarction and stroke continue to occur in up to two-thirds of all patients. Because many cardiovascular events have not been explained by genetics or other risk factors, and multiple epidemiologic studies have consistently suggested an infectious component, the introduction of entirely novel approaches for diagnostics and treatment that target infections are acutely needed. These complementary novel approaches addressing additional manageable risk factors such as infections will be based on the concept of personalized medicine to control CVD and achieve longevity, while also increasing the quality of life. There are a variety of avenues that could enable such novel approaches. These focus on the discovery and characterization of the infective component of atherosclerosis, the atherosclerosis microbiome. Specifically, we provide an update of the latest developments in the oral microbiome and its relation to CVD.

Keywords: Cardiovascular disease, atherosclerosis, stroke, inflammation, bacterial infection, periodontitis, periodontal pathogens, atheroma, *Porphyromonas gingivalis*



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INTRODUCTION

Cardiovascular disease (CVD) is the commonest cause of mortality and morbidity globally^[1,2]. Compared with the past, global progress in extending life expectancy is forecasted to be slower from 2016 to 2040. This trend resulted from predicted slowed advances on key drivers including stagnated gains on cardiovascular diseases, which was a major factor in historical improvements in life expectancy^[3].

Atherosclerosis is a chronic vascular inflammation associated with hypercholesterolemia, accumulation of lipids, hypertension, diabetes, smoking, smooth muscle cell proliferation, cell apoptosis, necrosis, fibrosis, and genetic factors. Atherosclerosis causes plaque accumulation, obstructing blood flow and contributes to acute ischemic events such as myocardial infarction or stroke. In these events, the arterial wall inflammatory lesion becomes destabilized, leading to plaque rupture and discharge of its necrotic core in the circulation, triggering coagulation and thrombosis. Such vulnerable plaques present the highest risk of acute events. The risk of atherosclerotic disease has been observed to be significantly higher in patients with periodontal disease, independently of other established risk factors.

Many CVD patients do not present any of the classical risk factors. Between 60%-70% of individuals with multiple cardiovascular disease risk factors have not experienced a cardiovascular event^[4] and only 50% of the CVD patients have been shown to have elevated serum cholesterol^[5]. While major statin trials report an average 28% reduction in low-density lipoprotein (LDL) cholesterol and a 31% reduction in relative risk, patients still have significant residual risk^[6]. Likewise, myocardial infarction and stroke continue to occur in up to two-thirds of all patients, even after many of these factors are addressed^[7]. This “forgotten” majority of patients leave wide open the door for exploration of risk factors that have not been adequately addressed to date.

While the importance of the traditional risk factors is well established, the data indicate additional factors contributing to atherogenesis. Infectious processes and products of the endogenous microbiome are capable to modulate atherosclerosis and its complications either directly, or indirectly, by eliciting local and systemic responses that potentiate atherogenesis. Here we will focus on bacterial infections as potential contributors to vascular inflammation, with an emphasis on periodontal pathogens as an established component of the atherosclerosis microbiome.

CURRENT VIEW OF THE INFECTIOUS COMPONENT OF ATHEROSCLEROSIS. ASSOCIATION OF PERIODONTITIS WITH CVD

The initiation of atherogenic process is typical for a chronic inflammatory disease. This process starts with recruitment of leukocytes from blood flow, mediated by a range of endothelial surface-expressed adhesins (more details in the study by Libby^[8]). The endothelial activation and subsequent leukocyte recruitment/transmigration in tissue is in response to an activating stimulus, which includes microbial constituents.

Gingivitis and its advanced stage, chronic periodontitis, are the most prevalent microbial infections in man. Only in recent decades has the association between periodontal diseases and systemic conditions such as coronary heart disease and stroke became subject to investigation^[9-16].

The largest genome network analysis (63,746 cases and 130,681 controls) identified lipid metabolism and inflammation as main pathways involved in the genetic predisposition to coronary artery disease (CAD). Specifically, the four most significant pathways mapping to putative genes involved in CAD are linked to lipid metabolism and inflammation, underscoring the causal role of these activities in the genetic etiology of CAD. However, the genetic variants strongly associated with CAD explain approximately only at most 10.6% of CAD heritability^[17-19].

Infection is an understudied contributing factor in vascular inflammation. Nevertheless, infectious component as a risk factor is supported by an abundance of epidemiological evidence^[20,21] and animal models. Multiple independent pathways of evidence already pinpoint inflammation as a key regulatory process that links multiple risk factors for atherosclerosis and its complications with altered arterial biology^[22]. Vascular infection due to transient bacteremia, from leaky guts or periodontal lesions alike, can lead to persistent inflammation, including one due to an intracellular bacterial “privileged niche”. Indeed, blood levels of inflammation markers (e.g., C-reactive protein, CRP) have been associated with vascular risk factors and the prevalence and incidence of atherothrombotic CVD^[23]. Consequently, hsCRP (a high sensitivity CRP test) is an important prognostic factor for atherosclerosis^[24].

More critical evidence can be found in the Northern Manhattan study of stroke incidence and prognosis. This prospective cohort study (1625 participants, mean age 68.5 ± 10.1 years; 64.9% women) demonstrated that infectious burden is associated with established measure of risk of stroke, carotid plaque intima-media thickness^[25-27]. Interestingly, a measure of infectious burden associated with risk of atherosclerosis and stroke was independently associated in this study with cognitive performance. This demonstrated that infections may be a culprit in cognitive impairment as well^[28].

Similarly, the Oral Infections and Vascular Disease Epidemiology Study (INVEST) demonstrated a direct relationship between tooth loss and carotid plaque prevalence^[29]. Specifically, colonization with pathogenic periodontal pathogens was associated with carotid artery intima-media thickness (IMT), a measure of subclinical vascular disease^[30]. INVEST also demonstrated that severe periodontal bone loss was associated with a nearly 4-fold increase in risk for the presence of carotid atheroma [odds ratio (OR) 3.64, $P < 0.05$]^[31]. The study also provided data supporting an effect of the subgingival periodontal bacteria level and both systolic and diastolic blood pressure in addition to prevalence of hypertension^[32]. At a protein level, higher secretory phospholipase A2 activity (an inflammatory enzyme associated with atherosclerosis) at high tertile of etiologic presence presents a mechanistic explanation of the link between periodontal bacteria and CVD^[33].

In the Atherosclerosis Risk in Communities (ARIC) study of 8,363 men and women from four United States communities (aged 52 to 75 years), patients with both high attachment loss and high tooth loss [OR = 1.5, 95% confidence interval (CI): 1.1 to 2.0] and also edentulous individuals (OR = 1.8, 95%CI: 1.4 to 2.4) had elevated odds of prevalent coronary heart disease (CHD) in comparison with controls with low attachment loss and low tooth loss. A number of traditional risk factors for CHD were factored in. The ARIC results thus presented evidence that both tooth loss and periodontal disease (PD) are associated with prevalent CHD^[34].

The Periodontitis and Its Relation to Coronary Artery Disease compared 805 patients (< 75 years of age) with myocardial infarction (MI) and 805 age-, sex- (male 81%), and area-matched controls (mean age 62 ± 8) without MI. This study determined that periodontitis was more common (43%) in patients than in controls (33%; $P < 0.001$). A significant increased risk for MI was observed in periodontitis patients (OR adjusted for confounders, 1.28; 95%CI)^[35].

In a Polish case-control study, the level of PD was significantly associated with the risk of acute MI (OR = 2.4, 95%CI: 1.1 to 5.2, $P = 0.0203$). This was even after an adjustment for age, sex, smoking, hypertension, diabetes, body-mass index, education and income^[36]. Interestingly, severe infection was sometimes associated with MI only in females. Similarly in another study, severe periodontitis was more prevalent in female patients than female controls (14% vs. 4%, $P = 0.005$). An increased risk for severe periodontitis in female patients with a first MI was reported (adjusted OR = 3.72, 95%CI: 1.24 to 11.16, $P = 0.005$)^[37].

Furthermore, multivariate analysis of coronary heart disease (CHD) individuals demonstrated a higher prevalence of oral diseases and lower compliance to oral disease prevention compared to healthy controls. The analysis showed a positive association between edentulousness (OR = 1.37, 95%CI: 1.02 to 1.85), the number of endodontic lesions (OR = 4.37, 95%CI: 1.69 to 11.28), chronic periodontitis (OR = 5.87, 95%CI: 1.17 to 29.4), and CHD^[38].

Similarly, a 9-year follow-up study examined a possible correlation between the duration of periodontal disease state and cardiometabolic risk factors. The odds ratio for the presence of ≥ 1 cardiometabolic risk factor (hypertension, hyperglycemia, dyslipidemia or obesity) in individuals with a longitudinal presence of periodontal pockets for ≥ 6 years was significantly higher compared to individuals without periodontal pockets^[39].

Finally, the recent Malmö Offspring Study is a population-based study using multivariable regression models to analyze the presence of carotid plaque and asymptomatic carotid plaque as related to measures of periodontal disease. This study demonstrated that the risk of developing a carotid plaque in study subjects with periodontitis was significantly higher compared to periodontitis-free subjects, with odds ratio of 1.75 (95%CI: 1.11 to 2.78)^[40].

Not surprisingly, endodontic infections were also associated with vascular inflammations. For example, endodontic infection was associated with higher prevalence of CHD and initial endothelial damage^[41,42]. In a cross-sectional study of the association between apical periodontitis (AP) and CVD using noninvasive methods, flow-mediated dilatation (FMD) was found to be significantly impaired in AP patients (mean = $4.9\% \pm 2.05\%$) in comparison with healthy individuals (mean = $9.74\% \pm 2.59\%$, $P = 0.000$). There was a statistically significant difference observed between carotid IMT of the AP group (mean = 0.64 ± 0.12 mm) and control group (mean = 0.54 ± 0.08 mm) ($P = 0.000$). Furthermore, there was a significant inverse correlation observed between c-IMT and FMD ($r_s = -0.381$, $P < 0.001$). This indicated an impaired FMD and greater carotid IMT in AP patients, supporting an association between endodontic infection and cardiovascular inflammation^[43].

Seroepidemiology

Serological animal and cell culture studies provided evidence that bacterial infection, often by the red complex pathogen *Porphyromonas gingivalis*, a major etiologic agent of PD, emerges as a new, important factor for atherosclerosis^[44]. Based on the accumulated epidemiological data, the infection hypothesis for initiation/exacerbation of atherosclerosis has already been established^[45,46]. Specifically, periodontal inflammatory mediators were recognized as contributors to or triggers for systemic inflammatory responses. Subgingival periodontal infection demonstrated an increased risk of developing atherosclerosis in periodontal patients by 168%^[47].

The ARIC study also presented an association between systemic antibody response to periodontal pathogens and coronary heart disease in ever and never smokers^[48]. The latest ARIC data presented significant association between high gingival inflammation, tooth loss, severe tooth loss, and severe periodontitis with diabetes, coronary heart disease, hsCRP, and IL-6, while only severe disease was associated with stroke^[49].

Furthermore, coronary disease was more common among seropositive for *P. gingivalis* subjects, relative to the seronegative (14.0% and 9.7%, respectively; $P = 0.029$). Hence, CHD was more prevalent in individuals with a high combined antibody response against *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* than in those with a low response (17.4% and 11.1%, $P = 0.026$). When adjusted for age and several CHD risk factors, the subjects with a high combined antibody response had an OR of 1.5 (95%CI: 0.95 to

2.50, $P = 0.077$) for prevalent coronary disease. The combined antibody response was directly associated with prevalent CHD ($P = 0.046$) and inversely associated with the concentration of serum high-density lipoprotein (HDL) cholesterol ($P = 0.050$). This demonstrated that serum antibodies to major periodontal bacteria were associated with CHD^[50].

In addition, the same group demonstrated that systemic exposure to *P. gingivalis* predicts incident stroke. Investigating seropositive subjects, it was found that they had a multivariate odds ratio of 1.6 (95%CI: 1.0 to 2.6) for stroke, compared with the seronegative subjects. Additionally, patients with a history of stroke or CHD at baseline contained more often *P. gingivalis* IgA than the controls, 79.7% vs. 70.2%. The seropositive subjects had an odds ratio of 2.6 (1.0 to 7.0) for secondary stroke, compared with the seronegative^[51]. In the CVD-free individuals ($n = 893$), systemic exposure to *P. gingivalis* increased the risk of stroke as follows: compared to seronegative subjects, men and women that were IgG-seropositive for *P. gingivalis* presented a multivariate OR (95%CI) of 1.63 (1.06 to 2.50) and 2.30 (1.39 to 3.78) for stroke, respectively. Interestingly, higher OR was observed in males, who had never smoked. Compared to seronegative men, *P. gingivalis* IgA-seropositive men had a OR of 3.31 (1.31 to 8.40, $P = 0.012$) for stroke. There was no association found between antibody titers to *A. actinomycetemcomitans* and stroke, suggesting that the systemic exposure specifically to *P. gingivalis* may contribute to incident stroke^[52].

These authors also presented data demonstrating that periodontitis also causes mild changes in HDL metabolism. These changes appear to be less severe than those occurring during the acute-phase response. Thus, periodontitis may reduce the anti-atherogenic properties of HDL, increasing the risk for CHD. Importantly, the HDL-mediated cholesterol efflux improved after periodontal treatment. More interestingly, this increase was significant ($P < 0.05$) among those patients whose CRP titers decreased (53.7% reduction, $P = 0.015$) and who were PCR-positive for *A. actinomycetemcomitans*^[53].

In comparison, *P. gingivalis* induces HDL oxidation, impairing the atheroprotective function of HDL. *P. gingivalis* likely makes it proatherogenic by raising a proinflammatory response via interaction with monocytes and macrophages^[54]. Overall, the presence of *A. actinomycetemcomitans* and *P. gingivalis*, major causative organisms in periodontitis was shown to be the strongest determinant of the systemic antibody response to these pathogens^[55].

Concerning serum antibodies, in coronary disease as well as in periodontal disease patients the antibody titers against *P. gingivalis* were the most prevalent. Hs-CRP test levels and antibody titers to *P. gingivalis* have been reported to be higher in periodontitis patients than in control subjects^[56]. Interestingly, while periodontal patients were seropositive for both studied *P. gingivalis* strains, FDC381 and Su63, higher antibody titers to *P. gingivalis* Su63 only was observed in coronary disease patients. This finding indicates that specific genomic virulence determinants present in particular *P. gingivalis* strains may affect atherogenesis^[57].

The association of *P. gingivalis* antibodies with mortality is however non-linear. In a specific study, mortality was highest for those just above the median anti-*P. gingivalis* response and a reduced risk was present among those with low or high titers of the antibody^[58], suggesting that the efficiency of the immune response itself may be the key to control of the infection.

In a first 27-year long-term study of association of chronic oral infections in childhood with subclinical carotid atherosclerosis in adulthood in 755 participants, the infections were associated with adulthood IMT. The relative risk (RR) found was 1.95 (95%CI), especially elevated in boys, RR 2.25 (95%CI). The associations were independent of cardiovascular risk factors^[59]. Specifically, the salivary IgA antibody levels to malondialdehyde acetaldehyde-modified low-density lipoprotein (MAA-LDL), Rgp44 (gingipain A

hemagglutinin domain of *P. gingivalis*), and Aa-HSP60 (heat shock protein 60 of *A. actinomycetemcomitans*) were discovered to be elevated in stable-CAD and acute coronary syndrome patients when compared to CAD-healthy subjects^[60]. Periodontal patients were characterized by higher levels of subgingival bacteria. The serum IgA/IgG burden indicated higher risk for acute coronary syndrome (OR = 1.84, 95%CI: 1.01 to 3.35 for IgA; OR = 1.87, 95%CI: 1.01 to 3.46 for IgG). This risk was independent of other cardiovascular risk factors (body mass index, number of teeth, subgingival bacterial levels and periodontal diagnosis)^[61]. The serological differences in periodontitis patients may present risk factors for atherosclerosis. These seroepidemiological findings are consistent with an association between periodontitis and cardiovascular disease.

Association of bacteria with atheromatous tissue

Identification of periodontal pathogens in vascular tissue. While oral tissues are the primary sites for *P. gingivalis* infection, it has been long shown it can also enter the circulation daily through the microvasculature following tooth brushing and other dental procedures^[62]. Routine procedures such as tooth extraction may also lead to transient bacteremia^[63-65]. Periodontal biofilm bacteria are thus disseminated to large vessels. Consequently, bacterial DNA was detected in atheromas by PCR^[66] where *P. gingivalis* was the most abundant pathogen compared to all others tested species^[67]. Similarly, a high content of periodontal pathogens were detected in atheromatous arterial specimens from atherosclerosis patients. The pathogens were specifically detected within primary atheromatous lesions. Critically, most patients had severe periodontitis^[68].

Using reverse transcription polymerase chain reaction, DNA from endodontic bacteria was identified in 20/36 (56%) of aortic aneurism tissue specimens and DNA from periodontal bacteria in 17/36 (47%) of these specimens^[69]. Compared to cardiac bypass control samples, both ruptured and unruptured aneurysm specimens presented significantly more bacterial DNA ($P = 0.003$ and 0.001 , respectively)^[70]. Further, quantitation of DNA from periodontopathic bacteria using universal and species-specific TaqMan probe/primer sets demonstrated total bacterial DNA in 94.9%, and periodontopathic bacterial DNA in 92.3 % of the atherosclerotic plaques from periodontal disease patients^[71]. Using sequence analysis of bacterial 16S rRNA libraries from atherosclerotic plaques, 23 bacterial species/phylotypes were identified, where 15 (60.9%) of the phylotypes were reported as yet uncultivable or as yet uncharacterized species^[72]. *P. gingivalis* DNA was found in 21 of 91 (23%) samples taken from carotid endarterectomies^[73].

More importantly, live invasive periodontal pathogens, *P. gingivalis* and *A. actinomycetemcomitans* were identified in a patient plaque^[74]. In the same line of investigation, a large number of strains were cultivated from patient plaques, belonging to different species, mostly associated with periodontal biofilm, including *P. gingivalis*^[75,76]. It will also be interesting to adapt to atheromas the recently communicated reverse-genomics-enabled cultivation and characterization of as-yet-uncultured species^[77].

It has also been shown that more than 90% of all infections in the head and neck region can have an odontogenic origin^[78]. Most recently, *P. gingivalis* proteinase gingipain was detected in 96% of the 53 brain tissue sections from Alzheimer's patients^[79], indicating overall systemic hematogenous spread of periodontal bacteria.

Effects of bacterial infection of vasculature

A variety of communications on animal experiments have suggested that bacterial infection may predispose to early atherosclerosis^[80] and plaque instability^[81]. In addition to passive dissemination by way of the bloodstream, bacteria may disseminate and cause low-grade focal infections due to their ability to invade and persist intracellularly. Low-grade infection presents mixed positive/negative results for infection, inflammation or pathogen identification since it requires prolonged culturing. Thus, using

quantitative polymerase chain reaction, it was shown that bacterial DNA was present in atherosclerotic plaque and - of note - the amount of this DNA correlated with the amount of leukocytes in the atherosclerotic plaque^[82].

Bacteria possess a profound ability to disrupt the host homeostasis. For example, infection with *P. gingivalis* induces procoagulant effects in human endothelial cells^[83]. Very important, an invasive, but not a non-invasive, *P. gingivalis* strain accelerated atherosclerosis in a murine model^[84], pointing to the significance of strain-specific genomic virulence determinants. Furthermore, it was found that *P. gingivalis* invasion (but not a non-invasive mutant) in ApoE (±) mice was critical for atherosclerosis progression^[45].

Bacterial infection can also cause apoptosis in endothelia^[85]. There is a large body of evidence that *P. gingivalis* has developed an elaborate proteolytic system composed of surface-located or secreted enzymes, Rgp and Kgp gingipains, which serve to provide these asaccharolytic bacteria with sole source of nutrients in the form of small peptides and amino acids, thus functioning as virulence factors leading to tissue destruction^[86-88]. Consequently, the proteolytic activities of this infectious agent may also contribute to vascular disruption and subsequent obstruction of the lumen.

At the same time, *P. gingivalis* cytotoxic activities have been well characterized^[89] and loss of cell adhesion properties with subsequent apoptotic cell death has been observed^[90-92]. Even more aggravating, *P. gingivalis* efficiently activates coagulation factors, thus promoting platelet aggregation^[93-96]. Thus, internalized destructive platelet-aggregating inflammatory agent such as *P. gingivalis* combined with macrophage infiltration in intimal regions would likely contribute to triggering apoptosis and formation of necrotic core, potentially leading to plaque weakening and rupture, followed by triggering of the coagulation cascade, thrombosis and acute ischemic events.

The Rgp gingipains of *P. gingivalis* lyse lipoproteins producing 2 apoE fragments, as well as 2 apoB-100 fragments, in LDL, while the Kgp gingipain lyses HDL, induce reactive oxygen species (ROS) and degrade antioxidants. In addition, both Rgp and Kgp gingipains induce lipid peroxidation. Thus, *P. gingivalis* may affect the lipoproteins expression in blood, another facet of its contribution to atherogenesis^[97]. Similarly, Pep19 from *P. gingivalis* HSP60 has a distinct ability to induce native-LDL oxidation which may serve as a plausible mechanism by which this peptide may drive epitope spreading to the neoantigen, i.e., oxidized LDL, in the pathogenesis of atherosclerosis^[98].

During endodontic (apical PD) infection, ligation of toll-like receptors (TLRs) on phagocytes' surface triggers activation of humoral and cellular responses and also phagocytosis, synthesis of ROS and production of inflammatory mediators, cytokines and matrix metalloproteinases. TLRs provide innate immune sensing of conserved pathogen-associated molecular patterns. TLR - mediated signaling also contribute importantly to cardiovascular disease. For a recent review of this particular subject, see^[99].

Specifically, oxidative stress has been strongly involved in the pathogenesis of atherosclerosis^[100]. The ROS-producing systems in the vasculature include reduced nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, the mitochondrial electron transport chain, and nitric oxide (NO) synthase. Oxidative stress due to ROS overproduction contributes to all stages of atherogenesis, from the plaque formation to the most critical stage, the plaque rupture^[101-103]. All cardiovascular risk factors such as hypercholesterolemia, hypertension, diabetes mellitus, and smoking increase ROS and decrease endothelial NO synthesis^[104].

Bacterial pathogens can cause oxidative stress via triggering LDL oxidation at the atherosclerotic lesion. In addition to hematogenous dissemination to the atheroma, bacteria may spread in the system while

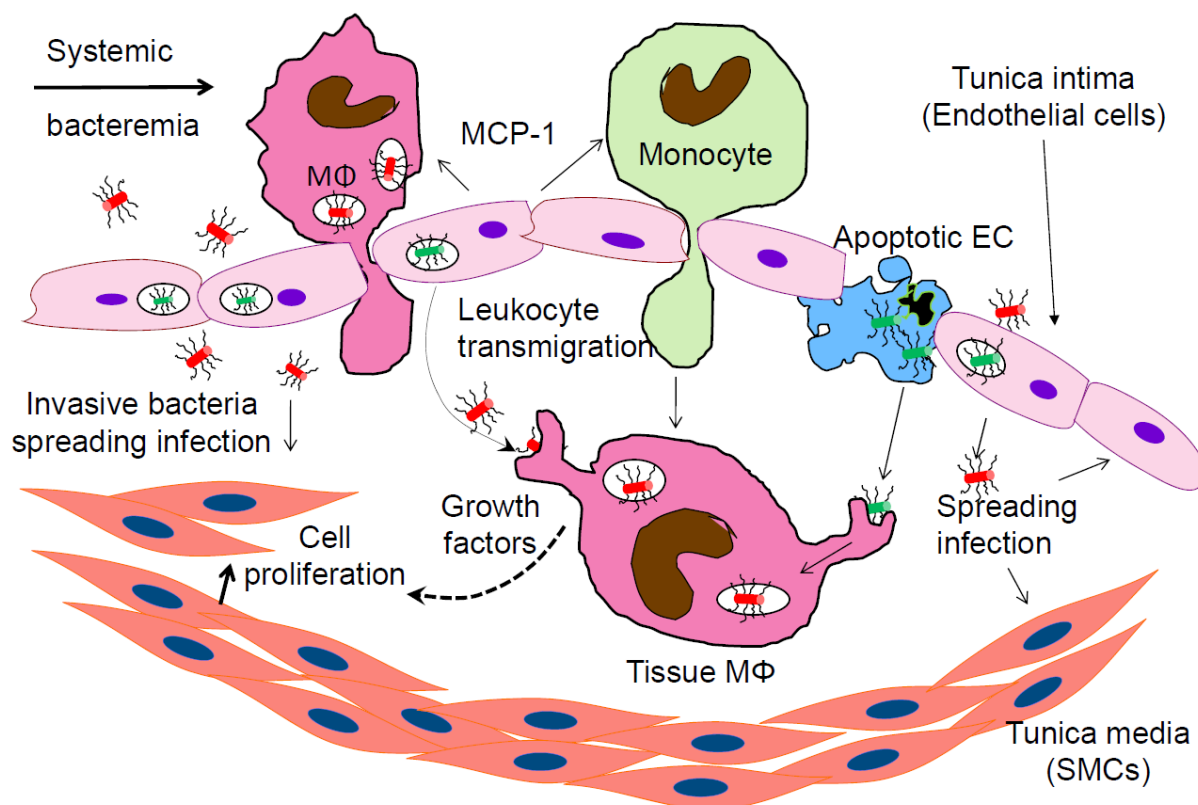


Figure 1. Bacterial component of atheromas representing transmutating from the vascular lumen and tissue-embedded macrophages (MΦ). The activated inflammatory leukocytes roll over the intima, adhere to the endothelial cells and transmute in the tissue. This includes intracellular bacteria-carrying macrophages extravasating from the vascular lumen into the arterial wall. Bacteria induce host cells to release chemotaxis molecules (such as MCP-1) and growth factors stimulating host cell division. Invasive bacteria multiply, causing persistent inflammation and apoptotic cell death, forming a necrotic core and eroding the vascular wall. MN: extravasating monocyte; EC: endothelial cell; SMC: smooth muscle cell; MCP-1: monocyte chemoattractant protein-1

intracellular, since they survive and multiply in peripheral blood mononuclear cells^[75]. They are also able to replicate and persist within vascular endothelial and smooth muscle cells (SMCs)^[105]. Intracellular *Chlamydomydia pneumoniae* infection has been shown to induce ROS in macrophages, endothelial and smooth muscle cells, causing oxidative stress^[106,107]. This can lead to endothelial dysfunction, foam cell formation, SMC proliferation, platelet aggregation as well as cytokine, growth factor, and cell adhesion molecule production^[101].

Periodontal bacteria are extensively studied in respect of oxidative stress. *P. gingivalis*-induced ROS production was shown to activate the NOD-like receptor family, thus increasing the aortic gene expression of Nod-like receptor family, pyrin domain containing 3 (NLRP3), pro-interleukin (IL)-1β, pro-IL-18 and pro-caspase-1^[108].

Further, *P. gingivalis* increases the uptake of oxidized LDL, promoting the foam cell formation^[109]. *P. gingivalis* also induces the synthesis and secretion into the vascular lumen of monocyte chemoattractant protein-1 (MCP-1), causing monocyte influx^[110] [Figure 1]. In addition, this organism induces apoptosis^[91,92,111], including in the presence of ox-LDL^[112]. The presence of an apoptotic core is a hallmark of the unstable plaque. It can eventually lead to plaque erosion, rupture and acute ischemic events. Finally, *P. gingivalis* may destabilize the plaque also by enhancing matrix metalloproteinase-9 activity and oxidative stress through impairing the selective autophagic clearance of damaged mitochondria^[113].

The LDL oxidation leads to an increased expression of adhesion molecules such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin on the activated endothelia. This results in the tethering of the leukocytes to the endothelia, adhesion and diapedesis (extravasation) into the vascular wall [Figure 1]^[114-116]. Thus, in a vicious circle, oxidative stress multiplies the effects of inflammation and accelerates the atherogenesis^[117].

In another deleterious activity, the periodontal infection itself accelerates lipid deposition and atherosclerosis in animal models^[118,119], and therefore addressing the infection would - as an important added benefit - suppress the atherogenic effects of dyslipidemia.

Atherogenic dyslipidemia (hypercholesterolaemia and hyperlipidaemia) is defined as high plasma LDL and low plasma HDL cholesterol with elevated triglycerides. The increase of triglyceride and LDL cholesterol levels include alterations observed in lipid metabolism and lipoprotein composition. Infection and the concomitant inflammation induce acute-phase response contributing to atherogenic changes in lipid and lipoprotein metabolism^[120].

An early and consistent effect of infection/inflammation is increased serum triglyceride level, characterized by an increase in very low-density lipoprotein (VLDL) levels^[121,122]. High bacterial lipopolysaccharide serum activity (endotoxemia) has shown a strong correlation with serum triglyceride concentrations ($P < 0.001$)^[123]. The increase in serum triglycerides may be due to both an increase in hepatic VLDL production and a decrease in the clearance of triglyceride rich lipoproteins in chronic inflammation^[124].

Endotoxemia also modulates HDL composition and size^[122]. HDL is one of the plasma lipoproteins that neutralize Gram-negative bacterial LPS and Gram-positive bacterial lipoteichoic acid, thus favoring the clearance of these products^[125]. For example, the mean percentage of HDL cholesterol in *Helicobacter pylori*-seropositive patients was significantly lower than the one measured in seronegative ones ($P = 0.008$ and $P < 0.001$, respectively)^[126]. In another study, the Health 2000 Health Examination Survey, which included 8028 Finnish subjects aged 30 or older, no consistent association between serum lipid levels and periodontal infection among normoweight subjects was found. However, an association was found of high serum triglycerides and low HDL with periodontal infection among obese subjects^[127]. Overall, these authors were not able to present evidence that unfavorable lipid composition can be considered as an important risk for periodontal infection in a general adult population^[128].

Specifically, for periodontal infections, both total and LDL-cholesterol were significantly associated with antibody titer to *P. gingivalis* in non-obese patients^[129]. The latest meta-analysis suggested that periodontitis is significantly associated with reduced HDL ($P = 0.0005$), elevated LDL ($P = 0.003$) and triglycerides ($P < 0.0001$) compared to healthy controls, supporting the rationale that periodontal disease is associated with lipid metabolic control^[130].

Effect of periodontal treatment on atherosclerotic inflammation

Due to the extended amount of time during which the atherogenesis takes place and even more to obvious ethical reasons, it is virtually impossible to obtain an unambiguous demonstration of the effectivity of antibacterial treatment on atherosclerotic inflammation. This however should not prevent from exploring the alternative approaches to examine attenuation and moreover, reversal of atherogenesis via resolution of the vascular inflammation.

Current therapeutic strategies focus on anti-inflammation, i.e., on pharmacologic intervention in the inflammatory pathways. However, only resolution of inflammation will restore the homeostasis. The isolation and characterization of resolving agonist molecules using endogenous lipid mediators

of resolution as potential therapeutic agents for the management of inflammation has opened a new promising area of research^[131-134].

Thus, in an established rabbit model of aortic plaque development, it was shown that oral/topical application of a proresolution lipid mediator, Resolvin E1 (RvE1) diminished diet and periodontitis- induced aortic atherogenesis. Importantly, RvE1 not only significantly attenuated the arterial inflammation; the treatment also prevented periodontal inflammation ($P < 0.05$). In the absence of the latter, oral/topical administration of RvE1 led to a reduction of the arterial plaque and a lower intima-media thickness ratio. RvE1 also reduced the inflammatory cell infiltration in the animal model compared to non-treated controls ($P < 0.001$). In addition, local oral RvE1 application significantly diminished serum CRP levels ($P < 0.05$)^[135,136].

Multiple clinical investigations have also indicated a positive effect of periodontal disease treatment on systemic inflammation. Periodontal therapy of periodontitis patients demonstrated significant improvements in periodontal pocket depth, in brachial artery FMD and in serum IL-6. A trend toward reduction in serum CRP has been reported in this study as well^[137]. Similarly, after non-surgical treatment of periodontal patients, serum leptin, IL-6, and CRP levels were significantly decreased (mean \pm SD before and after, P value, respectively: leptin, 8.02 ± 5.5 , 7.10 ± 4.4 , $P = 0.015$; IL-6, 1.73 ± 1.02 , 1.36 ± 0.73 , $P = 0.048$; and CRP, 802.0 ± 1065 , 491.2 ± 479.3 , $P = 0.047$)^[138]. Further, in a study of 49 patients with moderate to advanced level of periodontitis, hsCRP and anti-*P. gingivalis* antibody levels were measured and the effect of periodontitis treatment, including surgery and use of antibacterials, was analyzed on both markers. The hsCRP levels and antibody titers were higher in the periodontitis patients than in the 40 periodontally healthy control subjects. Furthermore, periodontal treatment significantly decreased the CRP levels and the antibody titers ($P < 0.005$). A significant reduction of hs-CRP levels was communicated as a result of the treatment in patients with hs-CRP levels > 1 mg/L ($P < 0.005$)^[56].

More data supporting treatment were provided by the INVEST study of 420 participants (68 ± 8 years old) at baseline and 3-year follow-up. The longitudinal carotid artery IMT progression was recorded. Adjustments were made for age, sex, race/ethnicity, diabetes, smoking status, education, body mass index, systolic blood pressure, LDL cholesterol and HDL cholesterol. An attenuation of the IMT progression was reported with improvement in clinical or microbial periodontal status^[139].

A positive association has also been reported between severe periodontitis and oxidative stress^[140]. Introduction of periodontal therapy triggered a burst of inflammatory response before a progressive and consistent reduction of systemic inflammation followed by an endothelial function improvement^[141]. Furthermore, the reactive oxygen metabolites (d-ROMs) test values decreased and the biological antioxidant potential test values increased in patients with chronic apical periodontitis after endodontic therapy treatment. The oxidative stress levels in these patients exhibited a downtrend, returning to normal in 90 days post treatment^[142]. Periodontal treatment however did not improve vasodilation in coronary disease patients in a brief follow-up period, even though it maintained the titers of vascular inflammation markers^[143]. An in-depth review on oxidative stress in periodontal disease, focusing on the relationship between the local and systemic markers of oxidative stress and periodontal disease is in^[144]. More viewpoints on the subject of treatment are reviewed in^[145,146].

Finally, a meta-analysis was conducted investigating the literature on the association between carotid IMT (c-IMT), FMD and periodontitis. The effect of periodontal treatment on carotid IMT and FMD was assessed. Periodontal disease diagnosis was associated with a mean increase in c-IMT of 0.08 mm (95%CI: 0.07 to 0.09). The mean difference in FMD was 5.1% when compared to controls (95%CI: 2.08% to 8.11%). As a consequence of periodontal treatment, a mean improvement on FMD of 6.64% (95%CI: 2.83% to 10.44%) was observed between test and controls. This meta-analysis demonstrated an association between arterial

inflammation, increased c-IMT and impaired FMD and PD. The results suggested a beneficial effect of periodontal treatment on FMD leading to improvement in endothelial function^[147].

EXPLORING CUTTING-EDGE TECHNOLOGIES TO DISCOVER NEW THERAPEUTIC TARGETS AND APPROACHES FOR DRUG DEVELOPMENT. ATHEROSCLEROSIS MICROBIOME

Presenting major fiscal burden, CVD is the costliest disease in the US at \$555 billion (American Heart 2017, <http://bit.ly/2LfsC5A>) and €210 billion in the European Union (2017, <http://bit.ly/2UDXS2F>). This underscores the need for novel diagnostic and therapeutic developments since cost-effective and rapid approaches are lacking.

A major incentive for novel approaches is that many CVD events have not been explained by classical risk factors. To address a modifiable risk factor, such as microbial pathogens, Robert Koch's postulate must be satisfied, namely the pathogen must be isolated from the diseased tissue. Thus, *C. pneumoniae* was isolated from a carotid endarterectomy specimen^[148]. However, it took time before a polybacterial infectious component from atherosclerotic plaques was identified, and these clinical isolates cultivated. This achievement demonstrated the existence of atherosclerosis microbiome, a sample of the microbial community localized within human atheromatous tissues^[67,74,75,149]. Such advancement enabled an entirely new approach to CVD diagnosis and treatment, fulfilling Koch's postulate.

A natural approach to restore the homeostasis, reversing the atherogenic process, is via control of the inflammatory component, often originating from periodontal lesions. The latest network analysis confirmed inflammation and lipid metabolism as the two key biological pathways involved in the predisposition to CVD^[150]. Of note, there is still no approach successfully addressing the actual source of the inflammation. Taking the potential opportunities presented by the identification of prokaryotes in vascular inflammations, randomized placebo-controlled clinical trials using antibiotics in the context of CVD have been designed^[151,152]. However, the results were disappointing, since the administered treatment may not have reached its target (i.e., intracellular location sheltering bacteria from antibiotics as well as from immune response). The latest anti-inflammation trials (CIRT^[153], CANTOS^[154,155], TETHYS^[156], SOLID-TIMI 52^[157]) did not target the plausible origin of inflammation, intracellular bacteria. The PEGASUS-TIMI 54 trial, although also targeting ischemic events, was strictly thrombosis-related.

As mentioned before, both ethical considerations and the slow progress of atherosclerosis preclude conducting a clinical trial to establish causality. Importantly, the WIZARD trial design predicted that a positive effect of drugs would tighten the association between atherosclerosis and bacteria without proving causality^[158]. As stated by Peter Libby, renowned vascular disease physician, "A therapeutic trial of antibiotics still would not establish a causal relationship between any particular infectious agent and atherosclerosis. Yet, if antibiotic treatment could reduce atherosclerosis events, the public health implications could be enormous; hence the need to keep an open mind"^[159].

In addition, animal studies suggest that atherosclerosis is induced or aggravated by invasive infectious agents (i.e., persistent IC infection)^[45,84,160-163].

It is becoming clear that atherosclerosis represents chronic vascular inflammation partly of microbial etiology. Intracellular invasive pathogens induce a low-grade persistent inflammation that exacerbate the atherogenesis. Therefore, to address intracellular infections as etiologic factors for CVD, entirely novel anti-infectives and vaccines are required^[164]. Alleviating this key pathological feature (i.e., IC bacteria internalized in vascular tissue) could significantly improve the clinical outcome.

Evidence supporting this view is several-fold: (1) epidemiological data support an infectious component in atherosclerosis; (2) atherosclerosis has many of the characteristics of a chronic infectious disease; and (3) internalization of bacteria can produce a “privileged niche” (i.e., shelter from immune response and drugs)^[165].

Of note, a variety of species, mostly with periodontal origin were cultivated from atheromatous tissue of endarterectomy patients^[74-76]. These are promising targets for intervention, specifically *P. gingivalis*, a gram-negative anaerobe capable of invading a variety of non-phagocytic eukaryotic cells^[166-170]. *P. gingivalis* is a key periodontal pathogen^[171] causing inflammation and host tissue destruction. It becomes internalized and also persists in vascular cells^[105]. While oral tissues are the primary sites for *P. gingivalis* infection, it can also enter the circulation daily through the microvasculature and its role in periodontitis is established^[30]. Most importantly, an invasive *P. gingivalis* strain accelerated atherosclerosis in a murine model^[84] and as mentioned before its tissue invasion ability was critical for atherosclerosis progression^[45]. These advancements pave the way for further promising developments along the Alzheimer's treatment technology where *P. gingivalis* is identified as a key target^[79].

Addressing the atherosclerosis microbiome: a new approach to CVD risk modification

The cultivation and identification in atheromatous plaques of a variety of viable bacteria suggests that atherosclerotic lesions can be induced or exacerbated by these inflammatory pathogens. Importantly, it was shown that bacteria not only invaded both vascular cell types but also persisted intracellularly. Moreover, the bacteria were transmitted between both cell types and to healthy cells, explaining the chronicity of infection^[105]. Such intracellular polymicrobial flora has been well demonstrated^[172], pointing to a plausible contributor to premature atherosclerosis^[173].

A natural approach to restore the homeostasis is reversing the atherogenic process, via control of the inflammatory component, often originating from periodontal lesions. An important advantage of such approach is that the main problem in medical care can be addressed as initiated or exacerbated by prokaryotes. Targeting bacteria, thus minimizing the side effects of treatment, is inherently more attractive than the current complicated designs addressing metabolic pathways.

Moreover, the infection itself accelerates lipid deposition and atherosclerosis in animal models^[118,119], and therefore addressing the infection would also suppress the effects of hyperlipidemia.

A variety of available methodologies can be adapted to bring about development of vaccines and small molecule inhibitors of the identified pathogens. The emergence of infections, specifically from periodontal origin as a potential risk factor for CVD, is leading to a convergence in oral and medical care that will hopefully benefit the patients and public health^[174].

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Authors' contributions

Wrote and reviewed the manuscript: Kozarov E, Progulske-Fox 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

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Review

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Microvesicles and exosomes in pulmonary hypertension

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Abstract

Pulmonary hypertension is a serious disorder with a high morbidity and mortality rate. The juxtaposition of endothelial cells and smooth muscle cells maintains vascular homeostasis. Vascular injury results in endothelial dysfunction, leading to impaired vascular relaxation, cell proliferation, and altered immune and metabolic states. In addition, injury induces pulmonary arterial endothelium and other cells to release increased levels of extracellular vesicles, including exosomes and microparticles that may be involved in enhancing the proliferation of apoptosis-resistant smooth muscle cells. These extracellular vesicles carry proteins, lipids, RNA, miRNA, chemokines cytokines and modulate immune function, inflammation, embryogenesis, regenerative processes, and serve as intercellular messengers. Importantly, mesenchymal stem cells-derived extracellular vesicles exert inhibitory effects on inflammation and restore homeostasis. This article reviews the pathophysiological role of extracellular vesicles in pulmonary hypertension.

Keywords: Endothelial cells, extracellular vesicles, mesenchymal cells, pulmonary hypertension

INTRODUCTION

Pulmonary hypertension (PH) is a serious complication of a number of systemic diseases including cardiovascular, respiratory and hematological disorders, autoimmune diseases, genetic mutations, and as yet unidentified causes. Based on the underlying disease, PH can be classified into five major groups. The term pulmonary arterial hypertension (PAH) is applied to diseases in group 1, which includes idiopathic and heritable PAH (IPAH, HPAH), and PAH associated with congenital heart defects, inflammation, autoimmune diseases and drug toxicity. A number of genetic mutations are also associated with PAH.



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Table 1. Pulmonary hypertension classification (based on Ref #1)

Gr. I PAH
Idiopathic and heritable PAH, PAH associated with CHD, inflammatory, autoimmune diseases, drug toxicity, genetic mutations, HIV, portal hypertension, Gr I' - pulmonary capillary hemangiomatosis, pulmonary veno-occlusive disease, Gr I'' - Persistent PH of the newborn
Gr. 2 PH
Left ventricular diseases (congenital and acquired)
Gr. 3 PH
Chronic lung diseases, hypoxia, developmental lung diseases
Gr. 4 PH
Chronic thromboembolic pulmonary hypertension
Gr. 5 PH
Miscellaneous systemic disorders including hematological, myeloproliferative, metabolic and thyroid diseases, and chronic renal failure

PAH: pulmonary arterial hypertension; PH: pulmonary hypertension

Pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis are assigned subcategory 1' and persistent PH of the newborn is designated as 1''. The remaining four groups are designated as PH. Group 2 includes PH associated with left ventricular diseases and pulmonary venous hypertension. Included in group 3 are chronic lung diseases, alveolar hypoventilation disorders and developmental lung diseases while group 4 includes chronic thromboembolic PH. Finally, group 5 includes PH associated with miscellaneous diseases such as myeloproliferative, hematological, thyroid and renal diseases^[1] [Table 1].

During the sixth World Symposium on PH, the mean pulmonary artery pressure threshold was reduced to > 20 mmHg from ≥ 25 mmHg, and pulmonary vascular resistance maintained as > 3 Wood units^[2]. This change is based on the evaluation of 47 studies from 13 countries that showed normal mean pulmonary artery pressure rarely exceeded 20 mmHg, irrespective of age^[3]. The survival time for patients with PAH (Gr. 1) without treatment is reported to be 2.8 years^[4]. Modern treatment has improved the quality of life and 3-year survival in these patients is now around 58%-67%^[5,6].

Despite such treatment, the underlying vascular pathology continues to worsen^[7]. Regardless of the underlying disease, pulmonary endothelial cell (EC) disruption/dysfunction plays a pivotal role in the pathogenesis of PH. ECs maintain vascular homeostasis and regulate vascular tone, cell permeability, inflammation, and coagulation through several mediators such as nitric oxide (NO), endothelium-derived hyperpolarization factor, endothelin-1 (ET-1), cell adhesion molecules, cytokines and chemokines. Endothelial dysfunction, alterations in the expression of NO, ET-1, caveolin-1, serotonin, inflammatory cytokines and chemokines, and disordered proteolysis of the extracellular matrix all contribute to the pathogenesis of PAH^[8,9]. The increased expression of cytokines such as interleukin-1 (IL-1) and IL-6^[10,11] and chemokines such as CX(3)CL1 (fractalkine) and CCL5, also known as RANTES^[12,13], have all been observed in both human and experimental PH. Furthermore, plexiform lesions contain perivascular infiltration with inflammatory T and B cells^[14,15]. Disruption or dysfunction of endothelial caveolin-1 (a major protein constituent of caveolae) associated with the activation of proliferative molecules such as tyrosine phosphorylated signal transducer and activator of transcription 3 (py-STAT3), B cell lymphoma-extra-large (Bcl-xL) and β -catenin leads to smooth muscle cell (SMC) proliferation, medial hypertrophy and PH^[16,17].

SMCs are a heterogeneous cell population which exhibit different proliferative, inflammatory, and extracellular matrix production changes during vascular remodeling. In addition, the extension of pericytes into non-muscularized arteries has been documented in PH^[18]. Pericytes are heterogeneous cells in origin. The close interactions between pericytes and ECs are important for paracrine signaling involved in vascular development and stability. In addition, pericytes modulate immune responses^[19]. It has recently been shown that the dysfunctional EC in IPAH may partly contribute to the increased pericyte coverage

in distal pulmonary arteries, through the EC-derived fibroblast growth factor 2 (FGF-2) and IL-6^[20]. Pericyte-specific upregulation of CXCR (C-X-C chemokine receptor)-7 and transforming growth factor- β receptor II (TGF- β RII) in patients with PAH are considered critical for their proliferation/migration capacities and myogenic potentials. During the early phase, pericyte numbers increase in a CXCL (C-X-C motif chemokine ligand)-12-dependent manner and later, the activation of the TGF- β signaling pathway induces pericytes to differentiate into smooth muscle-like cells^[21]. Furthermore, reduced endothelial-pericyte interactions result in progressive loss of small vessels in PAH. Increased expression of pyruvate dehydrogenase kinase 4 (PDK4) gene and protein in PAH pericytes correlated with reduced mitochondrial metabolism, higher rates of glycolysis, and hyperproliferation. Reducing PDK4 levels improved endothelial-pericyte interactions, restored mitochondrial metabolism, and reduced cell proliferation^[22]. These studies underscore the importance of EC and pericyte interactions in maintaining vascular homeostasis.

The disruption/apoptosis of ECs and accompanying endothelial caveolin-1 loss followed by enhanced expression of caveolin-1 in SMCs, proliferation of antiapoptotic ECs and neointima formation have all been reported in experimental PH and human PAH^[23-26]. In a monocrotaline (MCT) + hypoxia model, the enhanced expression of caveolin-1 revealed the presence of tyrosine 14-phosphorylated caveolin-1 (p-cav-1) and the loss of polymerase 1 and transcription factor also known as cavin-1^[25]. Cavin-1 maintains the shape of caveolae and stabilizes caveolin-1 in caveolae. The loss of cavin-1 is indicative of the flattening of caveolar structure. Cavin-1 knockout mice exhibit pathological lung changes such as remodeled pulmonary vessels, PH and right ventricular hypertrophy (RVH). In addition, these mice have an altered metabolic phenotype with insulin resistance^[27]. It is worth noting here that in cancer, p-cav-1 has been shown to inactivate the growth inhibitory function of the caveolin-1 scaffolding domain and facilitate cell migration^[28-30]. These studies indicate that the disruption of endothelial caveolin-1 and dysfunction of SMC caveolin-1 participate in the progression of PH. In addition, other factors such as vascular endothelial growth factor (VEGF), epidermal growth factor, transforming growth factor β (TGF β), matrix metalloproteinases, bone morphogenic protein receptor type 2 (BMPR2) and Notch1 have all been implicated in the pathophysiology of PAH^[31]. Thus, a large number of deregulated transcription factors and proliferative pathways participate in the pathobiology of PH. Recent studies have shown that extracellular vesicles (EVs) may have an important role in the pathogenesis of PH.

EVs

EVs have been isolated from body fluids such as blood, urine, saliva and cerebrospinal fluid. Initially EVs were thought to be a means for cells to get rid of unwanted components. Currently, they are identified as important mediators of intercellular communication. EVs participate in the exchange of lipids, proteins and genetic material between cells, modulate immune, inflammatory and regenerative processes, and maintain homeostasis. EVs are released from a variety of cells including platelets, erythrocytes, leukocytes, and ECs maintain their different compositions and function. Most cell types generate EVs that play important roles in various biological processes, including embryogenesis, tissue regeneration and immunomodulation. They regulate the transfer of biological information both locally as well as remotely^[32-35]. EVs include exosomes (30-130 nm, in diameter), microparticles (MPs, also known as microvesicles, 100-1000 nm) and apoptotic bodies (50-4000 nm). Apoptotic bodies are generated following activation of the apoptotic pathway and cell death.

Exosomes

For exosome formation, endosomal membrane invagination captures cytosolic components within intraluminal vesicles. Early endosomes then mature into late endosomes and accumulate intraluminal vesicles, known as multivesicular bodies in their lumen. These multivesicular bodies either fuse with lysosomes for degradation or with the plasma membrane and are released into the extracellular space as exosomes^[36,37] [Figure 1].

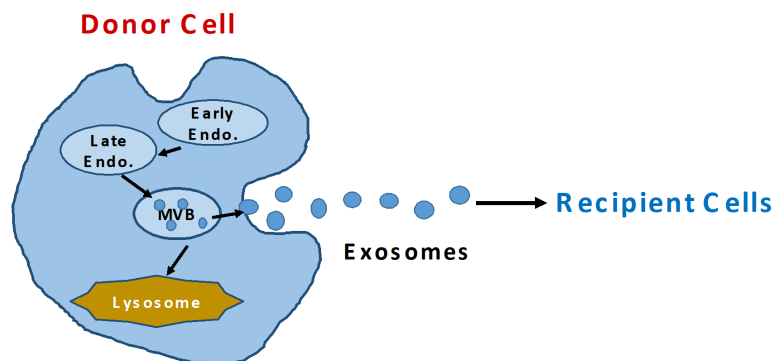


Figure 1. Early endosomes (Early Endo) mature into late endosomes (Late Endo) and accumulate intraluminal vesicles known as MVB. MVB release exosomes into the extra cellular space, or fuse with lysosome for degradation. These exosomes are then incorporated into recipient cells. MVB: multivesicular bodies

Reticulocytes were the first cells shown to release exosomes containing transferrin receptor during maturation^[38]. The main components of exosome membranes are lipids and proteins enriched with lipid rafts. In addition, they contain mRNAs, microRNAs (miRNAs), and other non-coding RNAs. The interaction between the transmembrane proteins of exosomes and the signaling receptors of target cells could either be direct, or indirectly after fusion with the plasma membrane of recipient cells to deliver the content into the cytosol. In addition, exosomes internalized into recipient cells may merge into endosomes, undergo transcytosis, move across recipient cells and released into neighboring cells. Alternatively, endosomes fused with engulfed exosomes mature into lysosomes and undergo degradation^[39]. EVs are engulfed into the cells via endocytosis. The lipid rafts, the known sites for endocytosis, are the specific microdomains within the plasma membrane that contain high concentrations of cholesterol and glycosphingolipids. Caveolins and flotillins are enriched with these lipid rafts. Interestingly, siRNA-mediated knockdown of caveolin-1 and flotillin-1, but not clathrin heavy chain, results in the inhibition of EV internalization^[40]. However, caveolin-1 localized in the plasma membrane negatively regulates exosome uptake, partly through the suppression of ERK1/2 signaling activation^[41]. *CAV1* gene knockout results in reduced caveolin-1 protein and impaired EV uptake. However, *CAV1* knockout in mouse embryonic fibroblast cells resulted instead, in increased EV uptake^[42]. Thus, EV uptake may depend on the cell type and its pathophysiological state. In addition, Rab proteins are essential regulators of EV transport^[43]. Endothelial exosomes are also involved in vascular development as they incorporate and transfer Delta-like 4 (Dll4) to neighboring ECs, resulting in inhibition of notch signaling and increased vascular branch formation^[44].

MPs

MPs are shed from various cell types as small fragments. In 1967, MPs shed during coagulation were regarded as platelet dust. Studies over the years have since shown that these MPs participate in cellular signaling, homeostasis, vascular injury and coagulation^[45]. MPs are formed by the plasma membrane pinching off and encapsulating cytosolic components, and they maintain surface markers and receptors of the plasma membranes of the parent cells [Figure 2].

They efficiently exchange biological information between cells and thus, participate in intercellular communication. In the healthy state, circulating MPs originate mainly from platelets and, to a smaller extent, from leukocytes, erythrocytes, granulocytes, monocytes, lymphocytes and ECs. Increased circulating MPs are biomarkers of vascular injury and inflammation. Cells exposed to different stimuli such as shear stress, physical agonists, pro-apoptotic stimuli or injury release MPs contributing to EC dysfunction in cardiovascular diseases^[46]. Stimulation of ECs by cytokines, reactive oxygen species, plasminogen activation inhibitor, thrombin or C-reactive protein leads to the formation of endothelial MPs.

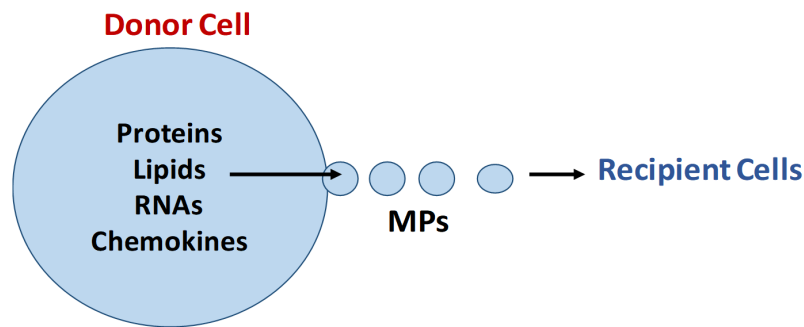


Figure 2. MPs are formed by the plasma membrane pinching off and encapsulating cytosolic components. MPs: microparticles

MPs play an important role in inflammation, EC function and cellular survival^[47]. Circulating MPs impair the atheroprotective function of ECs, in part, by decreasing NO synthesis. Increased levels of circulating MPs are useful biomarkers of vascular injury and a predictor of adverse cardiovascular events and mortality in patients with atherosclerotic disease. Atherosclerotic lesions contain a large number of MPs of leukocyte, SMC, EC and red blood cell origin^[48], and even patients with subclinical atherosclerosis exhibit increased levels of circulating leukocyte-derived MPs^[49].

In addition, MPs promote coagulation, inflammation and alter angiogenesis and apoptosis in ECs. Studies indicate that MPs induce EC dysfunction by disrupting NO release. Endogenous NO inhibits the release of endothelial MPs upon stimulation with C-reactive protein through a tetrahydrobiopterin-dependent mechanism^[50]. Endothelial MPs deliver C-reactive protein and participate in inflammation and coagulation^[51]. MPs from T- lymphocytes depress endothelial nitric oxide synthase (eNOS) activity and increase oxidative stress in ECs; however, T-lymphocytes carrying morphogen sonic hedgehog increase NO production^[46]. Furthermore, the release of MPs carrying lytic complement C5b-9 complex protects ECs from complement-induced lysis. MPs from leukocytes stimulate pro-inflammatory genes in ECs *in vitro*, resulting in the release of cytokines and leukocyte-EC adhesion molecules. On the other hand, MPs from polymorphonuclear leukocytes contain annexin-1, a functionally active anti-inflammatory protein. *In vivo*, annexin-1 inhibits the interaction between leukocytes and ECs^[52]. In *in vitro* studies, the inhibition of endothelial MP release has been shown to induce caspase-3 accumulation, leading to cellular apoptosis. Thus, endothelial MP release could protect EC apoptosis by reducing caspase-3 levels^[53]. Pulmonary microvascular ECs have higher levels of basal cAMP and pulmonary ECs release cAMP containing EVs. These EVs are thought to facilitate compartmentalized cAMP signals and thus, may strengthen the endothelial barrier^[54]. *In vitro* studies have shown that MPs derived from platelets stimulate cell proliferation, migration, and tube formation in ECs. In addition, metalloproteinases contained in endothelial MPs regulate proteolytic activity on the matrix and elicit angiogenesis. Furthermore, MPs derived from endothelial progenitor cells may induce apoptosis-resistant cell proliferation^[55]. MPs contain the cell surface protein and cytoplasmic components of the original cell, and exhibit phosphatidylserine on the surface that accounts for their procoagulant character. MPs from T cells induce EC dysfunction by altering NO and prostacyclin pathways. T-lymphocyte-derived MPs reduce eNOS expression and vascular relaxation, and exhibit increased expression of caveolin-1^[56].

EVs participate in physiological as well as pathological conditions depending on their cargo. EV biogenesis is dysregulated in pathological conditions. Release of EVs can induce inflammation, angiogenesis and thrombosis, and are implicated in many diseases such as cancer, chronic obstructive pulmonary disease, atherosclerosis and PH. During degranulation, activated polymorphonuclear leukocyte-derived exosomes (CD63⁺/CD66b⁺) acquire surface-bound neutrophil elastase that is resistant to α 1-antitrypsin (α 1AT). In chronic obstructive pulmonary disease and bronchopulmonary dysplasia (BPD), polymorphonuclear

leukocyte-derived exosomes acquire surface-bound elastase that degrade extracellular matrix^[57]. In sickle cell disease, inflammasome-dependent shedding of platelet EVs carrying IL-1 β and caspase-1 activate neutrophils and other vascular cells to form large platelet-neutrophil aggregates that occlude pulmonary arteries. The level of circulating platelet EVs correlate with disease severity. Increased circulating platelets and erythroid MPs were observed in untreated children with sickle cell disease; hydroxyurea therapy, however, normalized MP levels^[58,59]. *In vitro* studies have shown that high arterial stress results in platelet activation and release of prothrombotic EVs in the blood^[60]. In cell culture studies, platelet MPs have been shown to induce SMC proliferation in a platelet derived growth factor-independent manner^[61]. In addition, circulating platelet-derived EVs induced increased production of cytokines (IL-1, IL-6, and IL-8), leading to immune modulation, endothelial dysfunction and remodeling. In contrast, neutrophil-derived EVs enhance the biosynthesis of specialized pro-resolving mediators^[62]. MPs from patients with metabolic syndrome decrease NO production in ECs in culture, independent of oxidative stress. Furthermore, injection of MPs in mice resulted in reduced eNOS synthesis and impaired vascular relaxation^[63]. In addition, tissue factor containing MPs are proinflammatory mediators between increased glucose levels and diabetic vasculopathy^[64]. In cancer, EVs promote tumor growth, metastasis and resistance to therapy; and the activation of oncogene signaling pathway can increase EV production. These EVs transfer oncoproteins and facilitate tumor angiogenesis, immunosuppression and metastasis^[65,66]. *In vitro* studies have shown large EVs carrying caveolin-1 in cancer cells to be involved in metastasis^[67]. Thus, the function of MPs depends on the cell types that they originate from and their pathophysiological state.

Mesenchymal stem cells (MSC) are the best cell types for tissue regenerative therapies. They can be readily derived, propagated and differentiated into a variety of cell types. They modulate several biological functions such as tissue repair, downregulation of inflammatory responses, and modulation of the immune system. MSC-derived exosomes have protective effects on ischemia-reperfusion injury via inhibition of cell apoptosis and inflammatory responses^[68,69]. Furthermore, treatment with multipotent stem cells showed strong regenerative capabilities in animal models of myocardial ischemia^[70], stroke^[71], and diabetes^[72]. Recent studies have suggested that MSC-derived EVs containing miRNAs might promote cell and tissue repair, and regeneration^[73]. Similar to the biological activities of MSC, MSC-derived exosomes recover and regenerate the damaged tissues and restore homeostasis^[74]. MSC-exosomes reduce oxidative stress and prevent adverse remodeling in hearts subjected to ischemia-perfusion^[75]; and placental MSC-derived exosomes promote new blood vessel formation and angiogenesis within the placenta under low oxygen conditions^[76]. Interestingly, female bone marrow derived MSCs exhibit higher therapeutic efficacy compared with male MSCs in reducing neonatal hyperoxia-induced lung inflammation, vascular remodeling and PH in a rat model of BPD. Female MSCs express higher levels of VEGF and IL-10, and are better in attenuating PH and improving pulmonary vascular remodeling. The effects on angiogenesis and alveolarization however, were similar in female and male MSC treatment^[77]. In an ischemia-reperfusion injury model, female MSC infusion revealed a greater degree of myocardial recovery compared to male MSC. The protective effect of female MSCs appears to be related to lower levels of TNFR1^[78]. In addition, 17 β -estradiol-treated cardiac stem cells increased the expression of VEGF and SDF-1, and decreased caspase3 resulting in improved myocyte survival after acute ischemia perfusion injury^[79]. These studies suggest that the superior cell survival effect of female MSCs may be dependent on estrogen levels. In contrast, male muscle-derived stem cells display better cartilage regeneration potential compared with their female counterparts^[80]. Thus, these gender differences may be tissue-specific and female MSCs appear to provide better protection in cardiovascular and lung diseases.

PULMONARY HYPERTENSION AND EXTRACELLULAR VESICLES

Altered immunity, platelet activation, vascular inflammation, endothelial dysfunction and thromboembolic complications are well known features of PH. Platelet MPs have been shown to roll over ECs to deliver CCL5^[81]. Importantly, CCL5 is a chemo-attractant for monocytes and T cells. In severe PAH, ECs

were found to be the major source of CCL5^[13]. Circulating MPs have also been shown to stimulate ICAM expression in pulmonary arterial ECs during the late stage in a Sugen + hypoxia model of PH^[82]. Increased levels of platelet-derived MPs, defined as CD31⁺/CD41⁺, and endothelium-derived MPs as CD31⁺/CD41⁻, were observed in IPAH, HPAH and “associated” PAH compared to controls^[83]. Recent studies have shown increased levels of circulating MPs of platelet (CD31⁺/61⁺), leukocyte (CD11b⁺) and endothelial (CD62E⁺) origin in patients with PH. Furthermore, significantly increased endothelial MP levels were detected in patients with thrombo-embolic PH compared to non-embolic patients, indicating increased endothelial dysfunction in the former^[84]. In addition, elevated levels of endothelial MPs, but not leukocyte MPs, prior to treatment are associated with adverse clinical events. Increased CD62e⁺ levels are associated with an inflammatory state^[85]. EVs from animals with PH induce endothelial dysfunction. MPs from hypoxic rats impair EC-dependent relaxation in pulmonary arteries and aorta via reducing NO production and increasing oxidative stress^[86]. MPs bearing active tissue factor and CD105 (endoglin) were reported to be elevated in patients with PAH. Interestingly, in patients with PAH, a further increase in endothelium-derived CD105 MPs was observed in pulmonary arterial blood compared with venous blood. Furthermore, patients in functional class III and IV were found to have higher levels of MPs bearing active tissue factor^[87]. In addition, increased levels of circulating endothelial (CD62E-E-selectin) and CD3 (T cell)-derived EVs were observed in patients with different forms of PH^[88]. Interestingly, *in vitro* studies have revealed that upon exposure to injury, pulmonary arterial ECs release increased amounts of exosomes that induce apoptosis-resistant pulmonary arterial SMC proliferation^[89].

Disturbed blood flow has been shown to acutely induce both endothelial activation and apoptosis, resulting in the release of MPs from activated (CD62E⁺) and apoptotic (CD31⁺/CD42b⁻) ECs^[90]. Increased endothelial MPs have been reported in adult patients with congenital heart defects (atrial and ventricular septal defects) especially with ‘associated’ PAH. These endothelial MPs may contribute to inflammation, leading to endothelial dysfunction, impaired vasodilatation and inhibit angiogenesis via p39 MAPK^[91]. However, no differences in endothelial MP expression were observed in children with congenital heart defects with or without associated PAH^[92].

Apoptotic ECs release exosomes containing tumor susceptibility gene 101 and translationally controlled tumor protein (TCTP) with antiapoptotic function. Vascular SMCs, upon exposure to these nanovesicles, exhibit increased resistance to apoptosis and ERK1/2 activation. Silencing TCTP blocks the resistance to apoptosis and ERK1/2 activation^[93]. TCTP is also a potent mediator of inflammation. In patients with IPAH, HPAH and in a Sugen + hypoxia model, increased levels of TCTP were found in blood outgrowth EC. Knockdown of TCTP resulted in increased apoptosis in these cells in *in vitro* studies. These authors have further shown increased levels of blood outgrowth EC-derived exosomes and MPs in patients with PAH associated with BMPR2 mutation^[94,95]. Upregulation of miR143-5p has been reported in pulmonary arterial SMCs from experimental PH and human PAH. Pulmonary arterial SMC-derived exosomes exhibited enhanced expression of miR143-5p, which induced pro-migratory and pro-angiogenic effects on ECs^[96].

CD39, an ectonucleoside triphosphate diphosphohydrolase, is expressed in the lipid raft domain in plasma membranes of cells including ECs, monocytes and lymphocytes, and functions as an anti-inflammatory and thromboregulatory factor. The absence of CD39 on ECs results in increased susceptibility of ECs to stimulation^[97]. Importantly, ECs in IPAH exhibit downregulation of CD39. Furthermore, suppression of CD39 in *in vitro* studies results in apoptosis resistant pulmonary arterial ECs, and an increased ATP niche that stimulates SMC proliferation and migration^[98]. Visovatti *et al.*^[99] reported the presence of increased circulating endothelial and platelet MPs with CD39 on the surface in patients with IPAH. In addition, ATPase and ADPase activities were increased. In PAH, endothelial dysfunction/disruption and/or apoptosis is the underlying pathological event. Therefore, it is likely that CD39 is lost from ECs, which

could then facilitate proliferation of anti-apoptotic ECs. The loss of endothelial caveolin-1, PECAM-1, vWF and vascular endothelial cadherin, which is indicative of endothelial disruption, has been described in experimental and human PAH^[16,24,100]. Furthermore, increased levels of endothelial MPs carrying vascular endothelial cadherin and PECAM-1 were shown to be associated with hemodynamic severity of PAH^[101]. In addition, BMPR2 loss has been reported in experimental PH and in patients with IPAH without the BMPR2 mutation, and to a lesser degree, in patients with “associated” PAH^[102,103]. Interestingly, the loss of endothelial caveolin-1 and oxidative stress leads to reduced BMPR2 expression, increased TGFβ-derived Smad 2/3 signaling and pulmonary vascular remodeling^[104]. Oliveira *et al.*^[105] recently showed that in the Sugen + hypoxia model of PH in rats and human PAH, endothelial caveolin-1 loss accompanied by increased plasma levels of caveolin-1 EVs and TGFβ, indicating that the endothelial caveolin-1 loss contributes to increased TGFβ signaling, leads to EC proliferation, vascular remodeling and PAH. Caveolin-1 appears to be a plasma biomarker of vascular injury and a key determinant of TGFβ-induced vascular remodeling. It is possible then that the increased levels of EVs containing caveolin-1 in plasma could, in part, be responsible for enhanced expression of caveolin-1 in SMCs observed in IPAH, HPAH and PAH associated with drug toxicity and congenital heart defects^[23,24,100,106].

Recent studies have shown that human pulmonary arterial ECs can efficiently incorporate EVs transmitted by human pulmonary arterial SMCs and translate their mRNA cargo. These EVs enriched in Zeb1 and TGF-β superfamily ligands contribute to endothelial mesenchymal transition (EndMT), thus facilitating disease progression^[107]. However, partial EndMT is a physiological process necessary for angiogenesis. In partial EndMT, ECs do not separate from their neighboring cells^[108]. Figure 3 (as shown on page 9) depicts the inter-relationship between ECs and SMCs, and the role played by EVs in PH.

Interestingly, cigarette smoking results in the release of endothelial EVs with spermine enrichment both on the surface as well as in the cytosol and activates a Ca²⁺-sensing receptor leading to pulmonary vasoconstriction, SMC proliferation and PH^[109]. These results strongly support endothelial injury and disruption underlying the release of EVs. Depending on the cargo, EVs participate in EC-SMC crosstalk in physiological or pathological conditions.

PH, MSC AND MESENCHYMAL EVS

A number of studies have shown the beneficial effects of MSC therapy in experimental models of PH. Intravenous treatment with adipose-derived MSCs improved MCT-induced PH in rats. In addition, adipose-derived MSCs in co-culture with MCT-treated human pulmonary arterial ECs exhibit increased cell proliferation and expression of VEGF^[110]. Bone marrow-derived MSCs over-expressing eNOS attenuated MCT-induced PH in rats^[111], and MSCs expressing increased hemoxygenase (HO)-1 reversed hypoxia-induced PH in mice^[112]. In addition, transplantation with bone marrow-derived MSCs transduced with prostacyclin synthase, and therapy with adiponectin gene modified adipose MSCs significantly attenuated MCT-induced PH, RVH, pulmonary vascular thickening and survival in rats. In *in vitro* studies, the inhibitory effect of adiponectin on the proliferation of pulmonary arterial SMCs obtained from rats with MCT-induced PH was shown to be dependent on the regulation of the AMPK/BMP/Smad pathway^[113,114]. Interestingly, intravenous administration of bone marrow-derived MSCs from donor rats with MCT-induced PH to the recipient rat with MCT-induced PH resulted in attenuation of PH and RVH, and normalization of right ventricular function. Bone marrow-derived MSCs from MCT rats produced more VEGF compared to controls^[115]. In addition, adipose tissue-derived MSC therapy in rats with shunt flow-induced hyperkinetic PAH was attenuated via increased expression of hepatocyte growth factor and eNOS promoting angiogenesis in the injured lungs^[116].

Importantly, female bone marrow-derived MSCs were found to attenuate MCT-induced PH and RVH in mice better than male MSCs. Female MSCs had increased expression of glyceraldehyde-3-phosphate

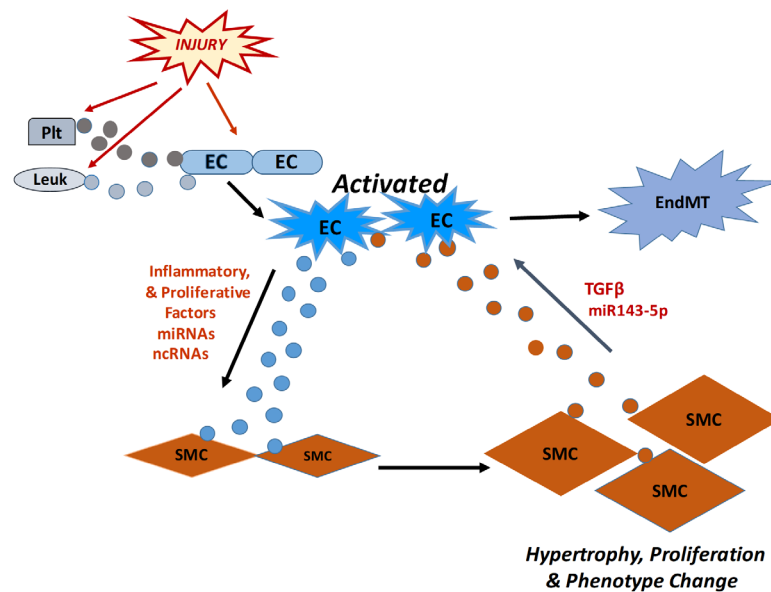


Figure 3. This figure depicts the possible mechanism of injury resulting in dysregulation of a number of vascular and inflammatory cells. Injury (inflammatory, oxidant, drug toxicity, increased pulmonary flow) activates Plt, Leuk and EC to produce increased amounts of EVs. EVs from platelets and leukocytes induce EC dysfunction. Endothelial EVs contain inflammatory and proliferative factors, and miRNAs are incorporated in SMCs, leading to hypertrophy, proliferation and phenotype change. These SMCs produce EVs containing TGFβ, miRNAs and other factors that are incorporated into ECs, leading to EndMT. EC: endothelial cell; EVs: extracellular vesicles; SMCs: smooth muscle cells; EndMT: endothelial mesenchymal transition; Plt: platelets; Leuk: leukocytes; TGFβ: transforming growth factor β

dehydrogenase that regulates (Ca^{2+})_i signal associated function, which might be responsible for the superior function of female bone marrow-derived MSCs^[117]. Furthermore, in an ischemia-reperfusion model, rat hearts treated with female MSCs demonstrated significantly greater recovery of left ventricular pressure compared to male MSC treated hearts. Importantly, male MSCs produced higher levels of TNFα and less VEGF than female MSCs^[118].

EVs from plasma and lung homogenates from mice with MCT-induced PH have been shown to induce PH and RVH in healthy mice. Interestingly, exosomes but not microvesicles from MCT-mice injured wild type mice; MSC-induced exosomes also prevented and reversed MCT-induced PH. Furthermore, exosomes from MCT-treated mice and patients with IPAH revealed increased expression of miRs-19b, -20a, -20b, and -145. In contrast, MSC-exosomes exhibited increased levels of anti-proliferative and anti-inflammatory miRs (-34a, -122, -124, -127)^[119]. Chen *et al.*^[120] have shown that both bone marrow-derived MSCs and MSC-EVs ameliorated MCT-induced PH and RVH, indicating that a cell free approach to stem cell therapy is effective. In addition, adipose-derived exosomes have been shown to transfer miR125a to ECs to promote angiogenesis by suppressing angiogenic inhibitor Dll4 and facilitate repair^[121]. Renin-angiotensin involvement in the pathogenesis of PH is well documented. Liu *et al.*^[122] have reported that bone marrow-derived MSC-microvesicles attenuated MCT-induced PH and RVH in rats accompanied by increased levels of ACE2 mRNA in lung tissue, increased plasma levels of Ang-(1-7) and decreased ACE compared with controls. Interestingly, the administration of adipose-derived MSC-EVs was shown to inhibit neointima formation in the vein graft model, accompanied by a significant decrease in the expression of IL-6, monocyte chemoattractant protein-1 and phosphorylation of Akt, Erk1/2^[123]. In mice with hypoxia-induced PH, intravenous treatment with MSC-derived exosomes attenuated PH and RVH, reduced STAT3 activation and upregulated the miR-17 superfamily of miRNAs. In addition, treatment with MSC exosomes increased the level of miR204. It is worth noting here that the expression of miR204 in the lungs is low in patients with PAH. These authors have further shown in *in vitro* studies that the activation

of py-STAT3 in human pulmonary arterial SMCs in response to hypoxia is inhibited by exosomes derived from the human umbilical cord^[124]. Klinger *et al.*^[125] have recently reported the prevention and reversal of the Sugen+ hypoxia model of PH in rats by MSC EVs. Similar to the cancer phenotype, in PAH, cells undergo a metabolic shift towards glycolysis and lactic acid formation which enables sustained ATP production and uncontrolled cell growth. MSC exosomes increase glucose oxidation and prevent a shift to glycolysis and mitochondrial damage. In addition, exosomes inhibit SIRT4 expression upstream of pyruvate dehydrogenase and glutamate dehydrogenase that contribute to the improvement of mitochondrial function^[126]. Thus, MSC-derived EVs can have beneficial effects on the pathophysiology of PH and mitochondrial function.

PH is a frequent and serious complication in preterm infants with BPD, a chronic lung disease. Treatment with conditioned media from cultured mouse bone marrow-derived MSCs showed significant improvement in hyperoxia-induced BPD in mice. It reversed hyperoxia-induced lung parenchymal pathological changes and PH^[127]. Chang *et al.*^[128] treated nine preterm infants (gestational age 25.3 ± 0.9 weeks) with intra-tracheal transplantation of human umbilical cord blood-derived MSCs. At 7 days after treatment, these infants had no adverse effects, and the severity of BPD was observed to be low. In addition, tracheal aspirates revealed lower levels of IL-6, IL-8, metalloproteinase-9, TNF α and TGF β 1. These studies showed the beneficial effects of MSCs on lung development.

In summary, EVs play a significant role in the pathophysiology of PH. Under normal conditions, EVs produced by different cells modulate immune responses, participate in intercellular communication and maintain homeostasis. Increased levels of EVs observed in PH are indicative of endothelial injury. These EVs facilitate cell proliferation, inflammation, and progression of the disease. MSCs and MSC-derived EVs are capable of modulating immune responses, repairing injured tissues and have regenerative properties. The beneficial effects of MSCs and MSC-EVs, including some genetically modified MSCs have been reported in several experimental models of PH. Treatment with MSC-EVs (naïve or genetically modified) may have an advantage over cell therapy.

DECLARATIONS

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Authors' contributions

Written the paper: Mathew R

Contributed to discussion: Dorai T

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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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Original Article

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Risk factors adversely impacting post coronary artery bypass grafting longer-term vs. shorter-term clinical outcomes

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Abstract

Aim: Coronary artery bypass grafting (CABG) patients' characteristics and surgical techniques associated with short-term (ST; < 1 year) mortality are well documented; however, the literature pinpointing factors predictive of longer-term (LT; ≥ 1 year) death rates are more limited. Thus, the CABG factors associated with ST vs. LT mortality were compared.

Methods: Using advanced PubMed search techniques, the factors associated with improved post-CABG mortality were compared for ST vs. LT prediction models; ST vs. LT models' results were compared across three time periods: until 1997, 1998-2007, and 2007-2017.

Results: Of 156 post-CABG mortality risk models ($n = 125$ publications), 133 ST and 23 LT models were evaluated. Important predictors consistently included age, ejection fraction, and renal dysfunction/dialysis. The ST models more commonly identified surgical priority, gender, and prior cardiac surgery; however, the LT models more frequently included diabetes and peripheral arterial disease. Compared to ST mortality, patterns also emerged for cerebrovascular disease and chronic lung disease predicting LT mortality. As modifiable risks, body mass index or another marker of body habitus appeared in 31/133 (23%) of ST models; smoking or tobacco use was considered in only 4/133 (3%). No models evaluated compliance with ischemic heart disease guidelines. No time period-related differences were found.

Conclusion: Different risk factors predicted ST vs. LT post-CABG mortality; for LT death, debilitating chronic/complex comorbidities were more often reported. As few models focused on identifying modifiable patient risks or ischemic heart disease guideline compliance, future CABG LT risk modeling should address these knowledge gaps.



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Keywords: Coronary artery bypass graft surgery, risk assessment, outcomes research, survival, mortality

INTRODUCTION

Over the past 60 years, much has changed in the healthcare field. Increasingly, attention is being paid to healthcare quality with the goals of improving clinical outcomes and increasing value of care delivered. A special emphasis in quality improvement has been placed on high volume procedures such as coronary artery bypass grafting (CABG). Although CABG volumes have declined from ~213,700 procedures (2011) to ~156,900 procedures (2016), it remains the most common cardiac surgical procedure performed in the United States^[1-3]. To evaluate the true value of CABG, longer-term outcomes are necessary to establish the durability of the procedure. Accordingly, the baseline patient risk factors associated with short-term (< 1 year) and longer-term (\geq 1 year) CABG mortality were compared.

Interpreting CABG clinical outcomes data can often be challenging, as there may be a wide range in pre-CABG patient's severity of coronary disease or comorbidity-related disease complexity, variations in CABG operative techniques used or post-CABG pre-discharge patient care management, as well as provider-based variations for annual CABG volumes performed. In 1972, the Department of Veterans Affairs (VA) healthcare system began internally reporting national unadjusted outcome rates (e.g., "observed" in-hospital mortality rates) for patients undergoing cardiac surgery at its institutions; these first VA reports focused upon observed CABG mortality and post-CABG complication rates^[4].

After US hospitals' CABG mortality reports were made publicly available by the Department of Health and Human Services in 1985, Congress in 1986 mandated that the VA report risk-adjusted cardiac surgery mortality rates and compare these VA rates to national standards^[5]. Given these legislation-driven mandates, VA clinicians and scientists began looking for ways to "level the playing field" using statistical risk models to permit more meaningful comparisons between centers and surgeons; these risk-adjusted outcome reports were used in their local VA medical centers' quality improvement endeavors.

Initiated in April 1987, the VA Continuous Improvement in Cardiac Surgery Program (CICSP) was founded; CICSP was one of the first registries to report risk-adjusted CABG 30-day operative mortality and major morbidity across all participating VA hospitals^[4]. The VA CICSP identified a set of Veteran risk characteristics associated with CABG adverse outcomes; based on gathering 54 patients' risk, cardiac surgical procedural details, and hospital-related outcomes, the VA CICSP calculated the "expected" mortality occurrence for each Veteran undergoing a CABG procedure. Across providers and "high-risk" patient sub-groups, therefore, "observed" to "expected" outcome rates were compared to identify opportunities to improve their local VA cardiac surgical care^[6].

Some of the earliest lists of pre-CABG patient risk factors associated with mortality were developed entirely based on expert consensus. As different national, regional, and state-wide databases originally gathered different sets of patient risk factors, an early consensus conference was held to identify the minimal set of "core" risk variables required to be captured^[7,8]. Given challenges encountered with CABG records' data completeness, however, these earliest mathematical approaches to calculate risk-adjusted outcome rates made use of Bayes theorem^[9]. Since the VA's programmatic expansions in 1992, dramatic improvements were made in the VA completeness of CABG data captured; thus, logistic regression emerged as the most common analytical approach used. Other approaches have been reported, including applications of neural networks and Cox regression^[10,11]. Given both the ease of clinical interpretation and superior statistical model performance, however, logistic regression remains the standard analytical approach used to predict post-CABG short-term (ST) and longer-term (LT) mortality^[12-14].

Historically, the process of choosing logistic regression eligible (“candidate”) risk variables was different for each CABG registry. As this pre-selection candidate variable approach may have introduced subjectivity and biased model results, CABG risk models (such as those developed by the VA, Society of Thoracic Surgeons, and EuroSCORE teams) have been derived in recent years using a standardized approach with a core set of model eligible variables. Beyond this core set, however, each database incorporates an expanded set of population-specific risk variables in their risk modeling processes.

Over the past 30 years, nearly countless CABG risk models with various designs and complexity have been developed to predict the likelihood of death at pre-specified time periods. As the standard ST endpoint used, operative mortality was defined as death within 30 days or within the index hospitalization. As operative mortality avoids any potential post-discharge referral bias (e.g., post-CABG hospital discharge to a separate sub-acute care facility), this endpoint was determined to be the most clinically relevant performance metric; it is commonly used to assess the quality of the surgical procedure. Other models have considered LT death during longer periods of follow-up, investigating the durability of the CABG procedure and importance of other risk factors. For ST and LT published risk models, therefore, this study describes the patterns in pre-CABG factors differentially impacting ST vs. LT mortality. Until this report, these patterns had not been previously described. Moreover, this novel report identifies additional opportunities to improve future CABG risk models.

METHODS

An advanced literature review was undertaken to document published risk factors associated with post-CABG mortality. In February 2019, PubMed was searched for all Medline publications using the following terms: “CABG” (Title) OR “coronary artery bypass” (Title) AND “mortality” (Title) OR “risk” (Title) OR “death” (Title) OR “survival” (Title). This yielded 1904 publications. Following a review of all articles for pre-stated inclusion/exclusion criteria, there were a total of 125 included articles with 156 CABG mortality models. Only papers reporting risk models for mortality after an isolated CABG procedure were included; inclusion criteria were otherwise left intentionally broad so as to gather a wide variety of models. Models requiring data from the postoperative period were excluded for the purpose of this review, whereas those employing only preoperative variables [as opposed to preoperative and intraoperative variables (e.g., cardiopulmonary bypass time)] were identified for sub-analysis review. For the 125 publications meeting all inclusion/exclusion criteria, their reference lists were also carefully reviewed for relevant publications to augment the original search strategy’s findings.

Working collaboratively under the senior co-authors’ guidance, the majority of literature search screening and data extraction were performed primarily by one author (BC). To permit meaningful model comparisons, risks were classified into 91 different common clinical categories. Clinically relevant composite variables were reported based upon database-specific definitions (e.g., “critical preoperative state” and “extra-cardiac arteriopathy”). Named risk indices (e.g., “Elixhauser Comorbidity Index”) were analyzed using their assigned name as a group, rather than being recorded based upon the indices’ subcomponents. For the 125 publications evaluated, the set of risk factors identified to be associated with post-CABG ST or LT mortality were compared. Time trends in models’ risk factors reported were evaluated across three time periods until 1997, 1998–2007, and 2007–2017.

RESULTS

One hundred fifty-six post-CABG mortality risk models were identified within 125 different papers. In Appendix A, the full listing of these papers and models can be found in [Supplementary Tables 1 and 2](#).

Of these models, 133 predicted ST CABG mortality. Operative mortality was the most commonly reported ST endpoint, defined as death occurring during the index hospitalization and/or up to 30 days after the

index surgical procedure. Twenty-three LT CABG mortality models were identified. The longest period of follow-up was seven years, reported by Wu *et al.*^[15] When looking at those models considering only preoperative (i.e., not intraoperative) risk factors, there were 75 ST models and 14 LT models (total = 89). As a pre-planned sub-analysis, risk models considering on-pump *vs.* off-pump CABG and only preoperative risk factors were also compared separately. This identified three ST and one LT models (total = 4). The complete listing of variables for the ST *vs.* LT models with frequency counts is included in [Table 1](#).

Overwhelmingly, age was the most common preoperative variable identified to be predictive of ST post-CABG mortality, reported in 115 of 125 (86%) of those models. Of the articles summarized, 22/156 (14.1%) did not report age as a risk factor. Across these 22 publications, the age-related variability in reporting observed appears to be due in part to their study-specific populations' inherent risk profile. For example, articles focused upon higher risk patient sub-groups (e.g., emergent CABG patients or those experiencing an acute myocardial infarction) commonly did not report age as a post-CABG mortality model finding. Despite this observed pattern, however, there was not a single, simple explanation for the observed inconsistency in age not being reported across all models.

Age was followed by left ventricular ejection fraction (included in 64% of ST mortality models), surgical case priority or status (59%), patient gender (57%), and having undergone a prior cardiac surgical procedure before the index procedure (55%); these represented the top five most common preoperative variables for predicting ST post-CABG mortality. For LT models, the top five risk factors were age, ejection fraction, diabetes mellitus, peripheral arterial disease, and renal failure. There appeared to be a trend toward cerebrovascular disease and lung disease being more commonly reported by CABG risk models focused upon mortality beyond one year (compared with other variables within that same subset of models), perhaps suggesting debilitating chronic and complex comorbidities are more useful in prediction of LT mortality.

When the results were grouped into early, mid, and late subgroups by year of publication [[Tables 2-4](#)], age and ejection fraction remained among the most common risk factors for models throughout those time periods. No definite trends over time were observed in risk factor prevalence for the overall group or the ST or LT model subgroups, although sample size may have impacted the ability to detect such trends, particularly within the subgroups. Results were also similar when considering models that included only preoperative risk factors [[Table 5](#)] or those that considered on-pump *vs.* off-pump CABG [[Table 6](#)].

DISCUSSION

Across the post-CABG follow-up periods, different pre-CABG risk factors predictive of mortality were documented. This literature search revealed dozens of logistic regression models, each reporting different patient risk factors associated with time-varying post-CABG mortality endpoints. As documented by the tables, the ST models found the patient's risk variables related to their severity of coronary disease (e.g., more commonly reported be important predictors), whereas patient's chronic comorbidities (e.g., diabetes, cerebrovascular disease, or pulmonary disease) appeared to be more frequently associated with LT post-CABG mortality. Following one-year post-CABG, life expectancy appears to be most strongly impacted by non-cardiac comorbidities than cardiac factors or surgical processes of care. While optimizing CABG patient selection and surgical techniques may be important ST, optimal management of non-cardiac comorbidities may improve post-CABG patients' LT survival. Moreover, across all follow-up time periods, a patient's age, ejection fraction, and renal function (e.g., creatinine or dialysis dependence) were important predictors of post-CABG mortality; these were consistently reported for the ST and LT mortality time periods.

A special sub-analysis was performed for the sub-group of models comprised of preoperative risk factors along with a variable indicating the on-pump *vs.* off-pump surgical technique. Although there were

Table 1. All data

All models			Short-term models			Long-term models		
Variable	n = 156	%	Variable	n = 133	%	Variable	n = 23	%
Age	134	86%	Age	115	86%	Age	12	52%
Left ventricular function	104	67%	Left ventricular function	85	64%	Left ventricular function	12	52%
Renal failure	88	56%	Urgency	78	59%	Comb. arterial disease	9	39%
Comb. heart failure variables	84	54%	Gender	76	57%	Diabetes	8	35%
Urgency	84	54%	Renal failure	73	55%	Peripheral arterial disease	8	35%
Gender	83	53%	Comb. heart failure variables	69	52%	Renal failure	7	30%
Comb. arterial disease	76	49%	Repeat operation	68	51%	Comb. heart failure variables	7	30%
Comb. CHF or NYHA	74	47%	Comb. arterial disease	61	46%	Comb. CHF or NYHA	7	30%
Repeat operation	73	47%	Comb. any MI variable	60	45%	Lung disease	5	22%
Comb. any MI variable	65	42%	Comb. CHF or NYHA	60	45%	Neurologic disease	5	22%
History of MI	63	40%	History of MI	59	44%	Congestive heart failure	5	22%
Comb. critical state	62	40%	Comb. critical state	58	44%	Comb. graft variables	5	22%
Peripheral arterial disease	62	40%	Peripheral arterial disease	49	37%	Postoperative variables	5	22%
Diabetes	54	35%	Lung disease	43	32%	Body size measurements	4	17%
Lung disease	52	33%	Comb. vessel disease	43	32%	Comb. vessel disease	4	17%
Comb. vessel disease	51	33%	Diabetes	40	30%	Type of graft(s)	4	17%
Neurologic disease	49	31%	Neurologic disease	39	29%	Smoking status	4	17%
Congestive heart failure	47	30%	Congestive heart failure	37	28%	Gender	3	13%
Body size measurements	39	25%	Body size measurements	31	23%	Urgency	3	13%
Left main disease	36	23%	Cardiogenic shock	30	23%	Comb. any MI variable	3	13%
Cardiogenic shock	34	22%	NYHA class	29	22%	Left main disease	3	13%
NYHA class	33	21%	Left main disease	29	22%	Repeat operation	2	9%
Number of diseased vessels	30	19%	Number of diseased vessels	27	20%	NYHA class	2	9%
Comb. ECG or arrhythmia variables	29	19%	Comb. ECG or arrhythmia variables	26	20%	History of MI	2	9%
Preoperative IABP use	27	17%	Concurrent procedure	26	20%	Valve disease	2	9%
Concurrent procedure	26	17%	Preoperative IABP use	24	18%	Hypertension	2	9%
Angina	26	17%	Angina	23	17%	Comb. HTN or BP	2	9%
Comb. HTN or BP	25	16%	Comb. HTN or BP	21	16%	Atrial arrhythmia	2	9%
Comb. PCI variables	24	15%	Comb. PCI variables	21	16%	Hypercholesterolemia	2	9%
Hypertension	24	15%	Hypertension	20	15%	Intraoperative variables	2	9%
Postoperative variables	22	14%	Pulmonary hypertension	19	14%	Ventricular wall motion	1	4%
Comb. graft variables	20	13%	Non-CABG surgery	18	14%	Calcified aorta	1	4%
Pulmonary hypertension	19	12%	Postoperative variables	17	13%	Angina	1	4%
Valve disease	19	12%	Any arrhythmia	16	12%	Active MI	1	4%
Non-CABG surgery	18	12%	Valve disease	15	11%	Number of diseased vessels	1	4%
Any arrhythmia	17	11%	Comb. graft variables	14	11%	Diffuse/severe disease	1	4%
Inotropic medication	15	10%	Extracardiac arteriopathy	12	9%	Number of grafts	1	4%
Prior/recent PCI or PTCA	15	10%	Inotropic medication	12	9%	Race or ethnicity	1	4%

Atrial arrhythmia	13	8%	Cardiopulmonary bypass time	12	9%	Preoperative IABP use	1	4%
Type of graft(s)	13	8%	Prior/recent PCI or PTCA	12	9%	Inotropic medication	1	4%
Extracardiac arteriopathy	13	8%	Nitroglycerin use	10	8%	Comb. critical state	1	4%
Cardiopulmonary bypass time	12	8%	Critical state	10	8%	Cardiogenic shock	1	4%
Race or ethnicity	11	7%	Preoperative diuretic use	9	7%	Immunosuppression	1	4%
Ventricular or unstable arrhythmia	10	6%	Type of graft(s)	9	7%	Date or order of surgery	1	4%
Preoperative diuretic use	10	6%	Liver disease	9	7%	Aortic cross-clamp duration	1	4%
Critical state	10	6%	Cardiomegaly	9	7%	On- vs. off-pump CABG	1	4%
Liver disease	10	6%	Atrial arrhythmia	9	7%	Prior/recent PCI or PTCA	1	4%
Smoking status	10	6%	Diffuse/severe disease	8	6%	Comb. PCI variables	1	4%
Nitroglycerin use	10	6%	PTCA failure/emergency	8	6%	Ventricular or unstable arrhythmia	1	4%
Diffuse/severe disease	9	6%	Ventricular or unstable arrhythmia	8	6%	Comb. ECG or arrhythmia variables	1	4%
Immunosuppression	9	6%	Preop intubation	8	6%	Preoperative thrombolysis	1	4%
Cardiomegaly	9	6%	Race or ethnicity	7	5%	Left ventricular hypertrophy	1	4%
PTCA failure/emergency	8	5%	On- vs. off-pump CABG	7	5%	Cachexia or malnutrition	0	0%
Preop intubation	8	5%	Endocarditis	7	5%	Pulmonary hypertension	0	0%
On- vs. off-pump CABG	8	5%	Dyspnea	6	5%	Extracardiac arteriopathy	0	0%
Number of grafts	8	5%	Number of grafts	6	5%	Dyspnea	0	0%
Intraoperative variables	7	4%	Immunosuppression	6	5%	Type of MI	0	0%
Endocarditis	7	4%	Aortic cross-clamp duration	5	4%	Pulmonary rates	0	0%
Aortic cross-clamp duration	7	4%	Intraoperative variables	5	4%	Preoperative diuretic use	0	0%
Dyspnea	6	4%	Pulmonary rates	4	3%	Killip classification	0	0%
Date or order of surgery	6	4%	Smoking status	4	3%	Blood pressure	0	0%
Digoxin or digitalis use	5	3%	Anticoagulation or antiplatelet use	4	3%	Nitroglycerin use	0	0%
Hypercholesterolemia	5	3%	Disaster, catastrophic state	4	3%	Liver disease	0	0%
Disaster, catastrophic state	4	3%	Anemia (hemoglobin, hematocrit)	4	3%	Cardiopulmonary bypass time	0	0%
Pulmonary rates	4	3%	Digoxin or digitalis use	4	3%	Cardiomegaly	0	0%
Active MI	4	3%	A published comorbidity index	4	3%	Preoperative CPR/cardiac arrest	0	0%
Ventricular wall motion	4	3%	Other preoperative comorbidities	4	3%	Location or type of surgical center	0	0%
Other preoperative comorbidities	4	3%	Ventricular wall motion	3	2%	Center's case frequency	0	0%
A published comorbidity index	4	3%	Active MI	3	2%	Endocarditis	0	0%
Anemia (hemoglobin, hematocrit)	4	3%	Preoperative CPR/cardiac arrest	3	2%	Abdominal aortic aneurysm	0	0%
Anticoagulation or antiplatelet use	4	3%	Location or type of surgical center	3	2%	PTCA failure/emergency	0	0%
Serum albumin	3	2%	Hypercholesterolemia	3	2%	Stent thrombosis	0	0%
Other preoperative labs	3	2%	Refused blood products	3	2%	Any family history variable	0	0%
Refused blood products	3	2%	Other preoperative labs	3	2%	Any arrhythmia	0	0%
Location or type of surgical center	3	2%	Serum albumin	3	2%	Antiarrhythmic agents	0	0%
Preoperative CPR/cardiac arrest	3	2%	Cachexia or malnutrition	2	2%	Other ECG abnormalities	0	0%
Preoperative thrombolysis	2	1%	Type of MI	2	2%	Non-CABG surgery	0	0%
Other ECG abnormalities	2	1%	Date or order of surgery	2	2%	Anticoagulation or antiplatelet use	0	0%
Stent thrombosis	2	1%	Abdominal aortic aneurysm	2	2%	PT or INR	0	0%

Type of MI	2	1%	Stent thrombosis	2	2%	Critical state	0	0%
Calcified aorta	2	1%	Any family history variable	2	2%	Disaster, catastrophic state	0	0%
Cachexia or malnutrition	2	1%	Other ECG abnormalities	2	2%	Anemia (hemoglobin, hematocrit)	0	0%
Recent admissions	2	1%	Steroid use	2	2%	Transfusion	0	0%
Patient education level/literacy	2	1%	Preoperative cardiac biomarkers	2	2%	Refused blood products	0	0%
Preoperative cardiac biomarkers	2	1%	Patient education level/literacy	2	2%	Digoxin or digitalis use	0	0%
Steroid use	2	1%	Calcified aorta	1	1%	Preop intubation	0	0%
Heart rate	2	1%	Killip classification	1	1%	Concurrent procedure	0	0%
Any family history variable	2	1%	Blood pressure	1	1%	A published comorbidity index	0	0%
Abdominal aortic aneurysm	2	1%	Center's case frequency	1	1%	Heart rate	0	0%
Transfusion	1	1%	Antiarrhythmic agents	1	1%	Steroid use	0	0%
PT or INR	1	1%	Preoperative thrombolysis	1	1%	Preoperative cardiac biomarkers	0	0%
Antiarrhythmic agents	1	1%	PT or INR	1	1%	Other preoperative labs	0	0%
Center's case frequency	1	1%	Transfusion	1	1%	Serum albumin	0	0%
Blood pressure	1	1%	Heart rate	1	1%	Other preoperative comorbidities	0	0%
Killip classification	1	1%	ACE inhibitor use	1	1%	ACE inhibitor use	0	0%
Acute mental status changes	1	1%	ASA classification	1	1%	Functional state	0	0%
Time from admission to procedure	1	1%	Insurance type or status	1	1%	Patient education level/literacy	0	0%
Left ventricular hypertrophy	1	1%	Recent admissions	1	1%	ASA classification	0	0%
Insurance type or status	1	1%	Time from admission to procedure	1	1%	Insurance type or status	0	0%
ASA classification	1	1%	Acute mental status changes	1	1%	Recent admissions	0	0%
Functional state	1	1%	Functional state	0	0%	Time from admission to procedure	0	0%
ACE inhibitor use	1	1%	Left ventricular hypertrophy	0	0%	Acute mental status changes	0	0%
Total variables (excl. combinations)	92		Total variables (excl. combinations)	90		Total variables (excl. combinations)	42	

Comb: combination variable; CHF: congestive heart failure; NYHA: New York Heart Association; MI: myocardial infarction; ECG: electrocardiogram; IABP: intra-aortic balloon pump; HTN: hypertension; BP: blood pressure; PCI: percutaneous coronary intervention; PTCA: percutaneous transluminal coronary angioplasty; CPR: cardiopulmonary resuscitation; ASA: American Society of Anesthesiology; ACE: angiotensin converting enzyme; INR: international normalized ratio; PT: prothrombin time

Table 2. All risk models by publication year

Variable	≤1997			1998-2007			2008-2017		
	n	%	Variable	n	%	Δa	n	%	Δa
Age	37	90%	Age	44	81%	-9%	53	87%	-3%
Repeat operation	24	59%	Left ventricular function	40	74%	18%	41	67%	11%
Comb. heart failure variables	24	59%	Renal failure	36	67%	25%	35	57%	16%
Left ventricular function	23	56%	Gender	31	57%	11%	34	56%	7%
History of MI	22	54%	Urgency	30	56%	7%	33	54%	8%
Comb. any MI variable	22	54%	Comb. heart failure variables	30	56%	-3%	32	52%	13%
Comb. CHF or NYHA	21	51%	Comb. arterial disease	28	52%	13%	30	49%	-9%
Urgency	20	49%	Repeat operation	25	46%	-12%	30	49%	20%
Gender	19	46%	Comb. any MI variable	25	46%	-7%	28	46%	-5%
Renal failure	17	41%	Comb. CHF or NYHA	25	46%	-5%	25	41%	2%

Peripheral arterial disease	16	39%	Diabetes	24	44%	22%	Repeat operation	24	39%	-19%	-7%
Comb. arterial disease	16	39%	History of MI	24	44%	-9%	Diabetes	21	34%	12%	-10%
Lung disease	15	37%	Lung disease	22	41%	4%	Comb. any MI variable	18	30%	-24%	-17%
Comb. vessel disease	15	37%	Neurologic disease	21	39%	5%	History of MI	17	28%	-26%	-17%
Neurologic disease	14	34%	Peripheral arterial disease	21	39%	0%	Cardiogenic shock	16	26%	12%	4%
Congestive heart failure	14	34%	Comb. vessel disease	21	39%	2%	Lung disease	15	25%	-12%	-16%
Angina	13	32%	Comb. critical state	20	37%	8%	NYHA class	15	25%	0%	10%
Comb. critical state	12	29%	Congestive heart failure	19	35%	1%	Comb. vessel disease	15	25%	-12%	-14%
Body size measurements	11	27%	Body size measurements	18	33%	7%	Neurologic disease	14	23%	-11%	-16%
Left main disease	11	27%	Left main disease	18	33%	7%	Congestive heart failure	14	23%	-11%	-12%
Comb. ECG or arrhythmia variables	11	27%	Comb. HTN or BP	14	26%	11%	Number of diseased vessels	12	20%	3%	-1%
NYHA class	10	24%	Hypertension	13	24%	9%	Preoperative IABP use	12	20%	3%	5%
Diabetes	9	22%	Cardiogenic shock	12	22%	8%	Concurrent procedure	11	18%	3%	1%
Number of diseased vessels	7	17%	Postoperative variables	12	22%	15%	Body size measurements	10	16%	-10%	-17%
Preoperative IABP use	7	17%	Number of diseased vessels	11	20%	3%	Comb. PCI variables	10	16%	2%	2%
Valve disease	6	15%	Comb. ECG or arrhythmia variables	11	20%	-6%	Atrial arrhythmia	10	16%	14%	13%
Hypertension	6	15%	Pulmonary hypertension	10	19%	9%	Valve disease	9	15%	0%	7%
Nitroglycerin use	6	15%	Concurrent procedure	9	17%	2%	Inotropic medication	9	15%	7%	9%
Cardiomegaly	6	15%	NYHA class	8	15%	-10%	Angina	8	13%	-19%	4%
Cardiogenic shock	6	15%	Preoperative IABP use	8	15%	-2%	Race or ethnicity	7	11%	11%	4%
Non-CABG surgery	6	15%	Comb. graft variables	8	15%	0%	Left main disease	7	11%	-15%	-22%
Concurrent procedure	6	15%	Comb. PCI variables	8	15%	0%	Postoperative variables	7	11%	4%	-11%
Diffuse/severe disease	6	15%	Preoperative diuretic use	7	13%	6%	Extracardiac arteriopathy	7	11%	11%	0%
Comb. graft variables	6	15%	Any arrhythmia	7	13%	1%	Comb. ECG or arrhythmia variables	7	11%	-15%	-9%
Comb. HTN or BP	6	15%	Prior/recent PCI or PTCA	6	11%	4%	On- vs. off-pump CABG	6	10%	10%	6%
Comb. PCI variables	6	15%	Non-CABG surgery	6	11%	-4%	Prior/recent PCI or PTCA	6	10%	3%	-1%
Cardiopulmonary bypass time	5	12%	Intraoperative variables	6	11%	11%	Non-CABG surgery	6	10%	-5%	-1%
Any arrhythmia	5	12%	Extracardiac arteriopathy	6	11%	11%	Critical state	6	10%	10%	2%
Pulmonary hypertension	4	10%	Type of graft(s)	6	11%	4%	Comb. graft variables	6	10%	-5%	-5%
Pulmonary rates	4	10%	Angina	5	9%	-22%	Pulmonary hypertension	5	8%	-2%	-10%
Number of grafts	3	7%	Endocarditis	5	9%	4%	Hypertension	5	8%	-6%	-16%
Liver disease	3	7%	Ventricular or unstable arrhythmia	5	9%	2%	Smoking status	5	8%	6%	1%
Aortic cross-clamp duration	3	7%	Valve disease	4	7%	-7%	Immunosuppression	5	8%	6%	3%
Prior/recent PCI or PTCA	3	7%	Race or ethnicity	4	7%	7%	Comb. HTN or BP	5	8%	-6%	-18%
Anemia (hemoglobin, hematocrit)	3	7%	Smoking status	4	7%	5%	Any arrhythmia	5	8%	-4%	-5%
Preop intubation	3	7%	Liver disease	4	7%	0%	Date or order of surgery	4	7%	7%	3%
Postoperative variables	3	7%	Cardiopulmonary bypass time	4	7%	-5%	Type of graft(s)	4	7%	-1%	-5%
Ventricular wall motion	3	7%	Aortic cross-clamp duration	4	7%	0%	Liver disease	3	5%	-2%	-2%
Preoperative diuretic use	3	7%	Hypercholesterolemia	4	7%	7%	Cardiopulmonary bypass time	3	5%	-7%	-2%
Type of graft(s)	3	7%	Critical state	4	7%	7%	Other preoperative labs	3	5%	5%	5%
Inotropic medication	3	7%	Preop intubation	4	7%	0%	Number of grafts	2	3%	-4%	-2%

Preoperative CPR/cardiac arrest	3	7%	Dyspnea	4	7%	7%	A published comorbidity index	2	3%	1%	1%
PTCA failure/emergency	3	7%	Number of grafts	3	6%	-2%	Preoperative cardiac biomarkers	2	3%	3%	3%
Ventricular or unstable arrhythmia	3	7%	Nitroglycerin use	3	6%	-9%	Patient education level/literacy	2	3%	3%	3%
Disaster, catastrophic state	3	7%	Cardiomegaly	3	6%	-9%	Recent admissions	2	3%	3%	3%
Serum albumin	3	7%	Immunosuppression	3	6%	3%	Dyspnea	2	3%	3%	-4%
Endocarditis	2	5%	Active MI	3	6%	6%	PTCA failure/emergency	2	3%	-4%	-2%
Anticoagulation or antiplatelet use	2	5%	Inotropic medication	3	6%	-2%	Stent thrombosis	2	3%	3%	3%
Digoxin or digitalis use	2	5%	Location or type of surgical center	3	6%	6%	Ventricular or unstable arrhythmia	2	3%	-4%	-6%
Cachexia or malnutrition	2	5%	PTCA failure/emergency	3	6%	-2%	Nitroglycerin use	1	2%	-13%	-4%
Smoking status	1	2%	Refused blood products	3	6%	6%	Hypercholesterolemia	1	2%	2%	-6%
Immunosuppression	1	2%	Date or order of surgery	2	4%	4%	Anticoagulation or antiplatelet use	1	2%	0%	0%
Any family history variable	1	2%	On- vs. off-pump CABG	2	4%	4%	Digoxin or digitalis use	1	2%	-3%	-2%
A published comorbidity index	1	2%	Abdominal aortic aneurysm	2	4%	4%	Preop intubation	1	2%	-6%	-6%
Other preoperative comorbidities	1	2%	Digoxin or digitalis use	2	4%	-1%	Other preoperative comorbidities	1	2%	-1%	-2%
ACE inhibitor use	1	2%	Heart rate	2	4%	4%	Insurance type or status	1	2%	2%	2%
Type of MI	1	2%	Steroid use	2	4%	4%	Time from admission to procedure	1	2%	2%	2%
Atrial arrhythmia	1	2%	Other preoperative comorbidities	2	4%	1%	Intraoperative variables	1	2%	2%	-9%
Antiarrhythmic agents	1	2%	Calcified aorta	2	4%	4%	Active MI	1	2%	2%	-4%
Other ECG abnormalities	1	2%	Diffuse/severe disease	2	4%	-11%	Diffuse/severe disease	1	2%	-13%	-2%
Race or ethnicity	0	0%	Atrial arrhythmia	2	4%	1%	Center's case frequency	1	2%	2%	2%
Date or order of surgery	0	0%	Preoperative thrombolysis	2	4%	4%	PT or INR	1	2%	2%	2%
On- vs. off-pump CABG	0	0%	Any family history variable	1	2%	-1%	Disaster, catastrophic state	1	2%	-6%	2%
Abdominal aortic aneurysm	0	0%	Anticoagulation or antiplatelet use	1	2%	-3%	Transfusion	1	2%	2%	2%
Hypercholesterolemia	0	0%	Anemia (hemoglobin, hematocrit)	1	2%	-5%	Cardiomegaly	0	0%	-15%	-6%
Critical state	0	0%	A published comorbidity index	1	2%	-1%	Aortic cross-clamp duration	0	0%	-7%	-7%
Heart rate	0	0%	Functional state	1	2%	2%	Endocarditis	0	0%	-5%	-9%
Steroid use	0	0%	ASA classification	1	2%	2%	Abdominal aortic aneurysm	0	0%	0%	-4%
Preoperative cardiac biomarkers	0	0%	Left ventricular hypertrophy	1	2%	2%	Any family history variable	0	0%	-2%	-2%
Functional state	0	0%	Acute mental status changes	1	2%	2%	Anemia (hemoglobin, hematocrit)	0	0%	-7%	-2%
Patient education level/literacy	0	0%	Ventricular wall motion	1	2%	-5%	Heart rate	0	0%	0%	-4%
ASA classification	0	0%	Type of MI	1	2%	-1%	Steroid use	0	0%	0%	-4%
Insurance type or status	0	0%	Killip classification	1	2%	2%	ACE inhibitor use	0	0%	-2%	0%
Recent admissions	0	0%	Blood pressure	1	2%	2%	Functional state	0	0%	0%	-2%
Left ventricular hypertrophy	0	0%	Other ECG abnormalities	1	2%	-1%	ASA classification	0	0%	0%	-2%
Time from admission to procedure	0	0%	Preoperative cardiac biomarkers	0	0%	0%	Left ventricular hypertrophy	0	0%	0%	-2%
Acute mental status changes	0	0%	ACE inhibitor use	0	0%	-2%	Acute mental status changes	0	0%	0%	-2%
Intraoperative variables	0	0%	Patient education level/literacy	0	0%	0%	Cachexia or malnutrition	0	0%	-5%	0%
Extracardiac arteriopathy	0	0%	Insurance type or status	0	0%	0%	Ventricular wall motion	0	0%	-7%	-2%
Calcified aorta	0	0%	Recent admissions	0	0%	0%	Calcified aorta	0	0%	0%	-4%
Dyspnea	0	0%	Time from admission to procedure	0	0%	0%	Type of MI	0	0%	-2%	-2%
Active MI	0	0%	Cachexia or malnutrition	0	0%	-5%	Pulmonary rates	0	0%	-10%	0%

Killip classification	0	0%	Pulmonary rates	0	0%	-10%	0	0%	Preoperative diuretic use	0	0%	-7%	-13%
Blood pressure	0	0%	Preoperative CPR/cardiac arrest	0	0%	-7%	0	0%	Killip classification	0	0%	0%	-2%
Location or type of surgical center	0	0%	Center's case frequency	0	0%	0%	0	0%	Blood pressure	0	0%	0%	-2%
Center's case frequency	0	0%	Stent thrombosis	0	0%	0%	0	0%	Preoperative CPR/cardiac arrest	0	0%	-7%	0%
Stent thrombosis	0	0%	Antiarrhythmic agents	0	0%	-2%	0	0%	Location or type of surgical center	0	0%	0%	-6%
Preoperative thrombolysis	0	0%	PT or INR	0	0%	0%	0	0%	Antiarrhythmic agents	0	0%	-2%	0%
PT or INR	0	0%	Disaster, catastrophic state	0	0%	-7%	0	0%	Other ECG abnormalities	0	0%	-2%	-2%
Transfusion	0	0%	Transfusion	0	0%	0%	0	0%	Preoperative thrombolysis	0	0%	0%	-4%
Refused blood products	0	0%	Other preoperative labs	0	0%	0%	0	0%	Refused blood products	0	0%	0%	-6%
Other preoperative labs	0	0%	Serum albumin	0	0%	-7%	0	0%	Serum albumin	0	0%	-7%	0%
Total variables (excl. combinations)	60		Total variables (excl. combinations)	75					Total variables (excl. combinations)	64			

Aa: change from < 1998; Ab: change from 1998-2007. Comb: combination variable; CHF: congestive heart failure; NYHA: New York Heart Association; MI: myocardial infarction; ECG: electrocardiogram; IABP: intra-aortic balloon pump; HTN: hypertension; BP: blood pressure; PCI: percutaneous coronary intervention; PTCA: percutaneous transluminal coronary angioplasty; CPR: cardiopulmonary resuscitation; ASA: American Society of Anesthesiology; ACE: angiotensin converting enzyme; INR: international normalized ratio; PT: prothrombin time

Table 3. Short-term risk model variables by publication year

Variable	≤ 1997			1998-2007			2008-2017		
	n	%	Variable	n	%	Δa	n	%	Δa
Age	36	92%	Age	36	80%	-12%	43	88%	-5%
Repeat operation	24	59%	Left ventricular function	32	71%	15%	31	63%	7%
Comb. heart failure variables	23	59%	Gender	30	67%	20%	29	59%	10%
Left ventricular function	22	56%	Renal failure	30	67%	25%	27	55%	9%
History of MI	22	54%	Urgency	29	64%	16%	27	55%	14%
Comb. any MI variable	22	54%	Comb. any MI variable	25	56%	2%	26	53%	24%
Urgency	20	49%	Repeat operation	24	53%	-5%	24	49%	10%
Comb. CHF or NYHA	20	51%	History of MI	24	53%	0%	23	47%	-12%
Gender	19	46%	Comb. heart failure variables	23	51%	-7%	21	43%	-8%
Renal failure	16	41%	Comb. arterial disease	22	49%	10%	20	41%	-13%
Lung disease	15	37%	Comb. critical state	20	44%	15%	18	37%	-2%
Peripheral arterial disease	15	39%	Comb. CHF or NYHA	19	42%	-9%	14	29%	7%
Comb. arterial disease	15	39%	Comb. vessel disease	19	42%	6%	13	27%	-27%
Comb. vessel disease	14	37%	Diabetes	18	40%	18%	13	27%	-27%
Neurologic disease	13	34%	Lung disease	18	40%	3%	12	24%	10%
Angina	13	32%	Neurologic disease	17	38%	4%	11	22%	-2%
Congestive heart failure	13	34%	Peripheral arterial disease	16	36%	-3%	11	22%	-12%
Comb. critical state	12	29%	Left main disease	16	36%	9%	11	22%	8%
Body size measurements	11	27%	Body size measurements	14	31%	4%	10	20%	-16%
Comb. ECG or arrhythmia variables	11	27%	Congestive heart failure	13	29%	-5%	10	20%	-20%
NYHA class	10	24%	Comb. HTN or BP	13	29%	14%	10	20%	-16%
Left main disease	10	27%	Hypertension	12	27%	12%	9	18%	-16%
Diabetes	8	22%	Cardiogenic shock	12	27%	12%	9	18%	1%

Number of diseased vessels	7	17%	Number of diseased vessels	11	24%	7%	Comb. PCI variables	7	14%	0%	-3%
Preoperative IABP use	7	17%	Pulmonary hypertension	10	22%	12%	Body size measurements	6	12%	-15%	-19%
Valve disease	6	15%	Comb. ECG or arrhythmia variables	10	22%	-5%	Valve disease	6	12%	-2%	6%
Hypertension	6	15%	Concurrent procedure	9	20%	5%	Non-CABG surgery	6	12%	-2%	-1%
Nitroglycerin use	6	15%	NYHA class	8	18%	-7%	Critical state	6	12%	12%	3%
Cardiomegaly	6	15%	Preoperative IABP use	8	18%	1%	Postoperative variables	6	12%	5%	-6%
Cardiogenic shock	6	15%	Postoperative variables	8	18%	10%	Extracardiac arteriopathy	6	12%	12%	-1%
Non-CABG surgery	6	15%	Comb. PCI variables	8	18%	3%	Inotropic medication	6	12%	5%	6%
Concurrent procedure	6	15%	Any arrhythmia	7	16%	3%	Atrial arrhythmia	6	12%	10%	8%
Diffuse/severe disease	6	15%	Prior/recent PCI or PTCA	6	13%	6%	Pulmonary hypertension	5	10%	0%	-12%
Comb. graft variables	6	15%	Non-CABG surgery	6	13%	-1%	Angina	5	10%	-22%	-1%
Comb. HTN or BP	6	15%	Extracardiac arteriopathy	6	13%	13%	On- vs. off-pump CABG	5	10%	10%	6%
Comb. PCI variables	6	15%	Preoperative diuretic use	6	13%	6%	Comb. ECG or arrhythmia variables	5	10%	-17%	-12%
Cardiopulmonary bypass time	5	12%	Angina	5	11%	-21%	Comb. graft variables	4	8%	-6%	-1%
Any arrhythmia	5	12%	Endocarditis	5	11%	6%	Any arrhythmia	4	8%	-4%	-7%
Pulmonary hypertension	4	10%	Intraoperative variables	5	11%	11%	Race or ethnicity	3	6%	6%	-3%
Pulmonary rates	4	10%	Race or ethnicity	4	9%	9%	Left main disease	3	6%	-21%	-29%
Number of grafts	3	7%	Liver disease	4	9%	2%	Cardiopulmonary bypass time	3	6%	-6%	-3%
Liver disease	3	7%	Cardiopulmonary bypass time	4	9%	-3%	Prior/recent PCI or PTCA	3	6%	-1%	-7%
Prior/recent PCI or PTCA	3	7%	Critical state	4	9%	9%	Type of graft(s)	3	6%	-1%	-1%
Anemia (hemoglobin, hematocrit)	3	7%	Preop intubation	4	9%	2%	Other preoperative labs	3	6%	6%	6%
Preop intubation	3	7%	Dyspnea	4	9%	9%	Hypertension	2	4%	-11%	-23%
Postoperative variables	3	7%	Comb. graft variables	4	9%	-6%	Smoking status	2	4%	2%	2%
Preoperative diuretic use	3	7%	Ventricular or unstable arrhythmia	4	9%	2%	Liver disease	2	4%	-3%	-5%
Type of graft(s)	3	7%	Valve disease	3	7%	-8%	Immunosuppression	2	4%	2%	-3%
Inotropic medication	3	7%	Nitroglycerin use	3	7%	-8%	A published comorbidity index	2	4%	2%	2%
Preoperative CPR/cardiac arrest	3	7%	Cardiomegaly	3	7%	-8%	Preoperative cardiac biomarkers	2	4%	4%	4%
PTCA failure/emergency	3	7%	Immunosuppression	3	7%	4%	Patient education level/literacy	2	4%	4%	4%
Ventricular or unstable arrhythmia	3	7%	Aortic cross-clamp duration	3	7%	-1%	Dyspnea	2	4%	4%	-5%
Disaster, catastrophic state	3	7%	Active MI	3	7%	7%	Comb. HTN or BP	2	4%	-11%	-25%
Serum albumin	3	7%	Type of graft(s)	3	7%	-1%	PTCA failure/emergency	2	4%	-3%	-3%
Aortic cross-clamp duration	2	7%	Inotropic medication	3	7%	-1%	Stent thrombosis	2	4%	4%	4%
Endocarditis	2	5%	Location or type of surgical center	3	7%	7%	Number of grafts	1	2%	-5%	-2%
Anticoagulation or antiplatelet use	2	5%	PTCA failure/emergency	3	7%	-1%	Nitroglycerin use	1	2%	-13%	-5%
Digoxin or digitalis use	2	5%	Refused blood products	3	7%	7%	Date or order of surgery	1	2%	2%	0%
Cachexia or malnutrition	2	5%	Number of grafts	2	4%	-3%	Hypercholesterolemia	1	2%	2%	-2%
Ventricular wall motion	2	7%	On- vs. off-pump CABG	2	4%	4%	Anticoagulation or antiplatelet use	1	2%	-3%	0%
Smoking status	1	2%	Abdominal aortic aneurysm	2	4%	4%	Digoxin or digitalis use	1	2%	-3%	0%
Immunosuppression	1	2%	Hypercholesterolemia	2	4%	4%	Preop intubation	1	2%	-5%	-7%
Any family history variable	1	2%	Steroid use	2	4%	4%	Other preoperative comorbidities	1	2%	0%	-2%
A published comorbidity index	1	2%	Other preoperative comorbidities	2	4%	2%	Insurance type or status	1	2%	2%	2%

	Other preoperative comorbidities	1	2%	Diffuse/severe disease	2	4%	-10%	Recent admissions	1	2%	2%	2%
Other preoperative comorbidities	ACE inhibitor use	1	2%	Atrial arrhythmia	2	4%	2%	Time from admission to procedure	1	2%	2%	2%
	Type of MI	1	2%	Smoking status	1	2%	0%	Center's case frequency	1	2%	2%	2%
	Atrial arrhythmia	1	2%	Date or order of surgery	1	2%	2%	Ventricular or unstable arrhythmia	1	2%	-5%	-7%
	Antiarrhythmic agents	1	2%	Any family history variable	1	2%	0%	PT or INR	1	2%	2%	2%
	Other ECG abnormalities	1	2%	Anticoagulation or antiplatelet use	1	2%	-3%	Disaster, catastrophic state	1	2%	-5%	2%
	Race or ethnicity	0	0%	Anemia (hemoglobin, hematocrit)	1	2%	-5%	Transfusion	1	2%	2%	2%
Preoperative cardiac biomarkers	Date or order of surgery	0	0%	Digoxin or digitalis use	1	2%	-3%	Cardiomegaly	0	0%	-15%	-7%
	On- vs. off-pump CABG	0	0%	A published comorbidity index	1	2%	0%	Aortic cross-clamp duration	0	0%	-7%	-7%
	Abdominal aortic aneurysm	0	0%	Heart rate	1	2%	2%	Endocarditis	0	0%	-5%	-11%
	Hypercholesterolemia	0	0%	ASA classification	1	2%	2%	Abdominal aortic aneurysm	0	0%	0%	-4%
	Critical state	0	0%	Acute mental status changes	1	2%	2%	Any family history variable	0	0%	-2%	-2%
	Heart rate	0	0%	Ventricular wall motion	1	2%	-5%	Anemia (hemoglobin, hematocrit)	0	0%	-7%	-2%
	Steroid use	0	0%	Calcified aorta	1	2%	2%	Heart rate	0	0%	0%	-2%
	Preoperative cardiac biomarkers	0	0%	Type of MI	1	2%	0%	Steroid use	0	0%	0%	-4%
Patient education level/literacy	Functional state	0	0%	Killip classification	1	2%	2%	ACE inhibitor use	0	0%	-2%	0%
	Patient education level/literacy	0	0%	Blood pressure	1	2%	2%	Functional state	0	0%	0%	0%
	ASA classification	0	0%	Other ECG abnormalities	1	2%	0%	ASA classification	0	0%	0%	-2%
	Insurance type or status	0	0%	Preoperative thrombolysis	1	2%	2%	Left ventricular hypertrophy	0	0%	0%	0%
	Recent admissions	0	0%	Preoperative cardiac biomarkers	0	0%	0%	Acute mental status changes	0	0%	0%	-2%
	Left ventricular hypertrophy	0	0%	ACE inhibitor use	0	0%	-2%	Intraoperative variables	0	0%	0%	-11%
	Time from admission to procedure	0	0%	Functional state	0	0%	0%	Cachexia or malnutrition	0	0%	-5%	0%
	Acute mental status changes	0	0%	Patient education level/literacy	0	0%	0%	Ventricular wall motion	0	0%	-7%	-2%
	Intraoperative variables	0	0%	Insurance type or status	0	0%	0%	Calcified aorta	0	0%	0%	-2%
Extracardiac arteriopathy	Extracardiac arteriopathy	0	0%	Recent admissions	0	0%	0%	Type of MI	0	0%	-2%	-2%
	Calcified aorta	0	0%	Left ventricular hypertrophy	0	0%	0%	Active MI	0	0%	0%	-7%
	Dyspnea	0	0%	Time from admission to procedure	0	0%	0%	Pulmonary rates	0	0%	-10%	0%
	Active MI	0	0%	Cachexia or malnutrition	0	0%	-5%	Preoperative diuretic use	0	0%	-7%	-13%
	Killip classification	0	0%	Pulmonary rates	0	0%	-10%	Killip classification	0	0%	0%	-2%
	Blood pressure	0	0%	Preoperative CPR/cardiac arrest	0	0%	-7%	Diffuse/severe disease	0	0%	-15%	-4%
	Location or type of surgical center	0	0%	Center's case frequency	0	0%	0%	Blood pressure	0	0%	0%	-2%
	Stent thrombosis	0	0%	Stent thrombosis	0	0%	0%	Preoperative CPR/cardiac arrest	0	0%	-7%	0%
	Preoperative thrombolysis	0	0%	Antiarrhythmic agents	0	0%	-2%	Location or type of surgical center	0	0%	0%	-7%
	PT or INR	0	0%	PT or INR	0	0%	0%	Antiarrhythmic agents	0	0%	-2%	0%
Refused blood products	Transfusion	0	0%	Disaster, catastrophic state	0	0%	-7%	Other ECG abnormalities	0	0%	-2%	-2%
	Refused blood products	0	0%	Transfusion	0	0%	0%	Preoperative thrombolysis	0	0%	0%	-2%
	Other preoperative labs	0	0%	Other preoperative labs	0	0%	0%	Refused blood products	0	0%	0%	-7%
	Total variables (excl. combinations)	60		Total variables (excl. combinations)	73		-7%	Serum albumin	0	0%	-7%	0%

Δa: change from < 1998; Δb: change from 1998–2007. Comb: combination variable; CHF: congestive heart failure; NYHA: New York Heart Association; MI: myocardial infarction; ECG: electrocardiogram; IABP: intra-aortic balloon pump; HTN: hypertension; BP: blood pressure; PCI: percutaneous coronary intervention; PTCA: percutaneous transluminal coronary angioplasty; CPR: cardiopulmonary resuscitation; ASA: American Society of Anesthesiology; ACE: angiotensin converting enzyme; INR: international normalized ratio; PT: prothrombin time

Table 4. Long-term risk model variables by publication year

Variable	≤ 1997			1998-2007			2008-2017		
	n	%	Variable	n	%	Variable	n	%	Δa
Age	1	50%	Age	8	89%	Age	5	42%	-8%
Diabetes	1	50%	Left ventricular function	8	89%	Left ventricular function	5	42%	-47%
Renal failure	1	50%	Comb. heart failure variables	7	78%	Gender	3	25%	14%
Left ventricular function	1	50%	Diabetes	6	67%	Diabetes	3	25%	-25%
Neurologic disease	1	50%	Renal failure	6	67%	Renal failure	3	25%	-42%
Peripheral arterial disease	1	50%	Congestive heart failure	6	67%	Peripheral arterial disease	3	25%	-42%
Congestive heart failure	1	50%	Comb. arterial disease	6	67%	Comb. arterial disease	3	25%	-31%
Left main disease	1	50%	Comb. CHF or NYHA	6	67%	Comb. any MI variable	3	25%	-42%
Aortic cross-clamp duration	1	50%	Peripheral arterial disease	5	56%	Comb. heart failure variables	3	25%	25%
Ventricular wall motion	1	50%	Body size measurements	4	44%	Comb. CHF or NYHA	3	25%	-53%
Comb. arterial disease	1	50%	Lung disease	4	44%	Urgency	2	17%	-42%
Comb. heart failure variables	1	50%	Neurologic disease	4	44%	Lung disease	2	17%	6%
Comb. CHF or NYHA	1	50%	Postoperative variables	4	44%	Neurologic disease	2	17%	-28%
Comb. vessel disease	1	50%	Comb. graft variables	4	44%	NYHA class	2	17%	-33%
Total variables (excl. combinations)	10		Smoking status	3	33%	History of MI	2	17%	17%
			Type of graft(s)	3	33%	Comb. vessel disease	2	17%	17%
			Left main disease	2	22%	Comb. graft variables	2	17%	-6%
			Hypercholesterolemia	2	22%	Atrial arrhythmia	2	17%	-28%
			Comb. vessel disease	2	22%	Body size measurements	1	8%	17%
			Gender	1	11%	Repeat operation	1	8%	-36%
			Urgency	1	11%	Angina	1	8%	-3%
			Repeat operation	1	11%	Congestive heart failure	1	8%	8%
			Number of grafts	1	11%	Number of diseased vessels	1	8%	-58%
			Valve disease	1	11%	Number of grafts	1	8%	8%
			Hypertension	1	11%	Valve disease	1	8%	-3%
			Date or order of surgery	1	11%	Hypertension	1	8%	-3%
			Aortic cross-clamp duration	1	11%	Race or ethnicity	1	8%	8%
			Digoxin or digitalis use	1	11%	Preoperative IABP use	1	8%	8%
			Heart rate	1	11%	Smoking status	1	8%	8%
			Functional state	1	11%	Left main disease	1	8%	-25%
			Left ventricular hypertrophy	1	11%	Cardiogenic shock	1	8%	-14%
			Intraoperative variables	1	11%	Immunosuppression	1	8%	8%
			Calcified aorta	1	11%	Date or order of surgery	1	8%	8%
			Preoperative diuretic use	1	11%	On- vs. off-pump CABG	1	8%	-3%
			Comb. HTN or BP	1	11%	Prior/recent PCI or PTCA	1	8%	8%
			Ventricular or unstable arrhythmia	1	11%	Intraoperative variables	1	8%	8%
			Comb. ECG or arrhythmia variables	1	11%	Postoperative variables	1	8%	-3%

Preoperative thrombolysis	1	11%	11%	Active MI	1	8%	8%
Total variables (excl. combinations)	31			Diffuse/severe disease	1	8%	8%
				Type of graft(s)	1	8%	-25%
				Comb. HTN or BP	1	8%	-3%
				Inotropic medication	1	8%	8%
				Comb. critical state	1	8%	8%
				Comb. PCI variables	1	8%	8%
				Total variables (excl. combinations)	35		

Δa: change from < 1998; Δb: change from 1998-2007. Comb: combination variable; CHF: congestive heart failure; NYHA: New York Heart Association; MI: myocardial infarction; ECG: electrocardiogram; IABP: intra-aortic balloon pump; HTN: hypertension; PCI: percutaneous coronary intervention; PTCA: percutaneous transluminal coronary angioplasty; CPR: cardiopulmonary resuscitation; ASA: American Society of Anesthesiology; ACE: angiotensin converting enzyme; INR: international normalized ratio; PT: prothrombin time

Table 5. Models containing only preoperative data

All models			Short-term models			Long-term models		
Variable	n = 89	%	Variable	n = 75	%	Variable	n = 14	%
Age	79	89%	Age	68	91%	Age	11	79%
Left ventricular function	62	70%	Left ventricular function	52	69%	Renal failure	11	79%
Renal failure	54	61%	Urgency	46	61%	Left ventricular function	10	71%
Comb. arterial disease	52	58%	Gender	45	60%	Diabetes	9	64%
Comb. heart failure variables	52	58%	Repeat operation	44	59%	Comb. arterial disease	9	64%
Gender	51	57%	Renal failure	43	57%	Comb. heart failure variables	9	64%
Urgency	50	56%	Comb. arterial disease	43	57%	Peripheral arterial disease	8	57%
Repeat operation	48	54%	Comb. heart failure variables	43	57%	Comb. CHF or NYHA	8	57%
Peripheral arterial disease	45	51%	History of MI	38	51%	Lung disease	7	50%
Comb. CHF or NYHA	44	49%	Comb. any MI variable	38	51%	Gender	6	43%
History of MI	42	47%	Peripheral arterial disease	37	49%	Neurologic disease	6	43%
Comb. any MI variable	42	47%	Comb. CHF or NYHA	36	48%	Comb. vessel disease	6	43%
Lung disease	41	46%	Comb. critical state	36	48%	Body size measurements	5	36%
Comb. critical state	40	45%	Lung disease	34	45%	Congestive heart failure	5	36%
Diabetes	37	42%	Comb. vessel disease	31	41%	Left main disease	5	36%
Comb. vessel disease	37	42%	Diabetes	28	37%	Urgency	4	29%
Neurologic disease	32	36%	Neurologic disease	26	35%	Repeat operation	4	29%
Left main disease	28	31%	Left main disease	23	31%	History of MI	4	29%
Congestive heart failure	27	30%	Cardiogenic shock	23	31%	Comb. any MI variable	4	29%
Cardiogenic shock	27	30%	Congestive heart failure	22	29%	Hypertension	4	29%
Body size measurements	26	29%	Body size measurements	21	28%	Comb. HTN or BP	4	29%
Number of diseased vessels	23	26%	Number of diseased vessels	20	27%	Race or ethnicity	4	29%
NYHA class	21	24%	NYHA class	18	24%	Comb. critical state	4	29%
Hypertension	19	21%	Comb. ECG or arrhythmia variables	17	23%	Smoking status	4	29%
Comb. HTN or BP	19	21%	Hypertension	15	20%	Cardiogenic shock	4	29%
Comb. ECG or arrhythmia variables	19	21%	Comb. HTN or BP	15	20%	NYHA class	3	21%

Angina	18	20%	Angina	15	20%	Angina	3	21%
Comb. PCI variables	18	20%	Comb. PCI variables	15	20%	Number of diseased vessels	3	21%
Valve disease	16	18%	Valve disease	13	17%	Valve disease	3	21%
Preoperative IABP use	15	17%	Preoperative IABP use	12	16%	Preoperative IABP use	3	21%
Prior/recent PCI or PTCA	14	16%	Prior/recent PCI or PTCA	11	15%	Inotropic medication	3	21%
Inotropic medication	13	15%	Any arrhythmia	11	15%	Immunosuppression	3	21%
Any arrhythmia	12	13%	Inotropic medication	10	13%	Date or order of surgery	3	21%
Pulmonary hypertension	10	11%	Pulmonary hypertension	10	13%	Prior/recent PCI or PTCA	3	21%
Race or ethnicity	10	11%	Nitroglycerin use	8	11%	Comb. PCI variables	3	21%
Preoperative diuretic use	8	9%	Preoperative diuretic use	7	9%	Atrial arrhythmia	3	21%
Nitroglycerin use	8	9%	Cardiomegaly	7	9%	Comb. ECG or arrhythmia variables	2	14%
Smoking status	8	9%	Race or ethnicity	6	8%	Extracardiac arteriopathy	1	7%
Atrial arrhythmia	8	9%	Extracardiac arteriopathy	6	8%	Preoperative diuretic use	1	7%
Extracardiac arteriopathy	7	8%	Liver disease	6	8%	Diffuse/severe disease	1	7%
Liver disease	7	8%	Atrial arrhythmia	5	7%	Liver disease	1	7%
Cardiomegaly	7	8%	Smoking status	4	5%	On- vs. off-pump CABG	1	7%
Immunosuppression	7	8%	Immunosuppression	4	5%	Any arrhythmia	1	7%
Diffuse/severe disease	5	6%	Diffuse/severe disease	4	5%	Ventricular or unstable arrhythmia	1	7%
Digoxin or digitalis use	5	6%	Digoxin or digitalis use	4	5%	Hypercholesterolemia	1	7%
Dyspnea	4	4%	Dyspnea	4	5%	Digoxin or digitalis use	1	7%
Pulmonary rates	4	4%	Pulmonary rates	4	5%	Functional state	1	7%
Date or order of surgery	4	4%	Critical state	4	5%	Recent admissions	1	7%
On- vs. off-pump CABG	4	4%	On- vs. off-pump CABG	3	4%	Cachexia or malnutrition	0	0%
Ventricular or unstable arrhythmia	4	4%	Ventricular or unstable arrhythmia	3	4%	Ventricular wall motion	0	0%
Critical state	4	4%	Ventricular wall motion	3	4%	Pulmonary hypertension	0	0%
Ventricular wall motion	3	3%	PTCA failure/emergency	3	4%	Calcified aorta	0	0%
PTCA failure/emergency	3	3%	Anticoagulation or antiplatelet use	3	4%	Dyspnea	0	0%
Hypercholesterolemia	3	3%	Anemia (hemoglobin, hematocrit)	3	4%	Type of MI	0	0%
Anticoagulation or antiplatelet use	3	3%	A published comorbidity index	3	4%	Active MI	0	0%
Anemia (hemoglobin, hematocrit)	3	3%	Other preoperative labs	3	4%	Pulmonary rates	0	0%
A published comorbidity index	3	3%	Hypercholesterolemia	2	3%	Killip classification	0	0%
Other preoperative labs	3	3%	Cachexia or malnutrition	2	3%	Number of grafts	0	0%
Cachexia or malnutrition	2	2%	Type of MI	2	3%	Type of graft(s)	0	0%
Type of MI	2	2%	Active MI	2	3%	Comb. graft variables	0	0%
Active MI	2	2%	Preoperative CPR/cardiac arrest	2	3%	Blood pressure	0	0%
Preoperative CPR/cardiac arrest	2	2%	Endocarditis	2	3%	Nitroglycerin use	0	0%
Endocarditis	2	2%	Stent thrombosis	2	3%	Cardiopulmonary bypass time	0	0%
Stent thrombosis	2	2%	Other ECG abnormalities	2	3%	Cardiomegaly	0	0%
Other ECG abnormalities	2	2%	Disaster, catastrophic state	2	3%	Preoperative CPR/cardiac arrest	0	0%
Disaster, catastrophic state	2	2%	Preop intubation	2	3%	Location or type of surgical center	0	0%
Preop intubation	2	2%	Steroid use	2	3%	Center's case frequency	0	0%

Steroid use	2	2%	Preoperative cardiac biomarkers	2	3%	Aortic cross-clamp duration	0	0%
Preoperative cardiac biomarkers	2	2%	Date or order of surgery	1	1%	Endocarditis	0	0%
Recent admissions	2	2%	Recent admissions	1	1%	Abdominal aortic aneurysm	0	0%
Calcified aorta	1	1%	Calcified aorta	1	1%	PTCA failure/emergency	0	0%
Killip classification	1	1%	Killip classification	1	1%	Stent thrombosis	0	0%
Location or type of surgical center	1	1%	Location or type of surgical center	1	1%	Any family history variable	0	0%
Any family history variable	1	1%	Any family history variable	1	1%	Antiarrhythmic agents	0	0%
Antiarrhythmic agents	1	1%	Antiarrhythmic agents	1	1%	Other ECG abnormalities	0	0%
Non-CABG surgery	1	1%	Non-CABG surgery	1	1%	Non-CABG surgery	0	0%
Preoperative thrombolysis	1	1%	Preoperative thrombolysis	1	1%	Anticoagulation or antiplatelet use	0	0%
PT or INR	1	1%	PT or INR	1	1%	Preoperative thrombolysis	0	0%
Transfusion	1	1%	Transfusion	1	1%	PT or INR	0	0%
Serum albumin	1	1%	Serum albumin	1	1%	Critical state	0	0%
ACE inhibitor use	1	1%	ACE inhibitor use	1	1%	Disaster, catastrophic state	0	0%
Functional state	1	1%	ASA classification	1	1%	Anemia (hemoglobin, hematocrit)	0	0%
ASA classification	1	1%	Insurance type or status	1	1%	Transfusion	0	0%
Insurance type or status	1	1%	Acute mental status changes	1	1%	Refused blood products	0	0%
Acute mental status changes	1	1%	Functional state	0	0%	Preop intubation	0	0%
Number of grafts	0	0%	Number of grafts	0	0%	Concurrent procedure	0	0%
Type of graft(s)	0	0%	Type of graft(s)	0	0%	A published comorbidity index	0	0%
Comb. graft variables	0	0%	Comb. graft variables	0	0%	Heart rate	0	0%
Blood pressure	0	0%	Blood pressure	0	0%	Steroid use	0	0%
Cardiopulmonary bypass time	0	0%	Cardiopulmonary bypass time	0	0%	Preoperative cardiac biomarkers	0	0%
Center's case frequency	0	0%	Center's case frequency	0	0%	Other preoperative labs	0	0%
Aortic cross-clamp duration	0	0%	Aortic cross-clamp duration	0	0%	Serum albumin	0	0%
Abdominal aortic aneurysm	0	0%	Abdominal aortic aneurysm	0	0%	Other preoperative comorbidities	0	0%
Refused blood products	0	0%	Refused blood products	0	0%	ACE inhibitor use	0	0%
Concurrent procedure	0	0%	Concurrent procedure	0	0%	Patient education level/literacy	0	0%
Heart rate	0	0%	Heart rate	0	0%	ASA classification	0	0%
Other preoperative comorbidities	0	0%	Other preoperative comorbidities	0	0%	Insurance type or status	0	0%
Patient education level/literacy	0	0%	Patient education level/literacy	0	0%	Left ventricular hypertrophy	0	0%
Left ventricular hypertrophy	0	0%	Left ventricular hypertrophy	0	0%	Time from admission to procedure	0	0%
Time from admission to procedure	0	0%	Time from admission to procedure	0	0%	Acute mental status changes	0	0%
Intraoperative variables	0	0%	Intraoperative variables	0	0%	Intraoperative variables	0	0%
Postoperative variables	0	0%	Postoperative variables	0	0%	Postoperative variables	0	0%
Total variables (excl. combinations)	76		Total variables (excl. combinations)	75		Total variables (excl. combinations)	39	

Comb: combination variable; CHF: congestive heart failure; NYHA: New York Heart Association; MI: myocardial infarction; ECG: electrocardiogram; IABP: intra-aortic balloon pump; HTN: hypertension; BP: blood pressure; PCI: percutaneous coronary intervention; PTCA: percutaneous transluminal coronary angioplasty; CPR: cardiopulmonary resuscitation; ASA: American Society of Anesthesiology; ACE: angiotensin converting enzyme; INR: international normalized ratio; PT: prothrombin time

Table 6. Models considering on- vs. off- pump CABG

All models			Short-term models			Long-term models		
Variable	n = 4	%	Variable	n = 3	%	Variable	n = 1	%
Age	4	100%	Age	3	100%	Age	1	100%
On- vs. off-pump CABG	4	100%	On- vs. off-pump CABG	3	100%	History of MI	1	100%
Gender	2	50%	Gender	2	67%	Comb. any MI variable	1	100%
Renal failure	2	50%	Renal failure	2	67%	On- vs. off-pump CABG	1	100%
Urgency	2	50%	Urgency	2	67%	Total variables (excl. combinations)	3	
History of MI	2	50%	Comb. critical state	2	67%			
Comb. any MI variable	2	50%	Body size measurements	1	33%			
Comb. critical state	2	50%	Diabetes	1	33%			
Body size measurements	1	25%	Left ventricular function	1	33%			
Diabetes	1	25%	Lung disease	1	33%			
Left ventricular function	1	25%	Pulmonary hypertension	1	33%			
Lung disease	1	25%	Repeat operation	1	33%			
Pulmonary hypertension	1	25%	Neurologic disease	1	33%			
Repeat operation	1	25%	Peripheral arterial disease	1	33%			
Neurologic disease	1	25%	Comb. arterial disease	1	33%			
Peripheral arterial disease	1	25%	NYHA class	1	33%			
Comb. arterial disease	1	25%	History of MI	1	33%			
NYHA class	1	25%	Active MI	1	33%			
Active MI	1	25%	Comb. any MI variable	1	33%			
Preoperative diuretic use	1	25%	Preoperative diuretic use	1	33%			
Comb. heart failure variables	1	25%	Comb. heart failure variables	1	33%			
Comb. CHF or NYHA	1	25%	Comb. CHF or NYHA	1	33%			
Number of diseased vessels	1	25%	Number of diseased vessels	1	33%			
Comb. vessel disease	1	25%	Comb. vessel disease	1	33%			
Hypertension	1	25%	Hypertension	1	33%			
Comb. HTN or BP	1	25%	Comb. HTN or BP	1	33%			
Race or ethnicity	1	25%	Race or ethnicity	1	33%			
Preoperative IABP use	1	25%	Preoperative IABP use	1	33%			
Inotropic medication	1	25%	Inotropic medication	1	33%			
Left main disease	1	25%	Left main disease	1	33%			
Cardiogenic shock	1	25%	Cardiogenic shock	1	33%			
Any arrhythmia	1	25%	Any arrhythmia	1	33%			
Comb. ECG or arrhythmia variables	1	25%	Comb. ECG or arrhythmia variables	1	33%			
Steroid use	1	25%	Steroid use	1	33%			
Total variables (excl. combinations)	26		Total variables (excl. combinations)	26				

Comb: combination variable; CHF: congestive heart failure; NYHA: New York Heart Association; MI: myocardial infarction; ECG: electrocardiogram; IABP: intra-aortic balloon pump; HTN: hypertension; BP: blood pressure; PCI: percutaneous coronary intervention; PTCA: percutaneous transluminal coronary angioplasty; CPR: cardiopulmonary resuscitation; ASA: American Society of Anesthesiology; ACE: angiotensin converting enzyme; INR: international normalized ratio; PT: prothrombin time

minor differences in the pre-CABG patients' risk factor frequency (which may have been associated with provider-based off-pump patient selection criteria), the pre-CABG patient risk factors identified were extremely similar to the overall findings, as reported above. Given the smaller number of on-pump *vs.* off-pump CABG mortality risk model comparisons reported, however, these findings may have limited generalizability.

When reviewing the frequency distribution of preoperative model risk variables, it is striking how very few modifiable (as opposed to non-modifiable) patient risk factors have been identified with a post-CABG mortality impact. As an inherently non-modifiable risk factor, the risk for post-CABG mortality increases as a patient's age increases. Perhaps by the time a patient is being evaluated for a CABG procedure, the negative prognostic impact for the most common preoperative risk factors, such as diabetes mellitus and poor left ventricular ejection fraction, may be difficult to reverse or otherwise counteract in the ST; however, these impacts can be seen in LT models.

In contrast, several of these reported patient risk factors have potential to be mitigated. As an example, body mass index or another marker of body habitus (e.g., height, weight, or body surface area) was included in 31/133 (23%) of ST models considering only preoperative risk factors. Similarly, a measure of smoking or tobacco use was considered in only 4/133 (3%). Although it is a well-known fact that these 2 risk factors represent important drivers for a patient developing ischemic heart disease, their significance in predicting post-CABG mortality risk appears likely confounded with presence of diabetes mellitus and poor renal function, which may also be sequela of obesity or diabetes.

Although these risk models may be helpful to enhance the providers' discussions with patients during the informed consent process or support provider discussions as to treatment-related risks for adverse events, the currently published CABG mortality risk models fall short of providing clinicians with useful information to optimize postoperative care consults, to ensure continuity of post-discharge care, or to enhance LT patients' survival. While it would likely not be surprising to most clinicians that these modifiable risk factors are important considerations, the manner presented in LT risks models may give the impression that LT post-CABG mortality risk is set in stone at the time of surgery, rather than an evolving risk that can be mitigated or exacerbated at any time. Using follow-up time-period-based risks (e.g., hemoglobin A1c management or continued tobacco use), therefore, future sequential modeling approaches may be needed to help better guide post-CABG follow-up care decisions and to optimize LT post-CABG survival.

One risk factor that is potentially modifiable, but not in the traditional sense, is operative urgency or priority, meaning whether a given procedure was performed in the elective *vs.* urgent or even emergent manner with an unstable patient. As clinically relevant examples, it is important to know when to intervene in patients with active angina or acute myocardial infarction. While operating in a time sensitive manner under potentially suboptimal conditions may be unavoidable, the fact that priority or status variables have been identified so frequently as ST mortality risk factors would suggest that future research funding should be prioritized to evaluate the impact of differential pre-CABG waiting periods^[16].

A limited number of CABG mortality models found preoperative medications such as nitrates, anti-platelet agents, angiotensin converting enzyme inhibitor, or anti-arrhythmic medication were associated with mortality. Given risk assessment inconsistencies, some of these medications (e.g., nitrates) may have been markers for the severity of coronary disease or preoperative instability. Other medications may, in fact, be markers of optimal medical management during the pre- and postoperative periods^[17].

Currently, no risk models incorporate direct measures of adherence with published clinical practice guidelines (e.g., the American College of Cardiology's guidelines for treatment of coronary artery disease) such as documenting the use of ischemic heart disease medications (e.g., pre-CABG statin use). As a potentially novel and important future enhancement to preoperative risk stratification, adherence to published guidelines should be considered. In general, adherence with published guidelines are increasingly becoming a marker used to identify high-quality, high-value care providers. Adherence to published guidelines has been shown to be suboptimal after CABG, yet adherence has been repeatedly associated with improved cardiovascular-related mortality in various populations^[18-20]. Applied proactively, guideline adherence may provide a useful direction for future cardiac surgery mortality risk modeling endeavors.

Interestingly, none of these CABG mortality risk models identified mental health-related (e.g., psychiatric) or socioeconomic risk factors as predictive; however, preoperative depression has been associated with increased 5- and 10-year post-CABG mortality^[21,22]. Similarly, one recent study showed a community-based marker of socioeconomic status (e.g., the Distressed Community Index) to be predictive of in-hospital mortality^[23]. Hence, these types of non-traditional CABG risk factors may be worthy of future exploration.

Limitations

Conducted as an advanced PubMed literature review in February 2019, this summary has identified knowledge "gaps", which are intended to foster future CABG risk modeling research. With collaborative team member oversight and guidance, the majority of these data extractions were performed by a single author (BC). Substantial overlap was documented among several risk variables (e.g., left ventricular ejection fraction vs. congestive heart failure vs. pulmonary rales vs. diuretic use); therefore, the relative impact of any individual risk factor could not be easily quantified. If standardized CABG quality improvement database definitions (e.g., the Society of Thoracic Surgeons' definitions) were uniformly utilized in the future, however, comparing variable-specific relative rankings (e.g., identifying the "top five variables impacting mortality" across all published models) would become possible.

Inherently, all risk variables reported were limited to the sub-group of patients' risk characteristics uniquely captured by each database. Although a common core of risk variables was captured, each dataset may have contained unique risk factors relevant specifically to their patient populations. Additionally, different risk modeling approaches (e.g., descending stepwise logistic regression) may have contributed to the variations documented for the risk factors associated with post-CABG mortality.

In conclusion, CABG maintains an important role in the management of coronary artery disease; thus, understanding patients' ST and LT surgical risk and risk factors remains important to optimizing CABG patient's selection, treatment, and follow-up care. A wide array of CABG mortality model findings and an equally vast diversity of analytic approaches were used, each prediction model having population-specific benefits and drawbacks. Over the past 20 years, it appears that the majority of CABG registries have come to a general consensus to utilize at least a core pre-CABG risk factor set. Beyond this core dataset, however, population-relevant risk factors are commonly reported.

As always, research continues to identify new risk factors that may affect post-CABG patients' risk; based on these data-driven findings, areas warranting further research were identified - such as incorporating modifiable risk factors and ischemic heart disease guideline compliance. Additionally, a new focus appears warranted to evaluate pre-CABG wait time impacts upon surgical priority, as well as CABG risk-adjusted outcomes. Applying the lessons learned, post-CABG mortality risk model findings may be quite different in the future from current findings - as the post-CABG care continues to improve and the field of statistical risk modeling advances forward.

DECLARATIONS

Authors' contributions

Wrote the initial study protocol, under the oversight and leadership of Grover FL: Carr BM, Shroyer ALW
 Prepared the research-related materials to obtain an official determination of “not research” by the Northport VA Medical Center’s Research and Development office: Shroyer ALW
 Performed the detailed data after implementing the advanced literature search strategy, acquisition with active involvement by Grover FL and Shroyer ALW: Carr BM
 Ran the initial data analyses and prepared the initial set of tables and figures: Carr BM
 Aided in the interpretation as well as the full co-author team worked collaboratively to assure a comprehensive search strategy: Grover FL, Shroyer ALW
 The first draft of this article was written jointly by Carr BM and Shroyer ALW, with revisions provided by Grover FL, all co-authors provided their final approval.

Availability of data and materials

This study’s data file, including data extracted for each reference listed, is available as an online-only supplement (Appendix A).

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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 Northport VA Medical Center’s Research and Development Office determined that this study was “not research”; this “not research” determination was dated September 12, 2019.

Consent for publication

Not applicable.

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Original Article

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Risk evaluation of abdominal aortic aneurysms based on both sex and morphology

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Abstract

Aim: To predict the peak wall stress in abdominal aortic aneurysms (AAAs) considering both morphological factors (maximum diameter, asymmetry index, and wall thickness) and sex differences, in order to assess the risk of AAA rupture more accurately.

Methods: Basic models of AAA focusing on different sexes with a range of morphological parameters were constructed. Using the Design-expert software for three-factor response surface methodology, 20 experimental models were built as well with the SolidWorks software. Fluid-structure interaction analysis was used to obtain stress distribution along the AAA wall. Polynomial regression equations were fitted to peak stresses in all experimental models.

Results: Based on fluid-structure interaction simulation data in the nonlinear polynomial regression model, separate equations for peak wall stress in AAA with regard to males and females were obtained. Morphological factors and sex differences have significant influence on peak wall stress. In some models, even when the maximum AAA diameter was relatively small, the peak wall stress became high. For the same maximal transverse measurement, when the AAA wall was thin and the asymmetry index large, or the former was thick and the latter small, the peak



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wall stress observed in males was higher than that in females.

Conclusion: To evaluate the risk of rupture of AAA more precisely and specifically, the present study proposes a new prediction method (based on equations) that includes more indicators such as sex and morphology, based on numerical biomechanical simulations, which were confirmed as such. This study provides a sex-specific clinical reference to assess the aforementioned risk of AAA rupture.

Keywords: Abdominal aortic aneurysm, sex difference, morphology, peak stress, rupture risk assessment

INTRODUCTION

Abdominal aortic aneurysms (AAAs) are a high-risk vascular disease characterised by local expansion of the abdominal aorta to more than 50% of its original diameter. If AAAs are not treated in time, it is likely to expand and rupture eventually^[1]. Its prevalence in individuals older than 60 years is at least four times higher in men than women^[2]. The mortality rate associated with a ruptured AAA is as high as 60%-80%^[3] and about 15,000 such deaths occur in the United States every year^[4]. Clinically, the treatment for AAA includes open repair and endovascular aneurysm repair.

Previously, indications for surgical treatment of AAA were based on the largest vessel diameter (greater than 5.5 or 5 cm)^[5]. However, relying mainly on maximal transverse measurement as a criterion to determine whether an AAA should be treated may delay the optimal timing for intervention in some patients or even lead to serious consequences such as rupture. In recent years, other biomechanical risk factors for AAA rupture have been used to more accurately predict its development, such as morphological factors (maximum diameter, asymmetry index, wall thickness, *etc.*)^[6,7]. Vorp *et al.*^[8] established a three-dimensional model of an AAA and found that an aneurysm which was asymmetric had a great influence on the distribution of wall stress, and peak wall stress increased nonlinearly with greater asymmetry. From a biomechanical point of view, a ruptured AAA occurs when local stress of the vascular wall exceeds the mechanical strength of its material. Thus, the higher local stress is along the AAA's wall, the higher the risk of a ruptured AAA^[9]. Besides peak wall stress, blood flow rheology has also been considered. For example, the wall shear stress induced by pulsatile flow, due to friction between blood flow and the inner wall of an AAA, was found to influence the rupture risk and function of AAA by damaging the endothelium and inducing AAA wall remodelling^[10-13].

Recent studies have found that the risk of AAA rupture has significant sex differences. The UK Small Aneurysm Trial, after a 10-year (1991-2001) follow-up of 496 patients, found that 5% of 411 males died of a ruptured AAA, while 14% of 85 females did, which suggests that the risk of rupture in affected women was three to four times higher^[14]. The medical literature has also reported a myriad of publications about the importance of sex differences^[15-18] with poorer outcomes in women. This observation has also been confirmed in a large number of observational studies and randomized controlled ones in recent years^[19,20]. As noted by Ash *et al.*^[21], there has been significant debate among vascular specialists regarding AAA between men and women. A much higher incidence is seen in men, with a male to female ratio of 4:1^[22]. Women though, are older at presentation, exhibit faster rates of AAA growth and thus have a higher risk of rupture at lower diameters^[23]. As the debate surrounding the definition, diagnosis and treatment of AAA in women continues, more specific guidelines are needed^[24].

The mechanism underlying sex-dependent differences in AAA rupture is still unclear however. The reason may be due to women having an aorta diameter that is generally smaller than in men. Therefore, for similarly sized AAAs, the degree of expansion in females is greater, and consequently, the risk of rupture increases accordingly, which suggests that the threshold for treatment of the maximum diameter in female

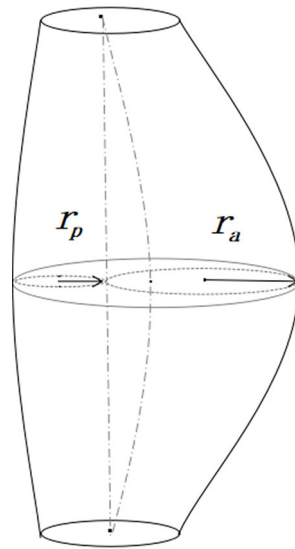


Figure 1. Three-dimensional model of abdominal aortic aneurysm. r_p : maximum posterior radius; r_a : maximum anterior radius

AAA patients should be reduced^[25,26].

In the present study, a series of quantitative simulations were conducted to investigate the relationships between the risk of a AAA rupture and patient sex and AAA morphology parameters. Based on these simulation results, equations for the peak wall stress for male and female patients were derived. A three-dimensional grid cube representing the risk comparison between both sexes has also been built considering wall thickness, asymmetry and maximum diameters of an AAA.

METHODS

Design of experiments

Based on the central composite design in response surface methodology, the maximum diameter, asymmetry index and wall thickness of AAA were selected as independent variables, and the peak wall stress measurement was used as the response value^[27-29]. Firstly, the morphology parameters for AAA in males and females over 70 years of age were determined according to clinical studies found in the literature^[30]. Measurements of the diameter in the upper and lower parts of the AAA were 2.8 and 2.3 cm for males, and 2.7 and 2 cm respectively for females. The length of the abdominal aorta was set at 12 cm for both sexes. The maximum diameters in AAA ranged from 3 to 6 cm for males and 2.7 to 5 cm for females. As shown in Figure 1, the asymmetry index β in AAA is defined as the ratio of the maximum posterior radius r_p to the maximum anterior radius r_a ($\beta = r_p/r_a$), which is identified to range from 0.3 to 1. Previous studies have revealed that the average wall thickness in AAA is generally 1.5 mm^[31]. Therefore, its variation range in the test design was set to 0.8 mm to 2.2 mm.

To define experimental groups, we selected a two-factor central composite design for this design as an example. Figure 2 represents the test points during a setup for a two-factor universal spiral combination design. For a full factorial one with $p = 3$ variables, there were total $m_c = 2^p = 8$ points in this work, including two test points of $\pm r$ on each axis, so their $m_r = 2^p = 6$. As for m_o , it was defined as how often the centre point test in which each factor takes a zero level^[32]. Therefore, the overall number of trials was $m = m_c + m_r + m_o$.

Finally, for the experiment design in this study, the coding factors of the models for males and females and

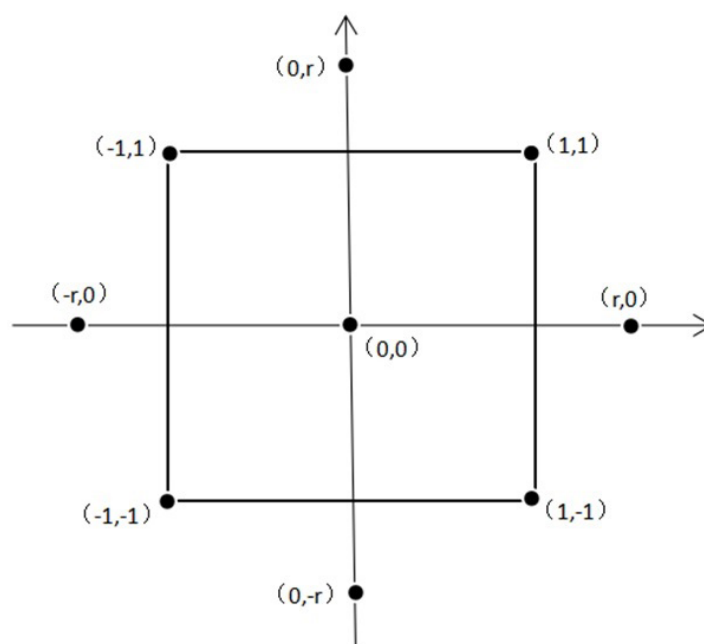


Figure 2. Distribution of test points for a two-factor general rotating combination design

Table 1. Coding factors for abdominal aortic aneurysm models designed for males and females and corresponding values

Factors		Coded levels				
		-r (-1.682)	-1	0	+1	+r (1.682)
Diameter (mm)	Male	30	36.1	45	53.9	60
	Female	27	31.7	38.5	45.3	50
Asymmetry index		0.3	0.44	0.65	0.86	1
Wall thickness (mm)		0.8	1.08	1.5	1.92	2.2

Table 2. Design matrix as well as simulated and predicted peak wall stresses for the abdominal aortic aneurysm models designed for males

Test model (male)	Maximum diameter D_{max} (mm)	Asymmetry index β	Wall thickness t (mm)	Simulated peak wall stress (KPa)	Predicted peak wall stress (KPa)
1	1 (53.9)	1 (0.86)	1 (1.92)	273.85	276.29
2	1 (53.9)	1 (0.86)	-1 (1.08)	531.13	533.65
3	1 (53.9)	-1 (0.44)	1 (1.92)	415.19	415.67
4	1 (53.9)	-1 (0.44)	-1 (1.08)	822.74	823.11
5	-1 (36.1)	1 (0.86)	1 (1.92)	167.33	178.41
6	-1 (36.1)	1 (0.86)	-1 (1.08)	326.61	337.61
7	-1 (36.1)	-1 (0.44)	1 (1.92)	267.29	276.23
8	-1 (36.1)	-1 (0.44)	-1 (1.08)	576.48	585.51
9	r (60)	0 (0.65)	0 (1.5)	487.86	489.81
10	-r (30)	0 (0.65)	0 (1.5)	225.48	207.67
11	0 (45)	r (1)	0 (1.5)	257.02	245.41
12	0 (45)	-r (0.3)	0 (1.5)	574.79	571.11
13	0 (45)	0 (0.65)	r (2.2)	228.5	220.15
14	0 (45)	0 (0.65)	-r (0.8)	820.63	817.73
15	0 (45)	0 (0.65)	0 (1.5)	374.61	375.67
16	0 (45)	0 (0.65)	0 (1.5)	374.73	375.67
17	0 (45)	0 (0.65)	0 (1.5)	371.05	375.67
18	0 (45)	0 (0.65)	0 (1.5)	372.17	375.67
19	0 (45)	0 (0.65)	0 (1.5)	378.92	375.67
20	0 (45)	0 (0.65)	0 (1.5)	379.23	375.67

Table 3. Design matrix as well as simulated and predicted peak wall stresses for the abdominal aortic aneurysm models designed for females

Test model (female)	Maximum diameter D _{max} (mm)	Asymmetry index β	Wall thickness t (mm)	Simulated peak wall stress (KPa)	Predicted peak wall stress (KPa)
1	1 (45.3)	1 (0.86)	1 (1.92)	222.16	226.76
2	1 (45.3)	1 (0.86)	-1 (1.08)	455.46	460.90
3	1 (45.3)	-1 (0.44)	1 (1.92)	352.66	355.21
4	1 (45.3)	-1 (0.44)	-1 (1.08)	719.4	721.11
5	-1 (31.7)	1 (0.86)	1 (1.92)	146.29	151.73
6	-1 (31.7)	1 (0.86)	-1 (1.08)	286.3	290.90
7	-1 (31.7)	-1 (0.44)	1 (1.92)	234.01	235.72
8	-1 (31.7)	-1 (0.44)	-1 (1.08)	504.1	506.65
9	r (50)	0 (0.65)	0 (1.5)	416.54	411.55
10	-r (27)	0 (0.65)	0 (1.5)	203.25	198.25
11	0 (38.5)	r (1)	0 (1.5)	218.87	210.26
12	0 (38.5)	-r (0.3)	0 (1.5)	498.78	497.10
13	0 (38.5)	0 (0.65)	r (2.2)	191.94	186.79
14	0 (38.5)	0 (0.65)	-r (0.8)	689.14	684.00
15	0 (38.5)	0 (0.65)	0 (1.5)	315.72	316.63
16	0 (38.5)	0 (0.65)	0 (1.5)	315.86	316.63
17	0 (38.5)	0 (0.65)	0 (1.5)	312.13	316.63
18	0 (38.5)	0 (0.65)	0 (1.5)	314.27	316.63
19	0 (38.5)	0 (0.65)	0 (1.5)	318.85	316.63
20	0 (38.5)	0 (0.65)	0 (1.5)	319.94	316.63

their values are defined in Table 1. Accordingly, 20 model parameters were generated for men and women, respectively (columns 1-4 of Tables 2 and 3).

Geometric model

All geometric models in this study were built using a three-dimensional modelling software SolidWorks 2016 (Dassault Systèmes SolidWorks Corporation, Waltham, MA, USA) based on the parameters listed in Tables 2 and 3. In order to obtain relatively smooth blood flow, the diameter at the upper and lower parts of the model were extended by 1.5 times. The schematic diagrams of all AAA designs are shown in Figure 3.

Governing equations, boundary conditions and material properties

The fluid-solid coupling analysis was carried out with ANSYS Workbench 15.0 (Ansys, Inc., Lebanon, NH, USA). The pressure distribution obtained by the Fluid Flow calculation was transmitted to the vascular wall through a fluid-solid coupling system, and the solid structure was determined by using the Static Structural Analysis module to figure out how stress is distributed in the final vascular wall. The fluid-filled domain was stitched using tetrahedral meshes. In order to ensure convergence of the physical quantities calculated iteratively such as velocity and force on the fluid-solid coupling surface, the vascular wall was also stitched as above.

Numerical simulation calculations were based on the Navier-Stokes equation and the mass conservation continuity one, neglecting gravity force:

$$\rho (\vec{u} \cdot \nabla) \vec{u} + \nabla p - \mu \Delta \vec{u} = 0 \quad (1)$$

$$\nabla \cdot \vec{u} = 0 \quad (2)$$

where, u and p represent the velocity vector (m/s) and pressure (Pa) respectively, and ρ and μ are the density (kg/cm³) and viscosity (Pa·s) of the blood.

In order to facilitate convergence of the fluid-solid coupling calculation, a steady-flow analysis was used.

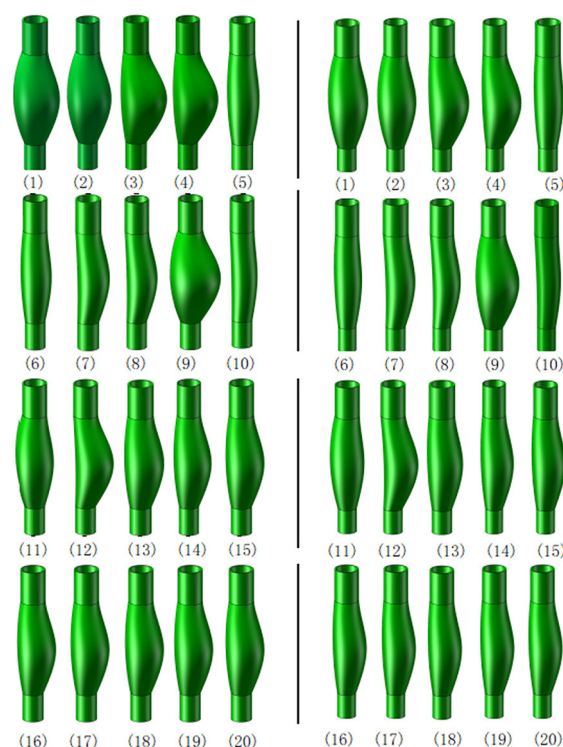


Figure 3. All geometric models (left: male; right: female, the number under each model represents the Test model number from Columns 1 of Tables 2 and 3)

Blood was assumed to be a uniform, continuous, isotropic, incompressible Newtonian fluid with a density value of 1050 kg/m^3 and a viscosity of $0.0035 \text{ Pa}\cdot\text{s}$. For boundary conditions, the inlet flow rate and the outlet pressure were fitted to the simulation with reference to the literature^[33]. The outlet pressure of models for males and females was equivalent to that of systolic blood at $16,414 \text{ Pa}$ ($\sim 123 \text{ mmHg}$), and the corresponding inlet flow velocity were 0.1614 m/s for men and 0.1797 m/s for women. The CFD software package which consisted of ANSYS Fluent 15.0 was used for simulation, and the SIMPLE algorithm was applied.

The wall of the AAA was assumed as a linear, isotropic elastic material. According to previous studies, the stiffness of the blood vessel wall in males is higher than that in females^[34,35]. Therefore, the Young's modulus obtained for women in this study was defined as 80% of men. Finally, the density was set to 2000 kg/m^3 , the Poisson's ratio 0.45, and the Young's modulus was 2.7 MPa for men and 2.16 MPa for women^[36]. Displacements along all directions at two ends were constrained.

After all the simulations, a multivariate regression analysis will be performed on the peak Von Mises stress values of the test models using Design-Expert software Version 10 to obtain cubic polynomial equations to predict the related stresses.

RESULTS

The simulated peak wall stress of each model has been shown in Table 2 and Table 3 (the penultimate column).

Two models with significant differences in the maximum diameter of AAA for the male group were selected, and the stress distribution contours are shown in Figure 4. Although the maximum diameter measured for model 2 was higher than that of model 8 (5.39 cm vs. 3.61 cm), the peak wall stress of model

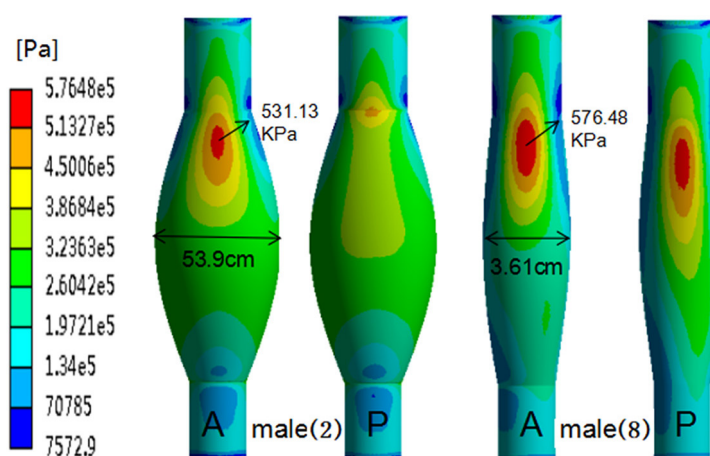


Figure 4. Stress distribution in models 2 and 8 of the male group. A: anterior; P: posterior

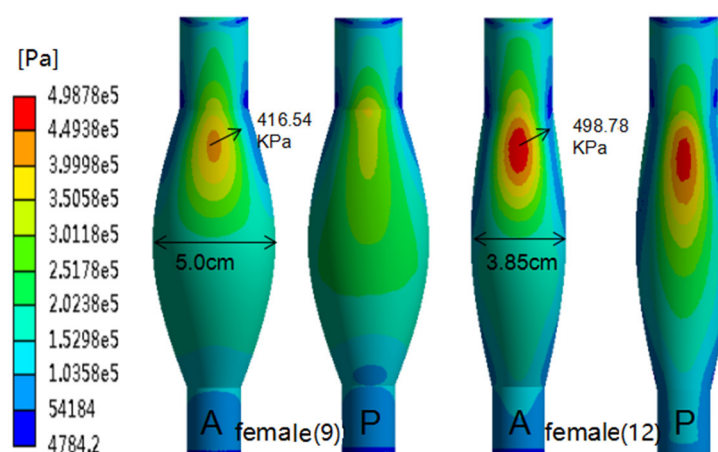


Figure 5. Stress distribution in models 9 and 12 of the female group. A: anterior; P: posterior

2 remained lower than that of model 8 (531.13 KPa vs. 576.48 KPa). Similar results were observed when model 2 was compared with models 9, 12, and 14, and when model 1 was compared with models 6, 8, 12, 14, and 15. In the female testing group, the stress distribution contours of models 9 and 12 are presented in Figure 5. The maximum diameter measured for the model 9 was higher than that of model 12 (5 cm vs. 3.85 cm), but the peak wall stress of model 9 turned out lower than that of model 12 (416.54 KPa vs. 498.78 KPa). This situation was also not unique in the female group, when model 9 was compared with models 2, 4, and 14. Comparison of results obtained for stress distribution showed that using only the maximum diameter to predict the risk of AAA rupture was not enough and might lead to non-optimal decisions.

Based on all simulation results, a multivariate regression analysis was performed on the peak Von Mises stress values of the test models in Tables 2 and 3 using Design-Expert software Version 10 to obtain cubic polynomial equations to predict the related stresses:

$$\begin{aligned} \text{Male_PWS} = & 375.67 + 83.87d - 96.82a - 177.64t - 10.39ad - 24.54dt \\ & + 37.52at - 9.52d^2 + 11.52a^2 + 50.64t^2 + 35.98d^2t \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Female_PWS} = & 316.63 + 63.06d - 86.05a - 149.16t - 11.11ad - 23.74dt \\ & + 32.94at - 4.1d^2 + 13.34a^2 + 42.76t^2 + 22.89d^2t + 9.31a^2d \end{aligned} \quad (4)$$

where a , d , t are coded dimensionless variables of the asymmetry index, the maximum diameter of the

Table 4. Analysis of variance for fit regression regarding male function

Source	Seq SS	DF	MS	F value	P value
Model	615,971.1	10	61,597.11	505.79	< 0.0001
A-Diameter	96,231.85	1	96,231.85	790.18	< 0.0001
B-Asymmetry	127,100	1	127,100	1043.33	< 0.0001
C-Wall thickness	175,300	1	175,300	1439.51	< 0.0001
AB	863.62	1	863.62	7.09	0.0259
AC	4819.66	1	4819.66	39.58	0.0001
BC	11,263.50	1	11,263.50	92.49	< 0.0001
A ²	1318.21	1	1318.21	10.82	0.0094
B ²	1859.77	1	1859.77	15.27	0.0036
C ²	35,937.61	1	35,937.61	295.09	< 0.0001
A ² C	4243.63	1	4243.63	34.85	0.0002
Residual error	1096.06	9	121.78		
Lack of fit	1039.04	4	259.76	22.78	0.0021
Pure error	57.01	5	11.40		
Total	617,100	19			

DF: degree of freedom; MS: mean square deviation

Table 5. Analysis of variance for fit regression regarding female function

Source	Seq SS	DF	MS	F value	P value
Model	458,400	11	41,675.00	994.59	< 0.0001
A-Diameter	22,746.31	1	22,746.31	542.85	< 0.0001
B-Asymmetry	100,400	1	100,400	2395.54	< 0.0001
C-Wall thickness	123,600	1	123,600	2949.85	< 0.0001
AB	988.35	1	988.35	23.59	0.0013
AC	4509.65	1	4509.65	107.62	< 0.0001
BC	8680.35	1	8680.35	207.16	< 0.0001
A ²	247.06	1	247.06	5.90	0.0413
B ²	2493.07	1	2493.07	59.50	< 0.0001
C ²	25,615.03	1	25,615.03	611.31	< 0.0001
A ² C	1718.25	1	1718.25	41.01	0.0002
AB ²	289.27	1	289.27	6.90	0.0303
Residual error	335.21	8	41.90		
Lack of fit	300.76	3	100.25	14.55	0.0066
Pure error	34.45	5	6.89		
Total	458,800	19			

DF: degree of freedom; MS: mean square deviation

AAA, and wall thickness, respectively.

To verify the two equations for predicting outcomes, the coefficients of determination were obtained: for $R_M^2 = 0.9982$ for *Male_PWS*, and $R_F^2 = 0.9993$ for *Female_PWS*, which indicates that the model is applicable. The analysis of variance [Tables 4 and 5] showed that the three selected factors (diameter, asymmetry and wall thickness) were significant for peak stress ($P < 0.05$). However, the lack of fit in this work was biased towards the other side of statistical significance ($P < 0.05$), because the computation was ideal when simulating a repeating model, instead of true replication of the simulation^[21]. Using the two predictive equations, the predicted peak stress in each model were obtained and presented in Tables 2 and 3 (the last column). Comparison of the simulated and predicted peak stresses in Tables 2 and 3 reveals good fit of the two regression models.

In Equations 3 and 4, the absolute value of the coefficient for first order from the regression equation reflects the contributions of each single factor to the response value. Then, we can sort out the three factors by their contribution to peak stress: AAA wall thickness, asymmetry index and maximum AAA diameter.

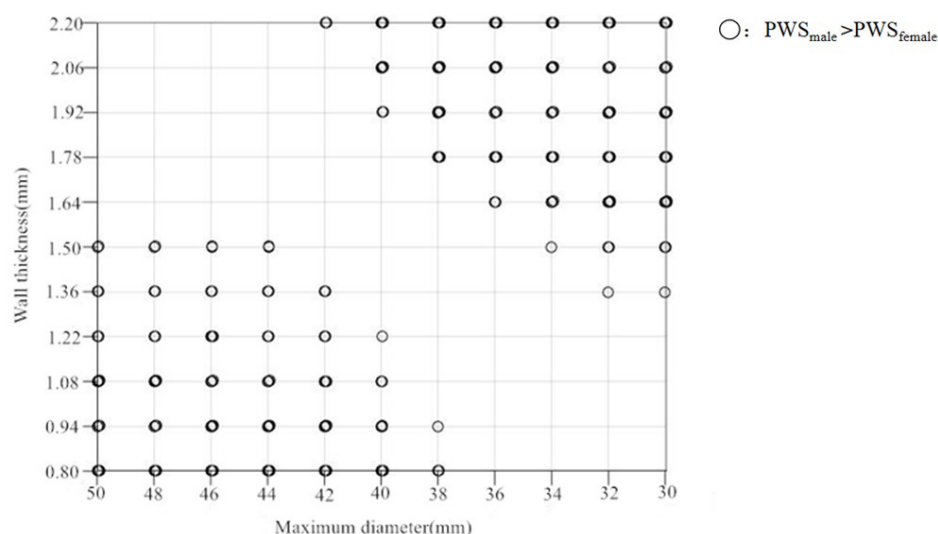


Figure 6. Scatter plot showing the correlation between peak stress and relative magnitude of the wall thickness in males and females with abdominal aortic aneurysm (into the plane of the wall thickness - maximum diameter)

This conclusion was consistent in both types of models for males and females. When the asymmetry index corresponded to 0.44 and the wall thickness to 2 mm, AAAs featuring maximum diameters of 5 cm for women and 5.5 cm for men had comparable risk of rupture. Similarly, according to Forbes *et al.*^[20], AAAs with maximal values of 5.2 cm for females and 5.5 cm for males presented a comparable risk. However, when the asymmetry index and wall thickness change, the peak stress of the AAA does the same, which means that the conclusions of Forbes *et al.*^[20] are not applicable in all cases^[37].

To more accurately reflect the relative magnitude of the risk of rupture in AAA, a range of values were selected for each variable in the models designed for males and females (a maximum diameter from 3 cm to 5 cm, an asymmetry index from 0.3 to 1, and a wall thickness from 0.8 mm to 2.2 mm) to determine their intersection. Eleven discrete points were uniformly taken within the variation range of each factor, and the dimensions were unified. Then, a scatter [Figure 6] and a 3D mesh [Figure 7] plots were obtained. As presented in Figure 6, the plot is projected onto the plane of the wall thickness-maximum diameter. It can be seen that the distribution trend of the points is obvious and there was no scatter in space. For AAAs featuring the same maximum diameter, $PWS_{male} > PWS_{female}$ occurred when the vascular wall was thin and the asymmetry index was large, or when the former was thick and the latter was small.

DISCUSSION

Traditionally, the maximum diameter of AAA has generally been used as a criteria for surgical treatment. Increasingly however, clinical studies have suggested a deficiency in this maximal transverse measurement, with greater consideration of morphology parameters in risk assessment of AAA over the last decades. In recent years, studies have revealed that male and female differences were also important and should be evaluated^[19,20,25]. In the present study, both sex differences and morphological factors were considered concurrently in predicting AAA rupture in high-risk patients. Nevertheless, it is of paramount importance to include biomechanical assessment of the structures involved in AAA for a more thorough evaluation. Collagen composition in the wall of AAAs in men and women are similar exception for its cross-linking^[38] but the risk of rupture is multifactorial from a biomechanical point of view^[39,40]. Sex affects AAA formation but the role of hormones is still poorly understood.

Based on a series of numerical FSI simulations that considered patient sex and morphological factors

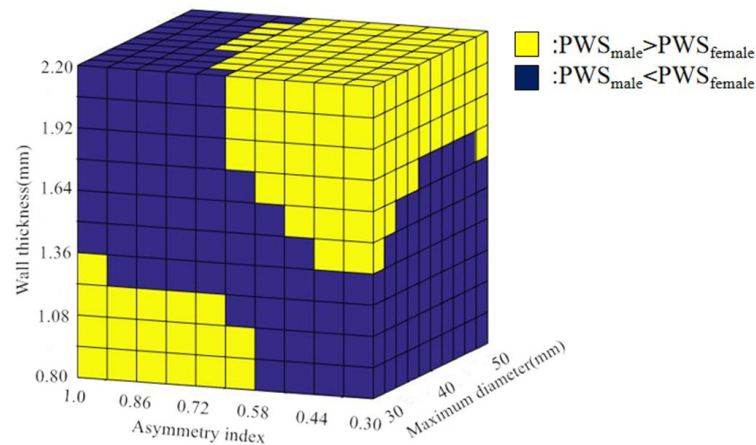


Figure 7. 3D grid cube presenting the relationship between peak wall stresses in males and females

(including maximum diameter, asymmetry index, and wall thickness), peak stress equations for males and females were developed to predict AAA rupture. According to these simulation results and the two equations developed above, some common and sex-specific features have been revealed.

For both sexes, the geometric features of the AAA have an obvious influence on peak wall stress. Among the three morphological factors, wall thickness is critical for developing a ruptured AAA in both men and women, which suggests that more attention should be paid to its distribution and change when predicting the risk of rupture. Regarding the specific equation for males or females, there were some models where even when the maximum diameter in AAA was relatively small, the corresponding peak wall stress conversely became large, which demonstrated the limitation of maximal transverse measurement as a criterion for AAA rupture risk assessment. In turn, this maximum diameter criterion may even result in delaying AAA repair.

The AAA peak wall stress also has sex differences. When the AAA wall is thin and the asymmetry index is large, or the vessel wall is thick and the asymmetry index is small, the peak wall stress observed in males would be higher than that in females with the same maximum diameter. When the asymmetry index was 0.44 and the corresponding wall thickness was 2 mm, AAAs with maximum diameters of 5 cm for women and 5.5 cm for men posed a comparable risk of rupture. This difference should alert surgeons to consider the influence of sex when assessing this risk of rupture of an AAA.

For ease of use in a clinical setting, equations to predict peak wall stress and a 3D grid have been developed in the present study (Equations 3 and 4, Figure 7). Both equations could assess the risk of AAA rupture in men and women separately. The 3D grid presented in Figure 7 revealed the relationship between peak wall stress in males and females with different maximum diameters, asymmetry index and wall thickness. Thus, the present study provides more biomechanical information with respect to the development and rupture of AAA to help with patient-specific assessment and treatment decision-making, i.e., open surgery or percutaneous deployment of a stent-graft.

As this is a preliminary study, there are several shortcomings. AAA models were simplified and idealised. Numerical calculations used unidirectional fluid-solid coupling. Material properties of the vascular wall were defined as linear, isotropic elastic, and its thickness was assumed to be uniform. These limitations would result in less accurate results than that using more realistic biomechanical properties, e.g., anisotropic, nonlinear, which entails residual stresses and implies statistically distributed heterogeneities^[41-43]. Despite these simplifications, the results still have qualitative significance. With the development of numerical simulation methodologies and further imaging technology, coupled with

consideration of blood flow rheology, predictive models of peak wall stress in AAA would be more accurate and complete.

In conclusion, to be more precise and specific in evaluating the risk of rupture in AAA, the present study developed a new prediction method (predictive equations) that included more patient sex and AAA morphology, based on quantitative biomechanical simulation. Results revealed that the risk of an AAA rupture is not only related to morphological factors, but sex differences as well. This study provides a sex-specific clinical reference tool to assess the aforementioned risk. Future studies should integrate more information from simulation and imaging technologies.

DECLARATIONS

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Authors' contributions

Conception and design: Sun AQ, Zhang YX

Data acquisition: Zhang YX

Analysis and interpretation of data: Sun AQ, Guidoin R, Zhang YX

Supervised the work: Sun AQ, Deng XY

Drafted the manuscript: Sun AQ, Zhang YX, Guidoin R, Xu ZP

Sustancially revised the manuscript: Sun AQ, Ren SQ, Zhang YX

Approved the manuscript: Sun AQ, Zhang YX, Xu ZP, Ren SQ, Deng XY, Guidoin R

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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Tumour vasculature targeted anti-cancer therapy

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Abstract

The tumour vasculature plays an important role in tumour growth and metastasis. Tumour angiogenesis provides more oxygen and nutrients to growing tumour cells, is not as tightly regulated as embryonic angiogenesis, and do not follow any hierarchically ordered pattern. The heterogeneity of the vasculature, high interstitial fluid pressure, poor extravasation due to sluggish blood flow, and larger distances between exchange vessels are potential barriers to the delivery of therapeutic agents to tumours. The prevention of angiogenesis, normalization of tumour vasculature, and enhancement of blood perfusion through the use of monoclonal antibodies against receptor proteins that are overexpressed on proangiogenic tumour cells, and improved, tumour-targeted delivery of therapeutic agents can all be achieved using nanocarriers of appropriate size. Nanomedicines such as polymeric nanoparticles, lipid nanoparticles, micelles, mesoporous silica particles, metal nanoparticles, noisomes, and liposomes have been developed for the delivery of anticancer drugs in combination with antiangiogenic agents. Amongst them, liposomal delivery systems are mostly approved by the FDA for clinical use. In this review, the molecular pathways of tumour angiogenesis, the physiology of tumour vasculature, barriers to tumour-targeted delivery of therapeutic agents, and the different strategies to overcome these barriers are discussed.

Keywords: Tumour, angiogenesis, antiangiogenic drug, targeted drug delivery, nanoparticle, normalization of tumour vasculature, sonoporation, hyperthermia



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ANGIOGENESIS

In general, there is an efficient vascular network that supplies blood to normal tissues. The hierarchical architecture and growth of blood vessels are maintained by the balance between pro-apoptotic and anti-apoptotic factors. This balance is controlled by the metabolic demands of the corresponding tissue. Lymphatic channels on the other hand, remove metabolic waste from the interstitium. Thus, the microstructure of the vascular network is capable of supplying adequate oxygen and nutrition to all associated cells^[1]. During tumour progression, there is rapid proliferation of tumour tissue. When the tumour reaches a critical size ($1\sim 2\text{ mm}^3$), tumour cells located further from the supplying blood vessel become starved of oxygen and nutrients, leading to the impairment of tumour growth by apoptosis or necrosis. In turn, this triggers angiogenesis, the formation of new blood vessels from existing ones^[2]. Although tumour angiogenesis provides for tumour growth and a route for metastasis, it is not as tightly regulated as embryonic angiogenesis^[2].

DIFFERENCES BETWEEN BLOOD VESSELS OF NORMAL AND CANCER TISSUES

The growth of tumour blood vessels does not follow any hierarchy. It is typically heterogeneous, tortuous, branches irregularly, and is enlarged circumferentially^[3-5]. The endothelial cells, pericytes (multifunctional mural cells that wrap around endothelial cells) and basement membrane of tumour blood vessels are all abnormal^[3]: endothelial cells have abnormally loose intracellular associations and focal intercellular openings that are $< 2\text{ }\mu\text{m}$ in diameter^[6] while their association with multiple layers of the vascular basement membrane is also loose due to high interstitial pressure, leading to hyper-permeable tumour blood vessels and vascular leakage^[7].

Tumour blood vessels also have a reduced surface area: volume ratio. The high interstitial pressure, coupled with a reduced surface area, impairs the delivery of oxygen, nutrients, and removal of metabolites. As such, the tumour microenvironment is typically characterized by hypoxia and acidosis which in turn, selects for apoptosis-resistant and metastasis competent tumour cells [Figure 1].

CELL SIGNALLING PATHWAYS IN HYPOXIA-INDUCED ANGIOGENESIS

Cell signaling pathways in hypoxia-induced angiogenesis is shown in Figure 2. HIF-1 α is the founding member of the hypoxia-induced factor (HIF) family^[8]. It regulates the genes associated with oxygen deprivation^[9]. The HIF activity pathway is regulated by prolyl hydroxylase enzymes (PHD1-3)^[10]. PHD acts as an oxygen sensor; in normoxia, PHD hydroxylates the proline residues of HIF-1 α . The hydroxylated HIF-1 α then binds to the von Hippel-Lindau E3 ubiquitin ligase complex leading to proteasomal degradation of HIF-1 α ^[11,12]. Under hypoxic conditions, oxygen and cofactor 2-oxo-glutarate substrates are depleted^[13] and PHD becomes inactivated, resulting in stabilization and intracellular accumulation of HIF-1 α . HIF-1 α is then translocated into the nucleus to bind with transcriptional factor Arnt (Aryl hydrocarbon nuclear translocator family protein)^[14]. Subsequently, a transcriptional complex is formed with p300/CBP which binds to HREs (hypoxia response elements) in the promoters and enhancers of target genes, leading to vasodilatation (for better delivery of oxygen), lowering of oxygen demand and upregulation of proangiogenic factors like fibroblast growth factor (FGF), insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF)^[15]. Vasodilatation is also caused by the upregulation of inducible nitric oxide synthase leading to increased production of nitric oxide and relaxation of vascular smooth muscle cells^[16]. Under hypoxic conditions, the demand for oxygen is lowered due to over expression of glucose transporter 1 enzyme (GLUT1). GLUT1 improves the uptake of glucose^[17] and induces glycolytic enzymes such as phosphoglycerate kinase^[18]. In turn, phosphoglycerate kinase is regulated by aldolase A and HIF- α . Aldolase A helps in better utilization of glycolysis, tumour epithelium mesenchymal cell proliferation^[19] and upregulation of pyruvate dehydrogenase kinase (PKD1) which inhibits mitochondrial respiration^[20]. HIF-1 α helps in cancer cell proliferation^[21] by regulating the expression of a number of proangiogenic genes like

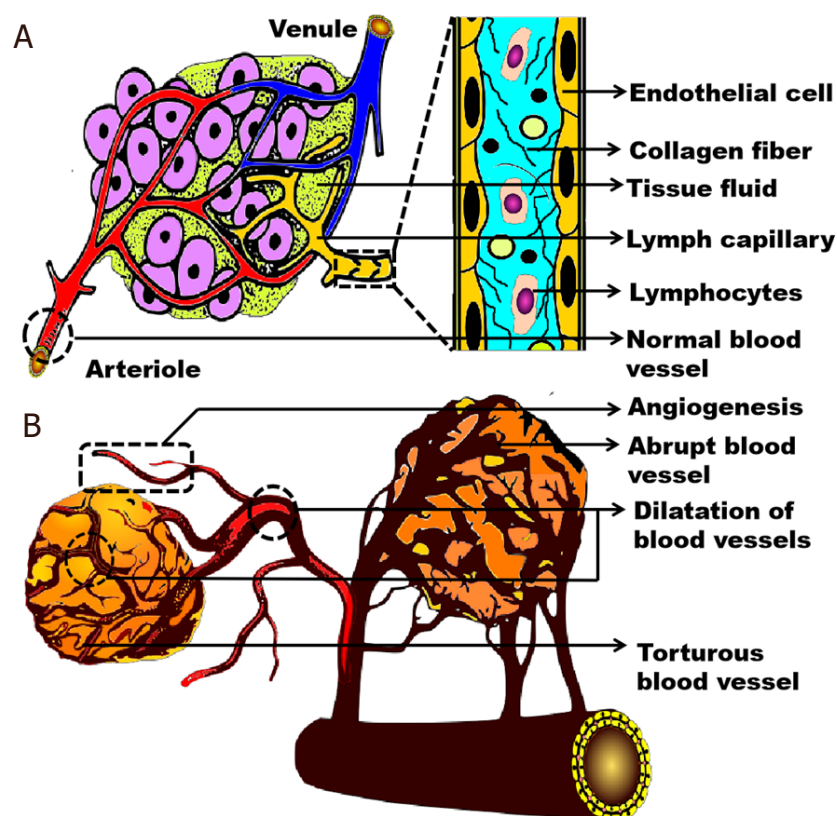


Figure 1. Schematic representation of the physiological differences between normal blood vessels (A) and the tumour vasculature (B)

VEGF, *Ang-1*, *Tie 2*, platelet-derived growth factor (*PDGF*), basic fibroblast growth factor (*bFGF*), monocyte chemoattractant protein-1 (*MCP-1*), *IGF* and epidermal growth factor (*EDGF*). These HIF regulated factors bind to corresponding receptors on the cell membranes of pericytes and increase vascular permeability, endothelial cell proliferation, sprouting, migration, adhesion, and tube formation. The angiogenic factors, their corresponding receptors, and functions are shown in Table 1. Vascular permeability is increased due to overexpression of *VEGF*^[22-25]. In endothelial cells and pericytes, *Ang-1* (angiopoietin-1) is induced by hypoxia. It is a *Tie-2* receptor agonist which recruits pericytes to mature vessels and promotes tumour angiogenesis^[22]. Despite active angiogenesis, the tumour microenvironments still have hypoxic domains that lead to sustained stabilization of *HIF-α*. *HIF-α* then promotes cap-dependent translation of selective mRNAs for angiogenesis through up-regulation of translational factor *eIF4E1*. In contrast, *4E-BP1* is a translation initiation repressor that sequesters *eIF4E1* and is thus a tumour suppressor protein. The activity of translational factor *eIF4E1* is also controlled by pathways such as *Ras* and *PI3K/AKT*. These pathways act by inhibiting *4E-BP1* and increasing the expression of *eIF4E1*.

The inducible enzyme cyclooxygenase-2 (*COX-2*) is also an important mediator of angiogenesis and tumor growth. It induces matrix metalloproteinases that have traditionally been associated with the degradation and turnover of most of the components of the extracellular matrix (ECM). Plasminogen activator inhibitor type 1 (*PAI-1*) though has the opposite effect of remodeling the ECM by regulating plasmin.

BARRIERS TO TARGETED DELIVERY OF THERAPEUTIC AGENTS TO TUMOUR

Spatial and temporal heterogeneities in blood supply

Vascular morphology and blood flow rate govern the movement of blood-borne particles through tumour vasculature. Depending on the tumour type, location and growth rate, the architecture of the tumour

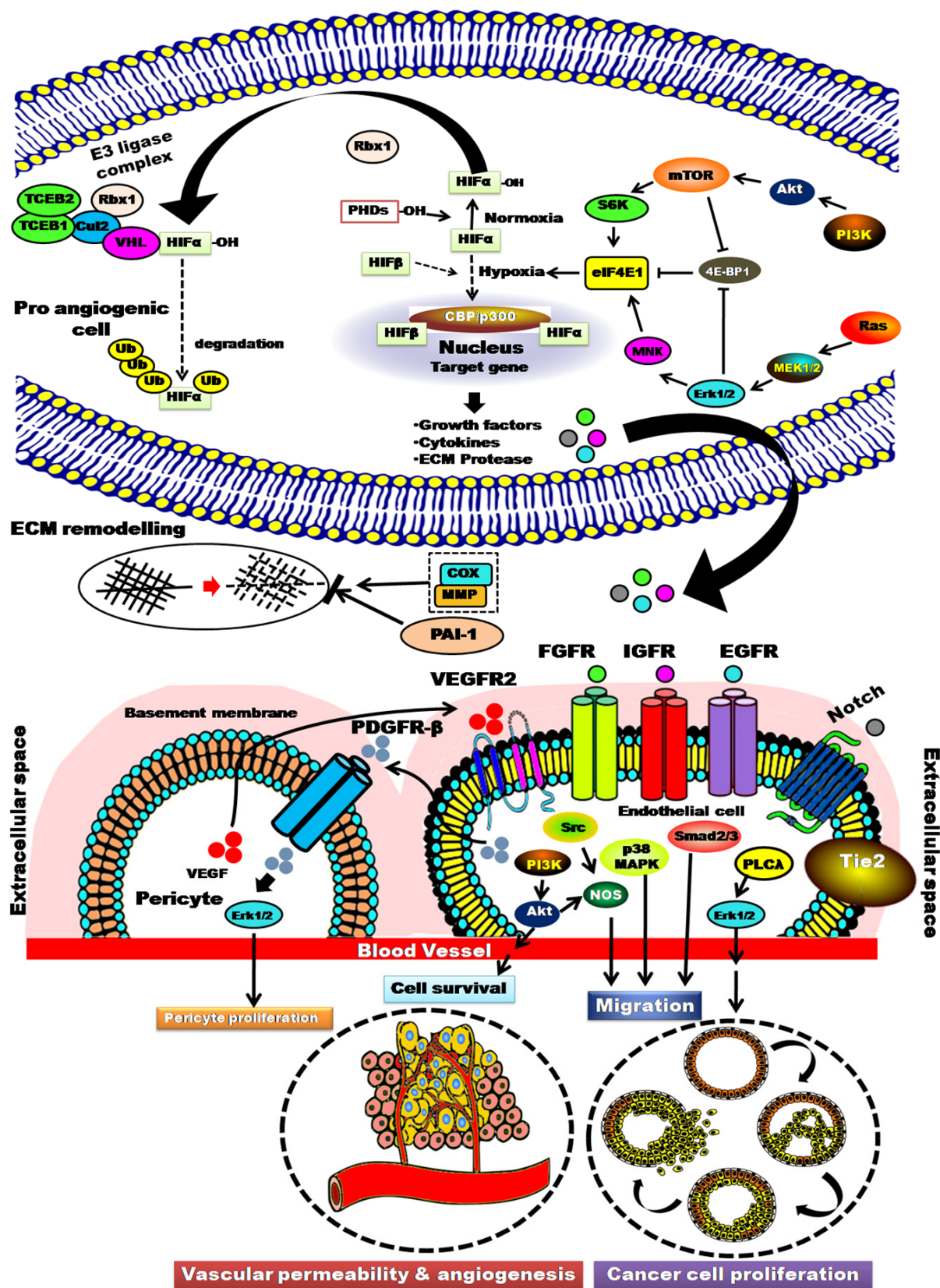


Figure 2. Cell signalling pathways of hypoxia-induced tumour angiogenesis. MNK: mitogen-activated protein kinase interacting protein kinases; EGFR: endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor type 2; PDGFR: platelet derived growth factor receptor; VEGF: vascular endothelial growth factor; ECM: extracellular matrix; MMP: matrix metalloproteinase; mTOR: mammalian target of rapamycin; TCEB: transcription elongation factor B; FGFR: fibroblast growth factor receptor; IGFR: insulin-like growth factor receptor

Table 1. List of angiogenic factors, corresponding receptors, and functions

Antigenic molecules	Receptors	Functions									
		Initiation of angiogenesis		Neovessel formation		Adaptation to tissue needs		Maturation			
		Enhancement of vascular permeability	Detachment of pericytes	Degradation of basement membrane	Endothelial cell proliferation and migration	Pericyte proliferation and migration	Regression of neovessels due to lack of flow or presence of growth factors	Attachment of pericytes	Deposition of basement membrane	Endothelial assembly and lumen acquisition	Vessel maintenance
VEGF	VEGFR1 (Flt1) VEGFR2 (Kdr)	✓	✓	✓	✓	✓					
Ang-2	Tie2		✓	✓			✓				
FGF	FGFR				✓	✓					
PDGFB	PDGFR				✓	✓		✓	✓		
PLGF	VEGFR1 (Flt1)				✓						
THBS 1	CD36, CD47, Integrins				✓						
Integrins	Extracellular matrix				✓					✓	
SDF1	CXCR4				✓						
DLL1-4	Notch				✓						
SCF	cKit				✓						
Interleukins	Interleukin receptors				✓						
Ang-1	Tie2							✓	✓	✓	✓

VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; PDGFB: platelet-derived growth factor subunit B; PLGF: placental growth factor; THBS 1: thrombospondin 1; SDF1: stromal cell-derived factor 1; DLL1-4: delta like1-4 (notch ligands); SCF: stem cell factor; Ang-1: angiopoietin-1; CXCR4: chemokine (C-X-C motif) receptor 4; VEGFR: vascular endothelial growth factor; PDGFR: platelet-derived growth factor receptor

vasculature may vary^[26]. Blood vessel distribution throughout the tumour mass is also not uniform and each region may have either peripheral or central vascularization. In other words, the central portion of some regions is well perfused whereas the periphery may have better perfusion elsewhere.

Microscopically, the tumour vasculature is highly heterogeneous. They are characterized by dilated, secular and tortuous blood vessels having tri-furcations, self-loops, and sprouts. The endothelial cell lining may even be absent. Blood flow is also chaotic and lacks a definite route between the arterial and venous systems. Therefore, in general, necrotic foci develop in a growing tumour. In turn, this decreases the average rate of perfusion.

Based on the rate of perfusion, there may be four regions in a tumour^[26]: (1) an avascular, necrotic region; (2) semi-necrotic region; (3) stabilized, microcirculation region; and (4) advancing front.

Regions I and II have a low blood flow rate whereas in regions III and IV, flow is more variable but still higher than that of surrounding normal host tissue. With tumour growth, the widths of regions I and II increase while that of III and IV remain unchanged, resulting in variation in vascular morphology at both

the macroscopic and microscopic levels. The resulting spatial and temporal heterogeneities in blood supply is thus responsible for non-uniform distribution of the therapeutic agent. Generally, the average uptake of a therapeutic agent decreases with an increase in tumour mass.

Poor extravasation and high interstitial fluid pressure limit transport across the microvascular wall

Diffusion and convection are the main mechanisms behind the transport of drug molecules across the vascular wall. The concentration gradient of the therapeutic agent across the plasma (C_p) and interstitial fluid (C_i) is the driving force for the diffusion process. This mass transfer process is proportional to the surface area; the proportionality constant is known as vascular permeability P (cm/s). Transfer of therapeutic agents by convection is associated with the leakage of plasma/fluid across the vascular wall due to differences in hydrostatic pressure of fluid in the blood vessel and interstitial space. The associated experimental constant is known as hydraulic conductivity, L_p (cm/mmHg-s). Similarly, the convection process is also proportional to the osmotic pressure difference between the blood vessel and the interstitial space^[27]. This proportionality constant is known as the osmotic reflection coefficient (σ). These three experimental constants (P , L_p , and σ) are used to describe the extent of transport of plasma content across tumour vessels. Tumour vessels have relatively high P and L_p values^[28,29] as they have wide endothelial junctions, a large number of fenestrae and trans-endothelial channels, discontinuous or absent basement membrane and significant spatial heterogeneities^[30,31]. Although these physiological characteristics increase vascular permeability, tumours also have poor extravasation, which is a significant barrier to the delivery of therapeutic agents. This can be explained as follows: tumour vessels have sluggish blood flow. The hydrostatic fluid pressure in the blood vessel (P_v) is less than that of fluid in the interstitial space (P_i). Of note, the P_i in animal/human tumours is even higher than that of normal tissue^[32]. Furthermore, it has been reported that P_i increases with the growth of a tumour. This is mainly due to high vascular permeability and poor, impaired lymphatic drainage^[32-35]. Both tumour hyperplasia around a blood vessel and increased production of extracellular matrix components contribute to high interstitial fluid pressure (IFP). In normal tissue, IFP is 0 mmHg but in tumour blood vessel, the IFP varies from 10-40 mmHg^[36]. The IFP is elevated throughout the mass of a tumour except at the periphery, where it becomes equal to normal physiological values. Therefore, intratumoral fluid may extravasate from the periphery of a tumour, resulting in non-delivery of a therapeutic agent. In different animal and human tumour models, it was found that 1%-14% of plasma entering the tumour leaked into the periphery^[28,37,38]. Again, the tumour interstitial space has a higher concentration of endogenous plasma protein, leading to higher interstitial osmotic pressure. Thus, the transfer of therapeutic agents by diffusion is further limited.

Resistance to transport through the interstitial space and distribution into the tumour microenvironment

Diffusion and convection are the main mechanisms behind the movement of therapeutic agents that have extravasated into the interstitial space^[39]. The concentration gradient is the driving force behind diffusion whereas fluid velocity determines the convection process. The interstitial diffusion coefficient (D) and hydraulic conductivity (K)^[32] are the experimental constants used for quantitative measurements of therapeutic agent distribution in the interstitial space. The interstitial space of a tumour is located at the TME (tumour microenvironment) and composed largely of a collagen and elastic fibre network, filled with a hydrophilic gel made up of interstitial fluid and macromolecular constituents^[40]. Its structural integrity is maintained by collagen and elastin whereas resistance to transport is provided by macromolecular constituents such as glycosaminoglycans and proteoglycans^[40,41]. Compared to normal tissues, tumours have a higher collagen content but lower concentrations of hyaluronate and proteoglycans^[32] due to increased activity of lytic enzymes such as hyaluronidase in the tumour interstitial space. Thus, the tumour interstitial space should provide lower resistance to the distribution of therapeutic agents, suggesting larger values of D and K . Paradoxically however, therapeutic agents are not distributed homogeneously in tumours. This

can be explained as follows: the time constant for a molecule with diffusion coefficient, D is proportional to the diffusion path length, raised to a power of two. Therefore, if the diffusion path length is doubled, the required time will be increased by four times. In solid tumours, the exchange vessels are at a large distance apart ($\sim 200 \mu\text{m}$)^[42,43]. Therapeutic agents will need a prolonged transit time for homogenous distribution. High interstitial pressures also slow down the distribution process. Thus, low molecular weight ($M_r < 1000 \text{ Da}$) anticancer drugs do not accumulate in the tumour because of their small size and hence, rapid clearance^[44]. The drug distribution process in a tumour may be further limited by the high affinity of the drug molecule for proteins present in interstitial fluid.

Growth induced solid stress

A tumour mass consists of proliferating cancer cells and stromal cells (i.e., fibroblasts, immune, and perivascular cells)^[45]. It is supplied by a dense ECM, and a tortuous and chaotic network of blood vessels^[45]. During tumour growth, there is rapid proliferation of cancer cells in a limited space resulting in the generation of mechanical forces from different structural components such as cancer cells, various host cells, and the ECM. Thus, there is also a growth induced solid stress, which commonly ranges from 10 to 142 mmHg^[46], that can deform the vascular and lymphatic structures and cause limited perfusion and hypoxia throughout tumour tissue. This creates a barrier to the penetration of therapeutic agents^[47] which restricts their flow to cells within the perivascular space, such that resistant cells in hypoxic regions are missed^[45]. Shear stress can also induce vascular endothelial growth factor receptor type 2 (VEGFR2) expression and ligand-independent phosphorylation. This causes activation of MAPK, PI3K, and Akt signalling pathways that are involved in promoting angiogenesis^[46]. Additionally, there is VEGFR2 membrane clustering and downstream signalling. Recently VEGFR3 has also been found to be a part of this mechanosensory complex. Depletion of VEGFR2 or VEGFR3 thus causes significant reduction in endothelial cell response to mechanical stress^[46].

Specific integrins can also contribute to tumour angiogenesis and tumour progression^[46]. In endothelial cells, VEGF upregulate the expression of $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins. The $\alpha 5\beta 1$, $\alpha \nu \beta 3$ and $\alpha \nu \beta 5$ integrins are also expressed in angiogenic vasculature to facilitate the growth and survival of newly forming vessels^[46].

Therefore, the general strategy to overcome the barriers to vascular and tumour tissue permeability is functionalization of the surface of nanoparticles with tissue and cell-penetrating peptides, such as the iRGD^[48]. It interacts with $\alpha \nu$ integrins on the endothelium and stimulates proteolytic cleavage. The released CendR peptide subsequently binds with neuropilin-1^[45] to ensure the homing of and penetration of tumour tissue by nanoparticles.

TARGETED DELIVERY OF THERAPEUTIC AGENTS BY EXPLOITING TUMOUR VASCULATURE

A therapeutic agent is delivered to the target tissue via supplying arterioles to that particular tissue. As discussed in the previous sections, there are a number of barriers that hinder the distribution process of therapeutic agents in the tumour. First, the tumour vasculature is highly heterogeneous in distribution. Unlike the tight endothelium of normal blood vessels, the vascular endothelium in tumour microvessels is discontinuous and leaky. Elevated levels of growth factors such as VEGF and bFGF cause vasodilatation and enhancement of vascular permeability. Therefore, the gap sizes between endothelial cells can range from 100 to 780 nm, depending on the anatomic location of the tumour^[49]. As such, low molecular weight anticancer drugs ($M_r < 1000 \text{ Da}$) can easily enter the tumour microenvironment but at the same time, they can also be easily removed because of their small size. Consequently, when delivered as an aqueous solution, small-molecule chemotherapeutic agents like paclitaxel^[50], gemcitabine^[51], cisplatin^[52], *etc.* do not accumulate in the tumour at the desired concentration for an adequate duration. These potent anticancer drugs undergo unwanted bio-distribution, leading to unfavourable pharmacokinetics characterized by a large volume of distribution, high renal clearance and short half-life^[53]. Furthermore, these cytotoxic agents

can cause severe dose-dependent side effects such as myelosuppression, neurotoxicity, mucositis, nausea, vomiting, and alopecia that may become fatal for patients^[54], or even, the development of drug resistance and relapse of cancer^[55].

This problem can potentially be solved by delivering anticancer drugs encapsulated within nanoparticles^[56,57] or as drugs conjugated to the nanoparticle's surface^[58-61]. Due to their size range, nanoparticles are inherently able to permeate through leaky tumour microvessels but impaired lymphatic drainage of the solid tumour, together with a higher interstitial fluid pressure, hinders clearance of nanoparticles from the TME. Thus, retention of anticancer drugs is enhanced when delivered as nanomedicine. This mechanism of passively targeting a solid tumour is known as the enhanced permeation and retention (EPR) effect, which was first described by Matsumura and Maeda^[62] in 1986.

The size of the tumour, degree of tumour vascularization, and angiogenesis are the main factors affecting EPR^[63-65]. Thus, the stage of the disease is critical for drug targeting using the EPR effect^[66]. Another factor is the challenge for the chosen delivery system to penetrate deep into tumour tissue due to the high interstitial fluid pressure at the centre of a tumour^[67]. This results in initial tumour regression, followed eventually by recurrence from residual cells in the non-accessible regions of the tumour^[68]. Therefore, the drug delivery system needs to be optimized for deep tumour penetration^[69-71]. This can be achieved by (1) enhancing blood perfusion to a tumour; (2) modulating the structure of tumour vasculature; and (3) destroying the mass of cancer cells to increase passage of nanoparticles.

Enhancing blood perfusion to a tumour

As discussed earlier, tumour blood vessels have sluggish blood flow. The hydrostatic fluid pressure in a blood vessel (P_v) is less than that of fluid in the interstitial space (P_i). This limits the distribution of therapeutic agents in the TME. Therefore, an increased rate of blood flow in tumour vessels will enhance the distribution of nanoparticles in the TME because of higher extravasation. Strategically there are two ways to increase the rate of blood flow in tumour vessels. First, vasoconstrictors such as angiotensin can be parenterally administered^[72]. This will constrict normal blood vessels but not tumour blood vessels which will remain unaffected because of their impaired muscular structure. As a result, more blood will be delivered to tumour blood vessels. Second, vasodilators like NO and CO should be delivered directly to tumour blood vessels without affecting blood vessels of normal tissue^[73].

In experimental rats with subcutaneously transplanted AH109A solid tumours, Suzuki *et al.*^[74] found a 5.7 fold enhancement of blood flow in the tumour after intravenous administration of angiotensin II. This enhanced the chemotherapeutic effect of mitomycin C on the main tumour and metastatic foci in lymph nodes. Nagamitsu *et al.*^[72] then successfully treated patients with SMANCS (neocarzinostatin, the anti-tumour antibiotics conjugated with a hydrophobic copolymer of styrene) under angiotensin induced hypertensive states. The induction of hypertension at ~15-30 mm Hg higher than normal blood pressure for 15-20 min resulted in remarkably enhanced and passively targeted delivery of neocarzinostatin to the tumour. This resulted in faster reduction of tumour size with the least toxicity to normal tissue.

Many research groups have developed nano-medicines that induce tumour-specific vasodilatation by releasing mediators such as NO^[75,76] and CO^[73] *in situ*. This helped in the accumulation of nanoparticles within the TME. Tahara *et al.*^[77] incorporated NONOate, a typical NO donor, into PEGylated liposomes. Its retention in blood was similar to that of empty PEGylated liposomes but its accumulation within the tumour was doubled. Due to successful augmentation of the EPR effect, this liposome could be a potential vehicle for the targeted delivery of potent chemotherapeutic agents.

Wei *et al.*^[78] then developed tumour vascular-targeted multifunctional hybrid polymeric micelles for the targeted delivery of doxorubicin [Figure 3]. Poly (D,L-lactide) (PLA) and poly (ε-caprolactone) (PCL)

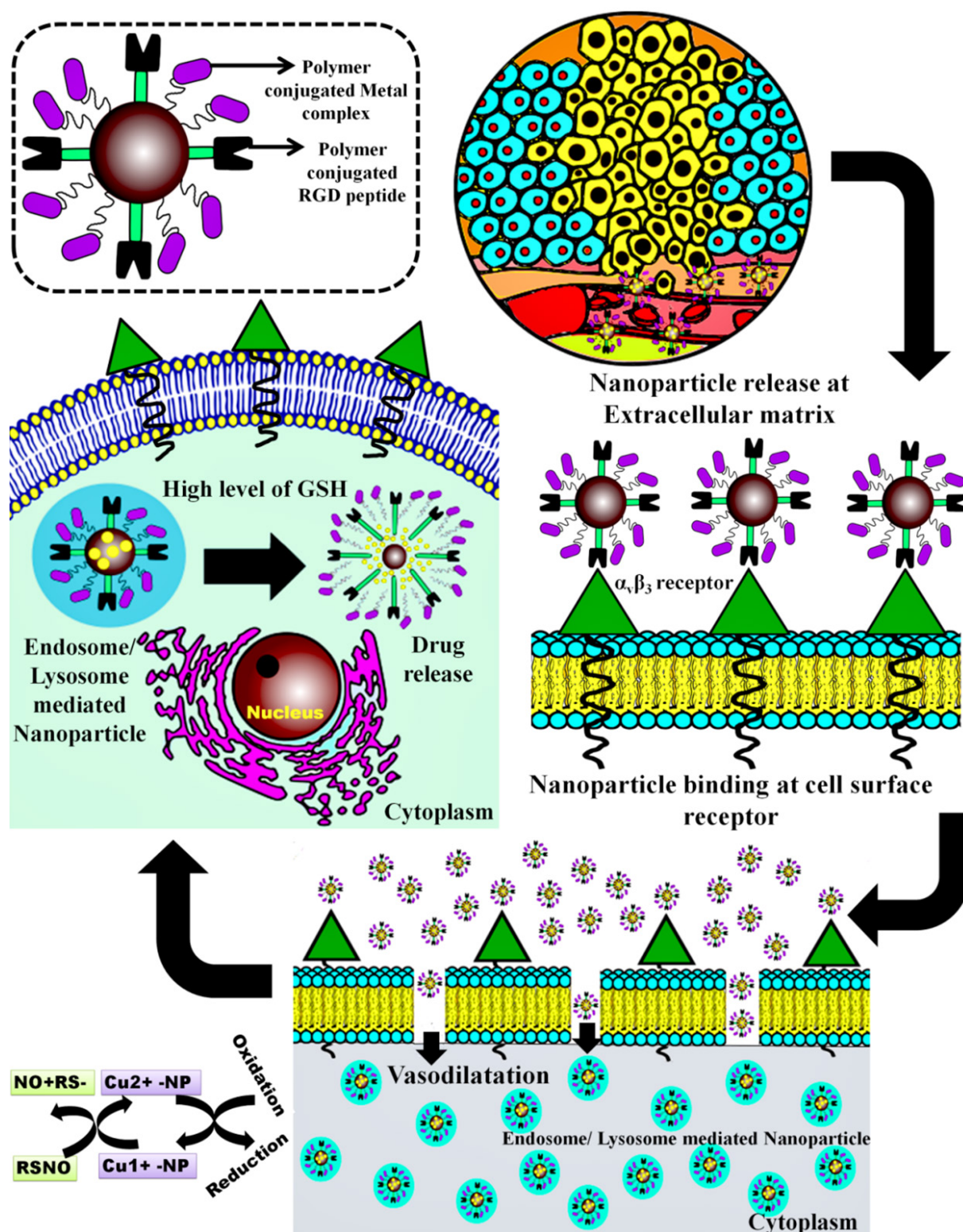


Figure 3. Schematic representation of NO generating tumour vasculature targeted drug delivery systems. Copper ion-chelated porphyrin triggers tumour vasculature specific release of NO causing local vasodilation, whereas RGD peptide causes $\alpha_v\beta_3$ mediated tumour cell-specific nanoparticle uptake. The drug is released specifically within the cancer cells where the cytoplasmic levels of GSH is higher than normal cells. NO: nitric oxide; GSH: glutathione; RSNO: S-Nitroso alkane NP: nanoparticle; RGD: arginylglycylaspartic acid

formed the inner core to encapsulate doxorubicin. The poly (ethylene glycol) (PEG) was linked to PLA with disulphide linkages to form the outer surface of the particle. Copper ion-chelated porphyrin (PpIX-Cu) was then added to the end of the PEG segment, providing a catalytic function to decompose endogenous NO donors like *S*-nitroso-glutathione (GSNO), *S*-nitrosocysteine, and *S*-nitrosoalbumin. Since these endogenous NO donors are also present in human plasma and all tissue fluid, 2-propionic-3-methyl-maleic anhydride (CDM)-modified methoxy polyethylene glycol (mPEG) (mPEG-CDM) was linked to the PpIX-Cu component as a pH-sensitive protective layer, in order to mask the positive charges of the micelles and avoid copper ion-catalysed NO production in the general circulation. Copper catalysed NO production occurred only in mildly acidic (pH 6.5) tumour tissue. Furthermore, cRGD grafted PCL-PEG-cRGD (PCE-cRGD) copolymer was added during the synthesis of micelles. The grafted cRGD peptide then effectively targeted the tumour vasculature and tumour cells, on which $\alpha v \beta 3$ integrin is overexpressed. Once taken up by the cancer cell, doxorubicin was immediately released due to the high cytoplasmic level of GSH. Thus, this complex hybrid polymeric micelle structure was very effective in treating tumours in an animal model.

Fang *et al.*^[79] reported augmentation of the EPR effect and efficacy of anticancer nanomedicine by CO generating agents. Haem oxygenase (HO) catalyses the degradation of haem to produce CO which causes vasodilatation similar to NO^[80-82]. Pegylated hemin is the HO inducer whereas tricarbonyl-dichloro-ruthenium (II) dimer (CORM2) is the CO-releasing molecule^[79]. The authors showed that in tumour-bearing mice, the accumulation of intravenously administered Evans blue-albumin complex (a macromolecule) in a tumour can be enhanced by the intradermal injection of recombinant haem oxygenase-1, intra-tumoral injection of tricarbonyl-dichloro-ruthenium (II) dimer (CORM2) and intravenous administration of PEGylated hemin. Thus CO plays a significant role in tumour uptake of macromolecular drugs by EPR^[83]. They have also developed polymeric micelles of CORM2 copolymer and styrene maleic acid. It had a prolonged plasma half-life and was able to maintain a sustained release of CO. They used it for photodynamic therapy with pyropheophorbide-a^[79].

Modulating the structure of tumour vasculature

The balance between pro-angiogenic (e.g., VEGF, PDGFB, IGF, PDGFRB, FGF-2, and TIE2) and anti-angiogenic factors (e.g., thrombospondin-1, angiostatin and endostatin) is responsible for the formation of normal tissue vasculature. This balance tips in favour of overexpression of pro-angiogenic factors in pathological conditions such as the progression of solid tumours^[84]. The purpose of such is to meet the high demand for oxygen and nutrients of tumour cells. Therefore, restoring this balance of factors may restore tumour vasculature back to normal. This process involves the inhibition of pro-angiogenic factors at a different level of their cell signalling pathways [Figure 2], which will reduce the diameter of tumour microvessels, prune immature vasculature, increase vasculature maturity with higher pericyte coverage, reduce tortuosity of microvessels, and decrease IFP. Although normalization of tumour vasculature is the rationale for inhibition of tumour growth^[85], it is not effective enough alone in clinical settings. Instead, it has been found in clinical trials that combinations of radiotherapy or chemotherapy together with anti-angiogenic agents are very effective^[84,86]. Ionizing radiation generates ROS that leads to DNA damage and cell death. Since the presence of oxygen helps in the generation of ROS, a well-vascularized and perfused tumour tissue would be more susceptible to radiotherapy^[86]. It has also been shown that under low-dose irradiation, cancer cells are induced to express proangiogenic factors (e.g., VEGF, PIGF) at a level sufficient to stimulate endothelial cell migration and sprouting. This is known as the vascular rebound effect^[87], which can be overcome by combining anti-angiogenic agents with radiotherapy. In one clinical trial on advanced pancreatic cancer patients, a combination of optimal dosages of bevacizumab, capecitabine and radiotherapy was found to be very effective^[88]. In another clinical study with rectal cancer patients, promising results were reported when radiotherapy was combined with bevacizumab, capecitabine, and oxaliplatin^[89]. In cases of chemotherapy used in combination with anti-angiogenic agents, normalization of tumour vessels will not only reduce vascular permeability but at the same time, enhance the trans-capillary

pressure gradient (due to lowering of IFP), resulting in better distribution of small molecule anticancer drugs and nanoparticles (< 60 nm) into the TME^[84].

Strategically, one may either block the pathways for synthesis of pro-angiogenic factors and their target receptor proteins, or neutralize the effects of these factors by inhibiting the corresponding target receptors with monoclonal antibodies. Such angiogenesis inhibitors can either target endothelial cells of the growing vasculature (known as direct inhibitors) or tumour cells and tumour-associated stromal cells (indirect inhibitors). Direct inhibitors like angiostatin^[90], endostatin^[91], arrestin^[92], canstatin^[93] and tumastatin^[94,95] bind with integrin receptor to prevent the proliferation and migration of endothelial cells in response to different pro-angiogenic factors. Indirect inhibitors prevent the expression of pro-angiogenic proteins (e.g., VEGF) expressed by tumour cells or block the expression of corresponding endothelial cell receptors (VEGFR). Many angiogenesis inhibitors have been approved by the FDA for cancer therapy including thalidomide^[96], bevacizumab^[97], pazopanib^[98] and everolimus^[99] amongst others. There are also many candidate anti-angiogenic drug molecules such as siRNA, shRNA, VEGF aptamer, KPQPRPLS-peptide currently under study.

Different types of nanomedicines such as polymeric nanoparticles, lipid nanoparticles, micelles, mesoporous silica particles, metal nanoparticles, noisomes, and liposomes have been developed for the delivery of anticancer drugs. Amongst them, liposomal delivery systems are mostly approved by the FDA for clinical use.

Therapeutic nucleic acids like small interfering RNA (siRNA) and short hairpin RNA (shRNA) are negatively charged and thus, frequently delivered with liposomes made up of cationic phospholipids. Cai *et al.*^[100] developed Bio-reducible fluorinated peptide dendrimers for efficient and safe delivery of VEGF siRNA. It improved physiological stability, serum resistance; promoted intratumoral enrichment, cellular internalization, as well as facilitated endosomal/lysosomal escape and reduction-triggered cytoplasm siRNA release. It had found to have excellent VEGF gene silencing efficacy (~65%) and a strong ability to inhibit HeLa cell proliferation. Upon intratumoral injection in mice with HeLa tumor xenografts, it significantly retarded tumour growth. Yang *et al.*^[101] developed strategy for co-delivery of VEGF siRNA and docetaxel. This dual peptide modified liposome binds specifically to glioma cells, undergoes specific receptor-mediated endocytosis and deep tissue penetration. Once within target cells, the siRNA silences the *VEGF* gene to inhibit angiogenesis while docetaxel kills tumour cells.

Chen *et al.*^[102] studied the effect of silencing the *VEGF* gene using siRNA for the treatment of breast cancer (MCF7 xenograft model) with doxorubicin. They prepared calcium phosphate/siRNA nanoparticles and further encapsulated it in a liposome. The liposome was injected intratumorally while doxorubicin was administered intraperitoneally. This combination therapy resulted in 91% tumour inhibition using only 60% of the standard dose of doxorubicin. In a more recent study, Zheng *et al.*^[103] utilized mesoporous silica nanocarriers (148.5 nm) for the co-delivery of sorafenib (a multikinase inhibitor) and VEGF targeted siRNA to treat hepatocellular carcinoma. The particles were further coated with lactobionic acid to target asialoglycoprotein receptors that are overexpressed on cancer cells. Taking one step further, Shen *et al.*^[104] co-delivered sorafenib and survivin shRNA with nano-complexes to reverse multidrug resistance in human hepatocellular carcinoma. Survivin is an angiogenesis promoting agent. Suppression of survivin with shRNA thus resulted in the reversal of drug resistance and promoted sensitization to sorafenib treatment, leading to cell cycle arrest and apoptosis.

While positively charged liposomes are best suited for the delivery of negatively charged RNA molecules, they undergo nonspecific electrostatic adsorption with blood components and are quickly recognized by the immune system, leading to rapid clearance from the blood by the reticuloendothelial system (RES). This limitation can be overcome by coating the positively charged liposomes with negatively charged anionic

Table 2. Strategies of tumour-targeted drug delivery exploiting tumour vasculature

Proangiogenic factor	Antiangiogenic agent	Anti-cancer drug	Formulation/delivery system	Mechanism of action	In vivo/ex vivo/clinical study	Year of study	Ref.
VEGF	siRNA	Not applicable	Liposome with two peptides (Angiopep and tlyP-1) attached on the surface	Angiopep ligand helps in brain tumour targeting, tlyP-1 ensures tumour penetration. siRNAs inhibit VEGF production	<i>In vivo</i> : nude mice bearing U87 MG glioblastoma	2014	[111]
	Not applicable	cis-di-amine-di-nitro-platinum (II)	Anti-VEGF mAb and anti-VEGFR2 mAb were attached on the liposome surface	The mAb targets the liposome to tumour cells. Cis-di-amine-di-nitro-platinum (II) kills cancer cells	<i>Ex vivo</i> : glioma C6 and U-87 MG cells <i>In vivo</i> : intracranial C6 glioma rat model using female Wister rat	2016	[112]
	Sorafenib and Cy3-siRNA	Not applicable	pH-sensitive carboxymethyl chitosan-modified liposomes	Inhibition of angiogenesis due to downregulation of VEGF	<i>Ex vivo</i> : HepG2 cell <i>In vivo</i> : H22 tumour-bearing mice	2019	[113]
	Not applicable	DOX	DOX-loaded Amino-triphenyl dicarboxylate-bridged Zr4+ metal-organic framework Nanoparticles gated with a duplex nucleic acid including an anti-VEGF aptamer in a caged configuration	VEGF overexpressed by cancer cells provides the mechanism to unlock the gate via the formation of the VEGF-aptamer complexes and the separation of the gating duplex. The released DOX kills the cancer cells	<i>Ex vivo</i> : MDA-MB-231 breast cancer cell line	2018	[114]
	siRNA	DOX HCl	Polycation liposome-encapsulated calcium phosphate nanoparticle	siRNA silences the expression of VEGF. DOX kills cancer cells	<i>Ex vivo</i> : MCG-7 cell line <i>In vivo</i> : MCF-7 xenograft tumour model in nude mice	2017	[115]
	Gambogic acid	Gambogic acid	PEGylated liposomes	Gambogic acid has both antiangiogenic and cytotoxic activity	<i>Ex vivo</i> : MDA-MB-231 cells <i>In vivo</i> : MDA-MB-231 orthotopic xenograft model	2016	[116]
	siRNA	Docetaxel	Liposome with two peptides (Angiopep and tlyP-1) attached on the surface	Angiopep ligand helps in brain tumour targeting, tlyP-1 ensures tumour penetration. siRNA inhibits VEGF production. Docetaxel kills cancer cells	<i>Ex vivo</i> : human glioblastoma cells (U87 MG) <i>In vivo</i> : male BALB/c nude mice with U87 MG tumours	2014	[117]
	siRNA	Etoposide	Cationic liposomes coated with PEGylated histidine-grafted chitosan-lipoic acid	siRNA silence <i>VEGF</i> gene. Etoposide kills cancer cells	<i>Ex vivo</i> : A549-Luc <i>In vivo</i> : nude mice bearing orthotopic A549-Luc tumour	2019	[105]
	Bevacizumab	Paclitaxel	Bevacizumab diluted with saline, paclitaxel dissolved in 1:1 mixture of cremophor el and ethanol solution	Inhibiting the binding of VEGF to its cell surface receptors with the anti-tubulin agent	<i>In vivo</i> : MX-1 human breast cancer xenograft model and A549 xenograft model	2010	[118]
	siRNA	Sorafenib	Lactobionic acid conjugated mesoporous silica nanoparticle	siRNA inhibits VEGF expression. Sorafenib has antiangiogenic and cytotoxic effects	<i>Ex vivo</i> : asialoglycoprotein receptor overexpressing hepatocellular carcinoma (HepG2, Huh7) cells	2018	[103]
	shRNA (Survivin)	Sorafenib	Pluronic P85- Poly-ethyleneimine/D- α -tocopheryl-PEG 1000 succinate nanocomplexes (nanomicelle)	shRNA inhibits VEGF expression. Sorafenib has antiangiogenic and cytotoxic effects	<i>Ex vivo</i> : multidrug resistance hepatocellular carcinoma cells (BEL-7402) <i>In vivo</i> : xenograft model in nude mice	2014	[104]
	Vatalanib	Not applicable	Oral tablet	Vatalanib is an angiogenesis inhibitor. It inhibits the tyrosine kinase domains VEGFR, PDGFR, and c-KIT	Clinical (Phase II): patients with metastatic pancreatic adenocarcinoma who failed first-line treatment with gemcitabine	2014	[119]
	Sorafenib	Paclitaxel	Hyaluronic acid conjugated D- α -tocopheryl polyethylene glycol 1000 succinate and polylysine-deoxycholic acid copolymer co-modified cationic liposome	Sorafenib is an angiogenesis inhibitor. It also inhibits cancer cell proliferation (by inhibiting RAF/MEK/ERK signalling pathways). Paclitaxel arrests cancer cells at G2/M phase	<i>Ex vivo</i> : multi-drug resistant MCF7 breast cancer cell line <i>In vivo</i> : xenograft model using BALB/c nude mice	2019	[120]

VEGF	Sunitinib	Near-Infrared dye-IR780	Liposome	Laser activated release of sunitinib inhibits tyrosine kinase associated with VEGF and PDGF receptors, whereas IR780 dye kills cancer cells by hyperthermia	Ex-vivo: 4T1 cell line <i>In vivo</i> : BALB/c mice bearing 4T1 tumours	2018	[121]
	Sunitinib	Paclitaxel	Paclitaxel loaded pH-responsive micelle was coated with β -cyclodextrin via MMP-2 sensitive peptide that was cleavable in the tumour matrix. Sunitinib was loaded in this cyclodextrin layer	Drugs were released at the tumour microenvironment (low pH, presence of MMP). Sunitinib inhibits angiogenesis and paclitaxel arrests cancer cells at the G2/M phase	<i>Ex vivo</i> : C6 glioma cell <i>In vivo</i> : C6 tumour bearing nude mice	2019	[122]
	KATWLPFR peptide	Gold nanoparticle	Gold NP capped with monocarboxy (1-mercaptopoundec-11-yl) hexa (ethylene glycol)	Gold nanoparticle delivers the peptide within the cell, where it predominantly binds to neuropilin-1 receptor and inhibits angiogenesis	<i>Ex vivo</i> : human breast cancer cell lines (MCF-7 and MDA-MB-231)	2013	[107]
FGF	FGF1 (recombinant ligand for all FGFRs)	Gold nanoparticle (AuNP)	FGF1 conjugated gold nanoparticle	FGF1 helps in the targeted delivery of AuNP to FGFR positive cells to cause NIR induced photothermal destruction of cancer cells	<i>Ex vivo</i> : BJ cells and mouse fibroblast (NIH 3T3) cells	2012	[123]
	Cetuximab	Paclitaxel	Cetuximab conjugated paclitaxel loaded nanodiamond	Cetuximab helps in cancer cell-targeted delivery of paclitaxel that arrests cells at G2/M phase	<i>Ex-vivo</i> : human colorectal cell line (HCT116, SW620, and RKO) <i>In vivo</i> : a special strain of Balb/C mice bearing subcutaneous tumour	2017	[109]
Epidermal growth factor	Cetuximab	Gemcitabine	"2 in 1" nanoconjugates containing both cetuximab and gemcitabine on a single gold nanoparticle core	Cetuximab helps in the targeted delivery of gemcitabine to the EGFR positive cancer	<i>Ex vivo</i> : pancreatic cancer cell lines (AsPC-1, PANC-1, and MIA Paca-2) <i>In vivo</i> : orthotopic model of pancreatic cancer using nude mice	2008	[108]
	Lapatinib	Paclitaxel	Liposome	Lapatinib inhibits angiogenesis. Paclitaxel arrests cells at G2/M phase	<i>Ex vivo</i> : 4T1 mouse mammary carcinoma cells	2015	[124]
	Lapatinib	Paclitaxel	Poly(lactide-co-poly(ethylene glycol)) filomicelles of 100 nm length and spherical micelles of 20 nm diameter	Lapatinib inhibits angiogenesis and p-GP protein. Paclitaxel arrests cells at G2/M phase	<i>Ex vivo</i> : MCF-7 breast cancer cell	2019	[125]
	Gefitinib	DOX	Gefitinib complexed with dioleoyl-phosphatidic acid via ion pairing was loaded onto the nanoparticle made of DOX conjugated poly(L-lactide)-block-polyethylene glycol (PLA-b-PEG)	At first, Gefitinib was released, followed by DOX. Gefitinib inhibits EGFR tyrosine kinase and DOX kills cancer cells	<i>Ex vivo</i> : MDA-MB-468 (breast cancer cell line) <i>In vivo</i> : orthotopic breast cancer model using FVB female mice and R7 murine breast cancer cells	2017	[126]
	Gefitinib	Gemcitabine	Gemcitabine was administered intravenously in saline solution. Gefitinib was dissolved in water and administered as oral gavage	Gefitinib inhibits EGFR tyrosine kinases and gemcitabine kills cancer cells	<i>Ex vivo</i> : UMSCC-1 cell line <i>In vivo</i> : nude mice bearing UMSCC-1 xenografts	2006	[127]
	Erlotinib and Fedratinib	Not applicable	Poly(ethylene glycol)-poly (lactic acid) nanoparticle	Inhibition of EGFR and suppression of the JAK2/STAT3 signalling pathway	<i>Ex vivo</i> : nonsmall cell lung cancer (H1650, H1975) <i>In vivo</i> : subcutaneous tumour-bearing male athymic nude mice	2018	[128]
	Lapatinib	Paclitaxel	Poly(lactide-co-Poly(ethylene glycol)) micelles	Lapatinib inhibits EGFR and HER2 tyrosine kinase whereas paclitaxel arrests cancer cells at G2/M phase	<i>Ex vivo</i> : MCF-7 breast cancer cell line	2019	[129]

Epidermal growth factor	Lapatinib	Paclitaxel	Liposome	Lapatinib inhibits EGFR and HER2 tyrosine kinase whereas paclitaxel arrests cancer cells at G2/M phase	<i>Ex vivo</i> : 4T1 murine mammary cell	2016	[130]
	Afatinib	Paclitaxel	Afatinib was loaded in stearic acid-based solid lipid nanoparticles. This nanoparticle and paclitaxel were loaded in poly(lactide-co-glycolide)-based porous microspheres	Afatinib inhibits EGFR and HER2 tyrosine kinase whereas paclitaxel arrests cancer cells at G2/M phase	<i>Ex vivo</i> : drug-resistant NSCLC	2019	[131]
	Erlotinib	Paclitaxel	Both erlotinib and paclitaxel were encapsulated in glyceryl monostearate nanoparticles, which was coated with a PEGylated polymeric layer	Erlotinib inhibits EGFR tyrosine kinase whereas paclitaxel arrests cancer cells at G2/M phase	<i>Ex vivo</i> : NCI-H23 cell line	2018	[132]
	Erlotinib	Gemcitabine	Erlotinib (100 mg/d, orally), Gemcitabine (1000 mg/m ² , i.v. infusion)	Erlotinib inhibits EGFR tyrosine kinase whereas gemcitabine kills cancer cells	Clinical (open level phase II clinical trial): patients with locally advanced, inoperable, or metastatic pancreatic cancer	2013	[133]
	Erlotinib	DOX	pH-sensitive charge conversion nanocarrier: DOX was loaded in amino-functionalized mesoporous silica nanoparticles, which was coated with a synthetic zwitterionic oligopeptide lipid-containing erlotinib	Erlotinib and DOX were released sequentially and showed a synergistic effect. Erlotinib inhibits EGFR tyrosine kinase whereas DOX kills cancer cells	<i>Ex vivo</i> : A549 cell line <i>In vivo</i> : tumour xenograft model using SD rats	2016	[134]
Androgen receptor	Thalidomide	Not applicable	Methoxy poly(ethylene glycol)-poly(ϵ -caprolactone) nanoparticle	Thalidomide inhibits androgen receptor and TNF- α	<i>Ex vivo</i> : A549 cell line <i>In vivo</i> : A549 xenograft model in nude mice	2018	[135]
mTOR	Everolimus	Not applicable	Everolimus loaded 3'-(1-carboxy)ethyl sialyl LewisX mimic-decorated liposome	Sialyl LewisX (sLeX), the natural ligand of E-selectin directs the delivery of liposome to tumour endothelium. Everolimus inhibits angiogenesis	<i>Ex vivo</i> : human umbilical vein endothelial cells	2019	[136]
	Everolimus	Paclitaxel	Poly(ethylene glycol)-b-poly(lactide-co-glycolide) copolymer nanoparticle. Everolimus: Paclitaxel molar ratio = 0.5:1	Everolimus suppresses tumour growth by antiangiogenic effect. Paclitaxel kills the cancer cells	<i>Ex vivo</i> : different breast cancer cell lines like MDA-MB-231, MDA-MB-468, MCF-7, TrR1, MDA-MB-231-H2N and SKBR3	2018	[137]
	Rapamycin	Cisplatin	Nanoprecipitate of cisplatin was coated with di-oleoyl-phosphatidic acid. It was further encapsulated in PLGA nanoparticles. Rapamycin was dispersed in PLGA shell	Rapamycin inhibits tumour growth by the antiangiogenic effect. It promotes vascular normalization to improve tumour perfusion. Thus the tumour cells are sensitized to cytotoxic cisplatin molecule	<i>Ex vivo</i> : A375 melanoma cells <i>In vivo</i> : xenograft model of human melanoma	2014	[138]

VEGF: vascular endothelial growth factor; DOX: Doxorubicin; siRNA: small interfering RNA; BMVEC: brain microvascular endothelial cells; PDGF: platelet derived growth factor; MMP: matrix metalloproteinase; EGFR: endothelial growth factor receptor; VEGFR: vascular endothelial growth factor receptor; PDGFR: platelet derived growth factor receptor; c-KIT: a type of receptor tyrosine kinase and tumor marker, also called CD117 and stem cell factor receptor; RAF: rapidly accelerated fibrosarcoma; MEK: mitogen activated protein kinase; ERK: extracellular signal-regulated kinases; FGF: fibroblast growth factor; NSCLC: non-small cell lung cancer; mTOR: mammalian target of rapamycin; NP: nanoparticle; NIR: near infrared; FGFR: fibroblast growth factors receptor; BJ: Normal human fibroblasts cell line; SD: sprague dawley; PLGA: poly(lactic-co-glycolic acid)

polymers, which would then prolong circulation of the nanoparticles in blood and enhance the accumulation of nanoparticles within the tumour due to the EPR effect. In a recent study, VEGF siRNA and etoposide were loaded in a cationic liposome that was further coated with PEGylated histidine-grafted-chitosan-lipoic acid (PHCL), a pH triggered charge-controllable and redox responsive polymer [Figure 4]^[105].

In the TME, at low pH (6.5), protonation of the imidazole group in the histidine segment of PHCL causes a reversal of nanoparticle charge from negative

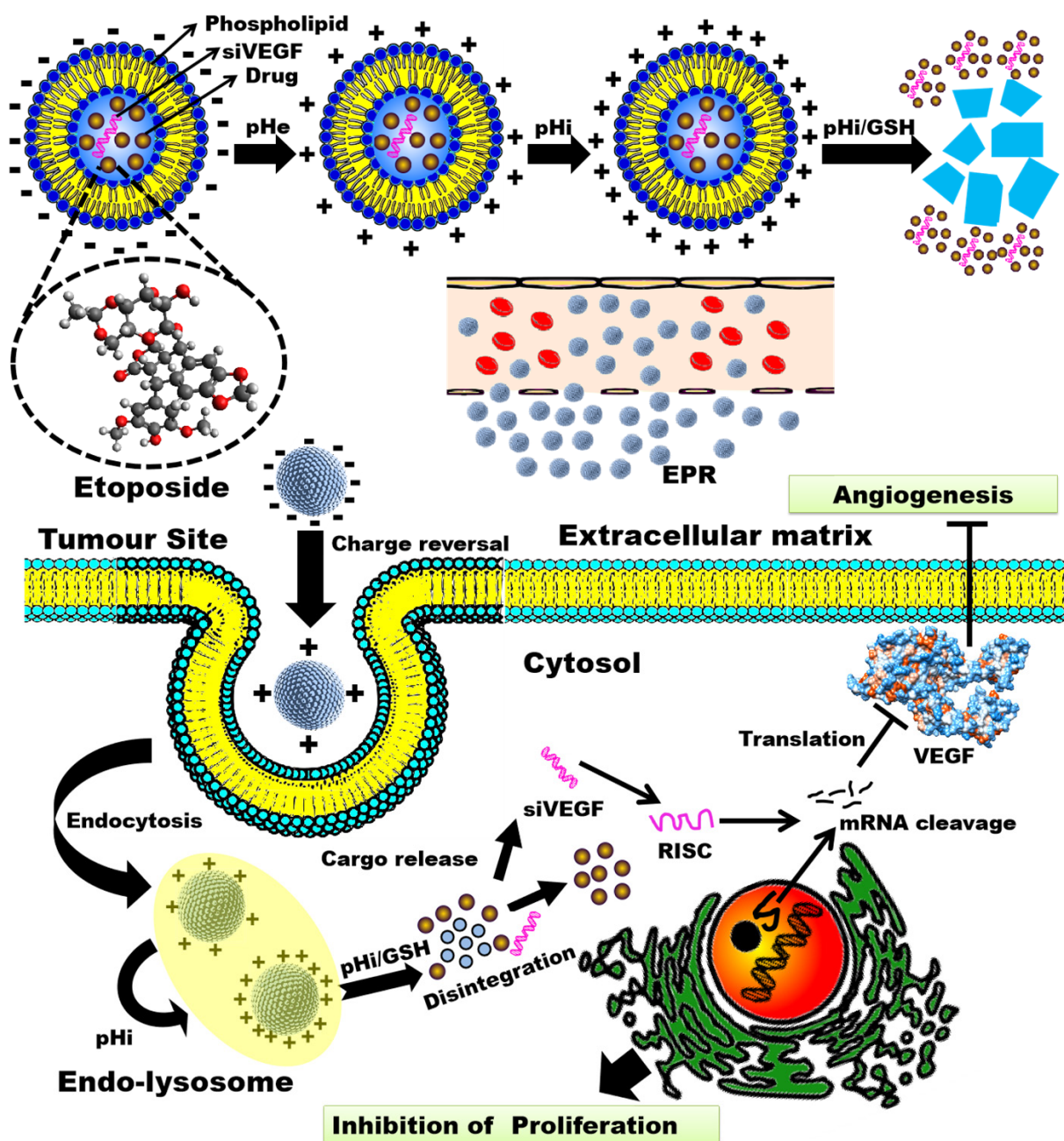


Figure 4. Schematic representation of using multifunctional nanoparticles for co-delivery of VEGF siRNA and etoposide (an anticancer drug) for enhanced anti-angiogenesis and anti-proliferation activity. RISC: siRNA induced silencing complex; VEGF: vascular endothelial growth factor; GSH: glutathione; EPR: enhanced permeation & retention

to positive, leading to deep tumour penetration and enhancement of internalization of nanoparticles. The positive charge is further enhanced in the lower pH of endo-lysosomes, where the disulphide bond of the lipoic acid segment in PHCL-liposomes undergo GSH induced redox-activated breakage, leading to the release of cargo within the liposome [Figure 4].

The antiangiogenic agent bevacizumab is a humanized monoclonal antibody that inhibits tumour growth and metastasis. When combined with a cytotoxic anticancer agent such as paclitaxel, therapeutic efficacy was significantly improved because of the targeted accumulation of paclitaxel within tumours^[106]. In a

preclinical study using the MX-1 human breast cancer xenograft model, different doses of paclitaxel were administered in combination with 5 mg/kg bevacizumab. 30 mg/kg paclitaxel in combination with bevacizumab was as effective as 100 mg/kg single dose of paclitaxel in inhibiting the growth of a tumour. This observation can be attributed to treatment with bevacizumab, which significantly enhances the effective concentration of paclitaxel within the tumour.

Gold nanoparticles have also been used for the targeted delivery of anti-angiogenic agents, either alone or in combination with an anticancer drug. Bartczak *et al.*^[107] synthesized gold nanoparticles of ~15 nm and capped them with mono-carboxy (1-Mercaptoundec-11-yl) hexa (ethylene glycol). These particles were then further functionalized through surface coating with a peptide (KATWLPPR) that specifically binds to neuropilin-1 receptor to inhibit angiogenesis. In an *in vitro* study using human endothelial cells, it was found that this peptide coated gold nanosphere could block capillary formation by endothelial cells without causing toxicity. Patra *et al.*^[108] then used gold nanoparticles for targeted co-delivery of cetuximab and gemcitabine. Cetuximab has been approved for the treatment of EGFR positive colorectal cancer whereas gemcitabine is used for pancreatic carcinoma. “2 in 1” nanoconjugates containing both cetuximab and gemcitabine on a single gold nanoparticle core were synthesized. Physically, this was more stable than a gold nanoparticle-containing either of the agents. This nanoconjugate could target metastatic EGFR expressing cells and inhibited 80% tumour growth and was significantly better than all other non-targeted groups.

EGFR tyrosine kinase inhibitors like cetuximab, lapatinib, afatinib, gefitinib, erlotinib, fedratinib are well studied for anticancer therapy when used in combination with different chemotherapeutic agents including doxorubicin, gemcitabine, paclitaxel, and carboplatin. They help in the normalization of tumour vasculature and sensitize tumour cells to cytotoxic drugs. Additionally, monoclonal antibodies such as cetuximab have been used as a targeting agent. Lin *et al.*^[109] conjugated both paclitaxel and cetuximab on the surface of carbon nano-diamond particles of 3-5 nm diameter. This was found to enhance the mitotic catastrophe and tumour inhibition in the drug resistance of colorectal carcinoma *in vitro* and *in vivo*. Among the other inhibitors, lapatinib also inhibits human epidermal growth factor receptor 2 (HER2) tyrosine kinases and ATP-binding cassette transporters, thereby sensitizing multidrug-resistant (MDR) cancer cells to chemotherapeutic agents. Lapatinib was clinically approved by the US FDA in 2007 for anticancer therapy. There have been many studies since where lapatinib has been used in combination with paclitaxel, and liposomes and polymeric micelles used as drug delivery vehicles. Li *et al.*^[110] developed stealth polymeric micelles using an amphiphilic diblock copolymer named poly (ethylene glycol) -block-poly (2-methyl-2-carboxyl-propylene carbonate-graft-dodecanol) which formed a core-shell structure by self-assembly. Hydrophobic molecules like paclitaxel, lapatinib are loaded into the hydrophobic core while the hydrophilic shell of PEG prevents their aggregation, restricts plasma protein adsorption, prevents recognition by the RES, and minimizes rapid elimination from the bloodstream. This ~60 nm particle successfully overcame multidrug resistance in an athymic nude mouse xenograft model established with DU145-TXT MDR prostate cancer cells. The strategies of tumour-targeted drug delivery exploiting tumour vasculature are summarised in Table 2. The FDA-approved anti-angiogenic agents for the treatment of cancer is summarized in Table 3.

Enhancement of vasculature permeability by physical treatment

EPR is a highly heterogeneous phenomenon. It is variable, even amongst different regions of the same tumour. In fact, within a single tumour, not all blood vessels are permeable to the same extent. Moreover, in many clinical settings, it has been found that tumours do not have a sufficient level of EPR to ensure the accumulation of nanomedicines. This is mainly because of the insufficient permeability of the vascular endothelium of tumour blood vessels. This problem can be addressed by local application of physical treatments such as sonoporation, hyperthermia, and radiotherapy that enhance tumour vasculature permeability, and aid in extravasation of nanomedicines uniformly throughout the TME.

Table 3. List of FDA-approved anti-angiogenic agents for the treatment of cancer

Serial No.	Agents	Marketed name	Mechanism	FDA approved therapy	Ref.
1.	Afatinib	Gilotrif®	Inhibits EGFR (ErbB1), HER2 (ErbB2), and HER4 (ErbB4) receptors	1st-line treatment of patients with metastatic NSCLC (Jan 12, 2018)	[139]
2.	Axitinib and pembrolizumab	Inlyta® and Keytruda®	Axitinib inhibits tyrosine kinase 1, 2 and 3 of VEGFR. Pembrolizumab binds to the Programmed cell death protein 1 (PD-1) receptor, blocking both immune-suppressing ligands, PD-L1 and PD-L2, from interacting with PD-1 to help restore T-cell response and immune response against cancer cells	Advanced renal cell carcinoma (Jan 27, 2017)	[140]
3.	Bevacizumab	Avastin®	It acts by selectively binding circulating VEGF, thereby inhibiting the binding of VEGF to its cell surface receptors. This inhibition leads to a reduction in microvascular growth of tumour blood vessels and thus limits the blood supply to tumour tissues	Avastin was approved for the most aggressive form of brain cancer (Dec 5, 2017), metastatic cervical cancer (Aug 14, 2014), and breast cancer (Nov 18, 2011). Avastin in combination with 5-FU was approved for metastatic carcinoma of the colon and rectum (Feb 26, 2004). Avastin plus chemotherapy has been approved for the initial treatment of metastatic non-squamous, NSCLC (Dec 6, 2018), women with advanced ovarian cancer following initial surgery (Jun 13, 2018), platinum-resistant recurrent ovarian cancer (Nov 14, 2014), first-line treatment of most common types of lung cancer (Oct 11, 2006). Avastin in combination with paclitaxel chemotherapy for first-line treatment of advanced HER2-negative breast cancer (Feb 25, 2008)	[141]
4.	Bosutinib	Busulf®	It is an ATP-competitive Bcr-Abl tyrosine-kinase inhibitor with an additional inhibitory effect on SRC family kinases (including Src, Lyn and Hck). It is also active against the receptors for PDGF and VEGF	Philadelphia chromosome-positive (Ph+) CML with resistance, or intolerance to prior therapy (Sep 5, 2012)	[142]
5.	Cabozantinib	Cabometyx®	It is a multiple tyrosine kinase inhibitor	Advanced renal cell carcinoma (Feb 15, 2018), renal cell carcinoma and hepatocellular carcinoma (Apr 25, 2016)	[143,144]
6.	Cetuximab	Erbix®	(c-Met, VEGFR2, AXL and RET receptor)	Squamous cell carcinoma of the head and neck (Mar 2016)	[145]
7.	Crizotinib	Xalkori®	Epidermal growth factor receptor inhibitor	NSCLC (Aug 26, 2011)	[146]
8.	Dasatinib	Sprycel®	Inhibitor of receptor tyrosine kinases including ALK, hepatocyte growth factor receptor (HGFR, c-Met), and RON	Paediatric patients with Philadelphia chromosome-positive (Ph+) CML in the chronic phase (Nov 9, 2017)	[147]
9.	Erlotinib	Tercava®	It is a dual Bcr-Abl and Src family tyrosine kinase inhibitor. It also targets tyrosine kinases of EPHA2, PDGFR, GFR, and c-KIT	Lung and pancreatic cancer (Nov 18, 2004)	[148]
10.	Everolimus	Afinitor®	It inhibits the intracellular phosphorylation of tyrosine kinase associated with the EGFR	Renal cell carcinoma, breast cancer, neuroendocrine carcinoma (Mar 30, 2009)	[149]
11.	Gefitinib	Iressa®	Inhibitor of mTOR	NSCLC (May 2003)	[150]
12.	Imatinib	Gleevec®	Selective inhibitor of the EGFR	Acute lymphoblastic leukaemia, chronic myelogenous leukaemia, myelodysplastic diseases, gastrointestinal stromal tumour (May 10, 2001)	[151]
13.	Lapatinib with Capecitabine	Tykerb®	Protein-tyrosine kinase inhibitor that inhibits the Bcr-Abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in CML	Breast cancer (Mar 13, 2007)	[152]
14.	Lenalidomide	Revlimid®	Dual tyrosine kinase inhibitor which interrupts the HER2/neu and EGFR pathways	Follicular lymphoma (May 28, 2019)	[153]
15.	Nilotinib	Tasigna®	Directly and indirectly by inhibition of bone marrow stromal cell support, by anti-angiogenic and anti-osteoclastogenic effects	CML (Mar 22, 2018)	[154]

16.	Nintedanib	Ofev® and Vargatef®	It binds to the intracellular ATP binding pockets of FGFR 1-3, PDGFRα/β, and VEGFR 1-3. This results in blockage of the autophosphorylation of these receptors and the downstream signalling cascades	Idiopathic pulmonary fibrosis (2014)	[155]
17.	Osimertinib	Tagrisso®	It targets the mutated EGFR T790M within the cancer cells	NSCLC (Apr 2018)	[156]
18.	Pazopanib	Votrient®	It inhibits VEGFR, PDGFR, c-KIT and FGFR	Advanced soft tissue sarcoma (Apr 27, 2012)	[157]
19.	Ponatinib	Iclusig®	It inhibits Bcr-Abl, an abnormal tyrosine kinase that is the hallmark of CML and Ph+ ALL	Adult patients with chronic phase, accelerated phase, or blast phase CML or Ph+ ALL for whom no other TKI therapy is indicated (Dec 14, 2012)	[158]
20.	Ramucirumab	Cyramza®	It is a direct VEGFR2 antagonist, that binds with high affinity to the extracellular domain of VEGFR2 and block the binding of natural VEGFR ligands (VEGF-A, VEGF-C and VEGF-D)	Gastric cancer, NSCLC, colorectal cancer, hepatocellular carcinoma (Apr 21, 2014)	[159]
21.	Regorafenib	Stivarga®	Dual targeted VEGFR2 and Tie2 tyrosine kinase inhibition	Hepatocellular carcinoma (Apr 27, 2017) Advanced gastrointestinal stromal tumour (Feb 25, 2013) Advanced colorectal cancer (Sep 27, 2012)	[160]
22.	Sorafenib	Nexavar®	Protein kinase inhibitor with activity against many protein kinases, including VEGFR, PDGFR and RAF kinases	Advanced renal cell carcinoma (Dec 20, 2005)	[161]
23.	Sunitinib	Sutent®	Multi-targeted RTK inhibitor	Renal cell carcinoma (Nov 16, 2017)	[162]
24.	Temsirolimus	Torisel®	Inhibitor of mTOR	Renal cell carcinoma (May 30, 2007)	[163]
25.	Thalidomide	Thalomid®	Inhibitor of Akt phosphorylation	Multiple myeloma (May 26, 2006)	[164]
26.	Vandetanib	Caprelsa®	It inhibits EGFR	Advanced thyroid cancer (Apr, 2011)	[165]
27.	Ziv-aflibercept	Zaltrap®	It is a recombinant protein that strongly binds with VEGFR and blocks all known ligands for this receptor	Colorectal cancer (Aug 15, 2012)	[166]

NSCLC: non-small cell lung cancer; EGFR: epidermal growth factor receptor; VEGFR: vascular endothelial growth factor receptor; PDGF: platelet derived growth factor; CML: chronic myelogenous leukaemia; RON: receptor d'Origine nantais; EPHA2: erythropoietin producing hepatocellular-carcinoma type A receptor 2; PDGFR: platelet derived growth factor receptor; c-KIT: a type of receptor tyrosine kinase and tumor marker, also called CD117 and stem cell factor receptor; GFR: growth factor receptor; mTOR: mammalian target of rapamycin; TKI: tyrosine kinase inhibitor; RAF: rapidly accelerated fibrosarcoma; RTK: receptor tyrosine kinase; FGFR: fibroblast growth factor receptor; ALL: acute lymphoblastic leukemia

Sonoporation

Sonoporation involves the application of ultrasonic sound to increase the gap between vascular endothelial cells. The mechanical effects can be further augmented with microbubbles and nanobubbles. The acoustic waves generate acoustic radiation force that causes bulk streaming and microstreaming. Bulk streaming is the movement of localized fluid current in the direction of propagation of ultrasonic sound while microstreaming involves localized eddies that are generated next to cavitating bodies. All these mechanical outputs may result in the release of drugs from carriers and the associated movement of drug molecules into targeted tissues. The efficiency of drug release is controlled by acoustic parameters like ultrasound frequency, power density, and pulse duration. Gas-filled micro-bubbles and nano-bubbles undergo violent collapse under large acoustic pressures. This phenomenon is known as inertial cavitation and is responsible for the generation of micro-streaming^[167,168], shock waves^[169-174], and jetting which are all responsible for enhancing the effect of EPR. The stability of bubbles is mainly affected by the transport properties of core gas. Air, and biologically inert heavy gases like sulphur hexafluoride, perfluorocarbon are used mainly. Though microbubbles are more responsive to ultrasonic radiation and undergo large changes in volume for the induction of EPR, they cannot escape the capillaries. In contrast, nanobubbles can easily penetrate the tumour via EPR. High-frequency ultrasound is thus suitable for targeted delivery of therapeutic agents to small and superficial tumours, whereas low-frequency ultrasound is beneficial for the treatment of large and deeply located ones.

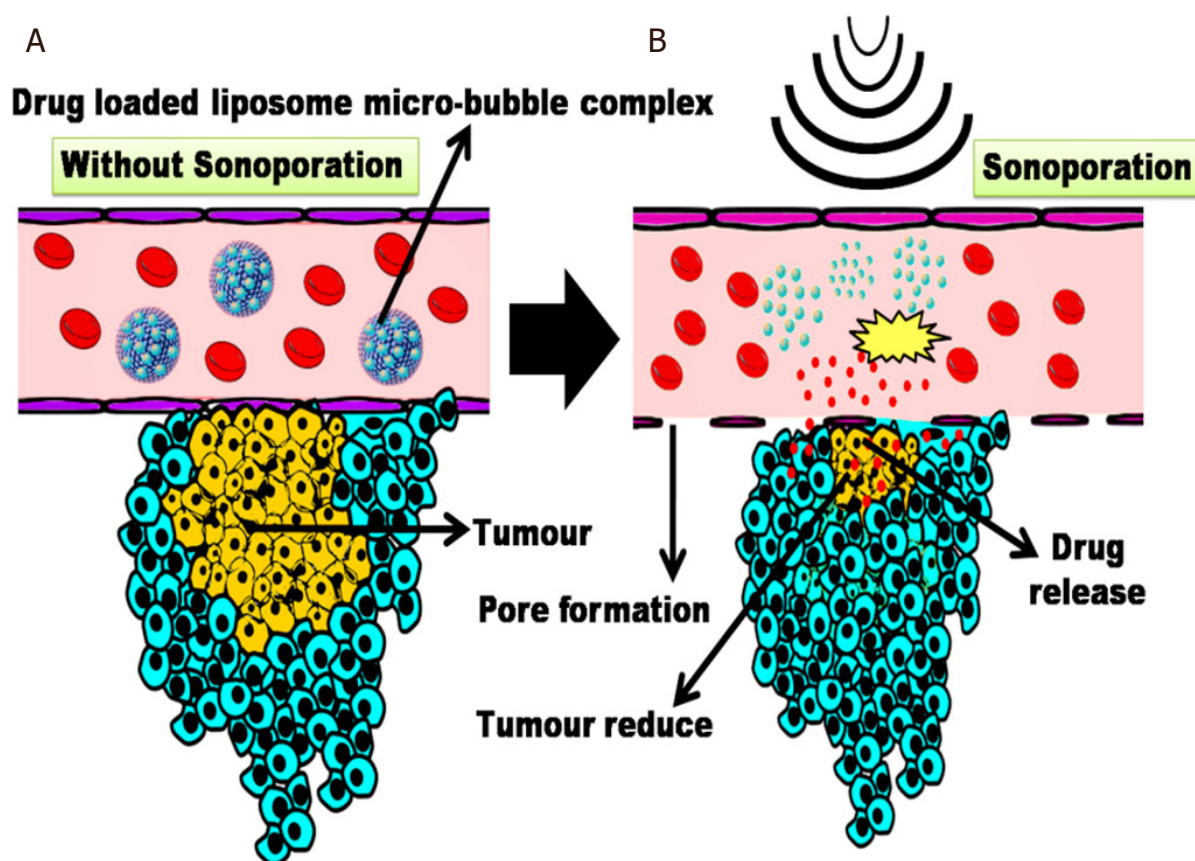


Figure 5. Schematic representation of cancer treatment with anticancer drug-loaded liposome-micro-bubble complexes (PLMC) assisted by ultrasound (US). A: when flowing through the target region, drugs remain attached to the lipid shells of MBs but are unable to cross the tumour vasculature by simple diffusion; B: application of high-intensity focused US bursts the micro-bubbles to release drugs. The cavitating and imploding MBs also enhance permeability of the plasma membrane, leading to higher uptake of released drugs. MBs: micro-bubbles

Theek *et al.*^[175] studied the effect of sonoporation and softshell/hardshell microbubbles on tumour accumulation of fluorophore-labelled 100 nm liposomes in mice bearing A431, BxPC-3 tumour. There was a 100% enhancement in tumour accumulation of liposome.

In another study, Yan *et al.*^[176] attached paclitaxel encapsulated liposomes to the lipid shell of microbubbles via avidin-biotin linkage. They achieved high encapsulation efficiency of doxorubicin and upon application of ultrasonic sound of optimized intensity for the optimal period of time, there was significant enhancement in the uptake of drug molecules in 4T1 breast tumours by EPR.

As an alternative approach, Meng *et al.*^[177] developed a doxorubicin loaded nanobubble [Figure 5]. It consisted of a core of a polymeric network where doxorubicin is dispersed. This core was encapsulated in a perfluoropropane gas bubble, the lipid shell of which was further stabilized with pluronic molecules. When delivered intravenously in combination with therapeutic ultrasonication, this ~170 nm diameter nanobubble showed higher accumulation and better distribution of doxorubicin in tumours, leading to significantly higher intracellular uptake and therapeutic efficacy.

Hyperthermia

In response to temperatures of 41-45 °C, there is increased tissue perfusion to dissipate heat. For healthy tissues like muscle and skin, this increase in perfusion can be as high as 10- and 15-fold respectively.

In tumour tissue, perfusion rates are increased by 1.5-2 folds only^[178,179]. Due to this insufficient perfusion, the temperature of tumour tissues raises further. This causes shut down of local blood flow due to (1) endothelial denaturation; (2) vasoconstriction in large pre-existing arterioles at the tumour periphery; and (3) increase in flow resistance because of high viscosity due to the formation of thrombus and fibrinogen gel. Ultimately, tumour cells are killed due to heat only.

Controlled, local heating of tumour tissue with radiofrequency^[180], microwave or ultrasound to temperatures between 40-45 °C has the following effects: (1) dilatation of tumour vessels leading to enhanced blood flow; (2) enhancement in microvascular permeability to macromolecules^[181] and nanomedicine^[181,182]. This further increases the EPR effect; and (3) triggering the release of cargo molecules (therapeutic agents) from thermoresponsive nanomedicine^[179].

There are different well-studied thermoresponsive nanomedicines such as liposomes^[183-188], nanogels^[189-192], hydrogel coated metal nanoparticles^[193], polymeric nanoparticles^[194-197] and elastin-like peptide-drug conjugates^[179]. Thermodox® is a doxorubicin loaded thermoresponsive liposome, approved for the treatment of liver cancer. It is capable of delivering 25 times more doxorubicin to tumour tissues compared to intravenous infusion, and 5 times more doxorubicin than standard/ordinary liposomal formulation^[23].

Again, to control drug release at mild hyperthermia, leucine zipper peptide was incorporated into the liposome^[24]. At ~42 °C, the leucine zipper gate dissociated to release the drug precisely.

The thermo-responsive bubble generating liposomes^[24] was also developed [Figure 6]. It consists of an ammonium bicarbonate loaded core, which generates CO₂ upon application of hyperthermia (42 °C) and increases the permeability of the liposome bilayer by triggering the release of the drug.

Gold nanoparticles coated with thermo-responsive hydrogel was developed for cancer therapy^[198,199]. Local hyperthermia enhances the accumulation of nanoparticles within the tumour^[200]. The gold nanoparticle has strong plasmon absorption, resulting in the generation of heat and removal of the polymeric shell. Thus, the gold nanoparticle acts as an anticancer agent^[201,202].

Sato *et al.*^[203] successfully applied threefold strategies to chemotherapy with Fe (Salen) nanoparticle. After intravenous injection, this magnetic nanoparticle was guided to the tumour site for delivery in a rabbit young tumour model. The nanoparticle, at the target site, was heated with an alternating magnetic field for the local induction of hyperthermia that helped in further distribution of the nanoparticle into the TME due to the EPR effect.

Hyperthermia by NIR laser irradiation causes shrinkage of blood vessels and tumour ablation. Combining hyperthermia and chemotherapy could be an efficient treatment approach. This is known as photothermal chemotherapy^[204]. Docetaxel loaded polypyrrole and hyaluronic acid-modified phospholipid nanoparticle were used for photothermal chemotherapy^[205]. There was complete inhibition of tumours in 4T1 tumour-bearing mice.

Whole-body hyperthermia at the mild fever range (39.5 °C, for 4-6 h) was found to help in the therapeutic efficacy of doxorubicin-loaded liposome in syngeneic CT26 colorectal mice carcinoma^[206]. There was a threefold increase in drug uptake in the tumour. It was also reported to be associated with decreased IFP and an increased fraction of perfused microvessels^[207].

CONCLUDING REMARKS

Hypoxia-induced formation of new blood vessels is the key factor in the progression of tumours. Tumour vasculature is heterogeneous, tortuous, irregularly branched, and hyperpermeable. Due to poor lymphatic

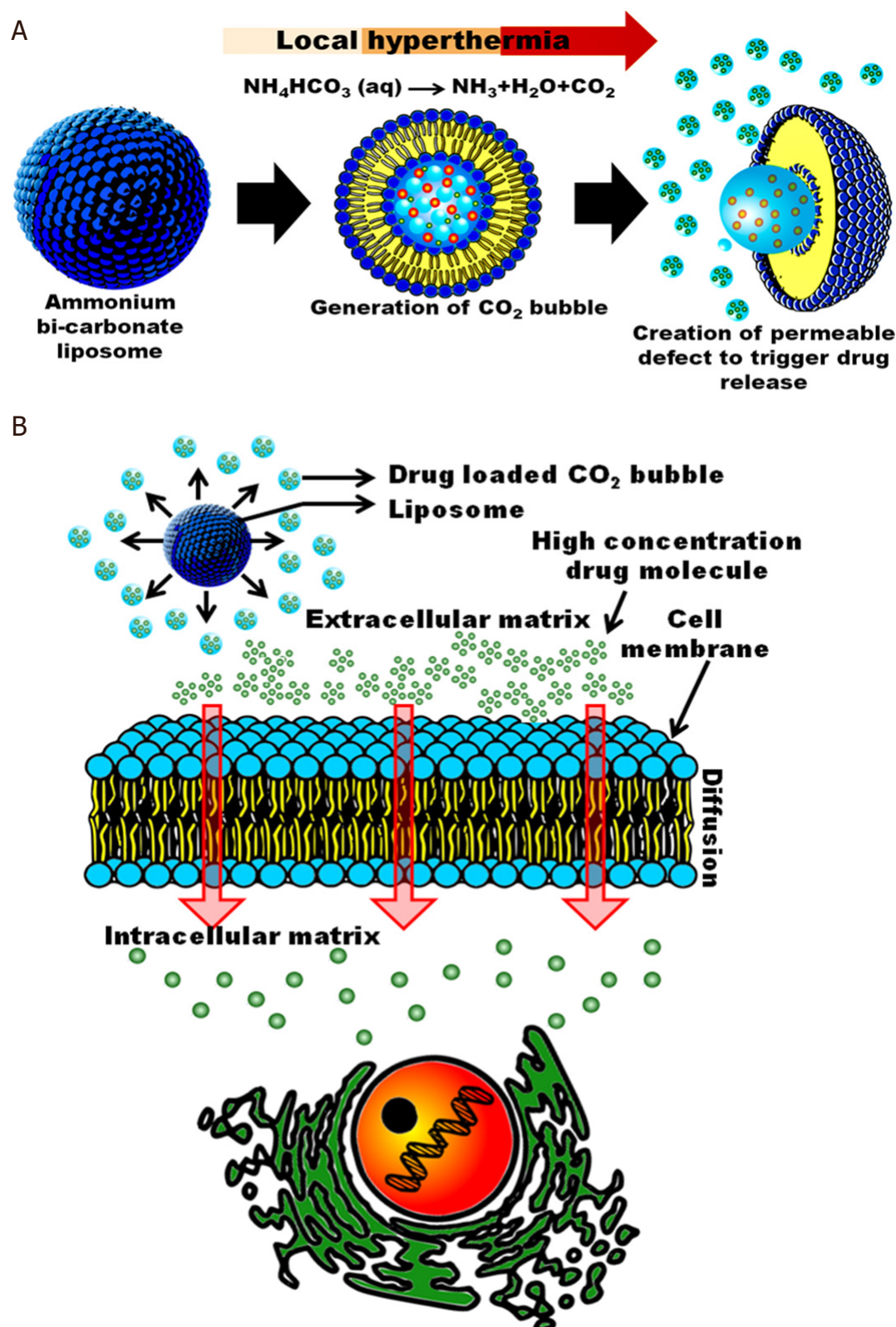


Figure 6. Schematic diagram showing the structure and function of thermoresponsive, bubble-generating liposomes and the mechanism of localized extracellular drug release triggered by heat. A: drug release mechanism upon application of hyperthermia; B: internalization of the released drug by the target cell

drainage, the TME has high IFP. This heterogeneity of the vasculature, high IFP, poor extravasation due to sluggish blood flow, and larger distance between exchange vessels are all potential barriers to the delivery of therapeutic agents to tumours. A rationally designed delivery system should overcome all these barriers to reach deep tumour tissue. As the endothelial cells of tumour vasculature have longer gaps, and the IFP is high, nanoparticles of proper size can inherently be accumulated in the tumour due to the EPR effect. This is known as passive targeting. The surface of nanocarriers can also be coated with monoclonal antibodies against receptor proteins overexpressed in proangiogenic tumour cells for active targeted drug delivery. The vascular barrier can be further reduced by enhancing blood perfusion in the tumour and normalization of tumour vasculature. Local delivery of mediators such as NO and CO enhance blood perfusion whereas inhibition of proangiogenic pathways and the use of antiangiogenic agents help in the accumulation of anticancer drugs loaded nanocarriers deep within tumour tissues. Furthermore, the use of sonoporation and hyperthermia boosts nanocarrier mediated tumour-targeted drug delivery.

DECLARATIONS

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Authors' contributions

Contributed in writing the manuscript: Dastidar DG
Contributed in editing the manuscript: Chakrabarti G
Did the literature survey and prepared the diagrams: Ghosh D

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

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Review

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Endothelial cell-derived extracellular vesicles in atherosclerosis: the emerging value for diagnosis, risk stratification and prognostication

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Abstract

Endothelial cell-derived extracellular vesicles are produced by both activated and apoptotic endothelial cells, and play a pivotal role in various physiological conditions such as inflammation, repair, programmed cell death, and immune responses. There is a large body of evidence on the dysregulation of synthesis and secretion of several types of endothelial cell-derived extracellular vesicles, which can then trigger microvascular inflammation, atherosclerotic plaque formation, plaque rupture, thrombosis and endothelial dysfunction. The development of atherosclerosis and cardiovascular events is associated with an increased number of apoptotic, endothelial cell-derived vesicles and a decrease in activated, endothelial cell-derived vesicles. This review depicts the role of endothelial cell-derived extracellular vesicles in the manifestation and progression of atherosclerosis. We also discuss the clinical use and benefits of altering the immune phenotypes of extracellular vesicles originating from endothelial cells, to function as predictive biomarkers in both asymptomatic and subclinical atherosclerosis.

Keywords: Atherosclerosis, cardiovascular events, extracellular vesicles, endothelial cells

INTRODUCTION

Atherosclerosis remains a leading cause of major cardiovascular events (MACEs) and cardiovascular (CV) diseases worldwide. It represents a serious economic burden on the healthcare system and is associated with high rates of mortality and morbidity^[1]. While there has been a steady trend towards decreasing CV mortality from conditions associated with atherosclerosis such as stroke and myocardial infarction in



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developed countries over the last decade, mortality trends from coronary artery disease (CAD) have been more varied^[2,3]. The decline in risk of potentially fatal complications from atherosclerosis in high-income countries has been reported to be closely connected with improved control of conventional CV risk factors such as hypertension, obesity and smoking, after implementation of current clinical guidelines^[4,5]. Overall, the impact of atherosclerosis on MACEs, CV disease manifestation and disability in both developed and developing countries continues to be under investigation because full control of traditional CV risk factors (i.e., dyslipidemia, and diabetes mellitus) did not achieve disease reversal.

Non-adaptive remodeling in atherosclerosis leads to plaque formation, which is a consequence of events including endothelial dysfunction, impaired vascular repair, systemic and microvascular inflammation, and the migration, proliferation and phenotypic switch of smooth muscle cells^[6]. There are numerous cellular and molecular mechanisms that contribute to the initiation and progression of vascular lesions in atherosclerosis including the infiltration of oxidized lipids into the sub-intima, transformation of macrophages from an anti-inflammatory into a pro-inflammatory phenotype, modification of the extracellular matrix due to imbalance between activities of matrix metalloproteinases and their inhibitors, the development of foam cells, over-production of inflammatory cytokines in the atherosclerotic plaque and within the sub-intima layer, expansion of the lipid core in the plaque and vascular tone dysregulation^[7-9]. In turn, endothelial and vascular integrity are ensured by interaction of genetic and epigenetic programs that play a pivotal role in the maintenance of vascular homeostasis^[10,11].

Previous pre-clinical and clinical studies have shown that extracellular vesicles (EVs) originate from progenitor and mature endothelial cells. EVs may act both locally and remotely as powerful regulators of vascular function and integrity through the transfer of biological information^[12-14]. EVs are also involved in several pathological processes underlying progression of atherosclerosis such as systemic and microvascular inflammation, immunity, signal transduction, cell proliferation, differentiation, survival and apoptosis, as well as neovascularization, angiogenesis, thrombosis, and autophagy^[14-16]. The purpose of this review is to summarize current knowledge on the role of endothelial cell-derived EVs in the manifestation and progression of atherosclerosis, and to discuss the clinical use and benefits of using altered immune phenotypes of these endothelial-cell derived EVs as predictive biomarkers in both asymptomatic and subclinical atherosclerosis.

DEFINITION AND NOMENCLATURE OF EVS

EVs are a heterogenic population of secreted, membrane-enclosed particles. This includes exosomes, ectosomes, microvesicles, small size microvesicles, micro particles, nano particles, apoptotic bodies and other subsets. Some (ectosomes and micro particles) are not distinct from each other, and several classification approaches (sedimentation speed-derived criteria, immune phenotype, origin, mechanism of release, and size) were applied to EV subsets to qualify them in some categories.

According to the last update of the Executive Committee of the International Society for EVs, EVs are defined as a mixture of particles ranging from 30-2000 nm in diameter, released by various types of viable cells through several mechanisms (blebbing and budding of endosomal or plasma membranes) and include exosomes, microvesicles and apoptotic bodies^[17]. EV subtypes are defined according to numerous physical characteristics however, such as size (small, medium and large EVs with diameters < 100 nm, 100-200 nm and > 200 nm), density (low, middle, and high, with each range defined), biochemical composition (CD63⁺/CD81⁺, Annexin A5-stained), and descriptions of conditions or cell of origin (e.g., podocyte EVs, hypoxic EVs, large oncosomes, apoptotic bodies). Although the terms “exosome” and “microvesicle” are historically burdened by both manifold and inaccurate definitions, [Table 1](#) reports both under the nomenclatures of EVs to easily understand the basic characteristics of several subtypes of EVs.

Table 1. Nomenclature and basic characteristics of several subtypes of EVs

Characteristics of EVs	Subpopulations of EVs			Ref.
	Exosomes (small EVs)	Microvesicles (ectosomes, medium/large EVs)	Apoptotic bodies	
Diameter, nm	40-100	100-1000	50-2000	[17]
Origin	Endocytic membrane	Cell membrane	Apoptotic cells	[18]
Mechanism of delivery	Ceramide-dependent, tetraspanin-dependent, and ESCRT-dependent exocytosis of multi vesicular bodies	Ca ²⁺ depending phospholipid redistribution and Rho-kinase-mediated myosin light chain phosphorylation, facilitating budding and blebbing	thin membrane protrusion and blebbing of the apoptotic cells' surface	[20,21]
Phosphatidylserine composition	Low	High	High	[22]
Complexity/granularity	High	High	Low	[26,27]
Components	Cytoplasmic and membrane molecules, proteins and lipids, tetraspanin's receptors	Adhesive molecules (ICAMs, PECAM-1, MCAM), membrane regulatory proteins (Rab), lipids (SpL, PL, LPS, LPS) and receptors (tetraspanin's receptors, LAIR-1, EGFR), enzymes (Rab GTPase, ERK, MLCK, TPI-1, HMGCL), immune system proteins (CD14, CD276, MiC-11), apoAII, SOD, β -actin, α -actin-4, HSP90AB1, cytochrome complex, SCP-2	Mitochondria, MHC II molecules, ICAM-3, phosphatidylserine, sialylated and glycosylated ligands	[28,29,31,35]
Nuclear fractions	mRNA and microRNA, other non-coding RNAs	non-coding RNAs	non-coding RNAs	
Specific surface markers	Tetraspanins (CD9, CD63, CD81), ESCRT machinery proteins (Alix, tumor susceptibility gene 10), flotillin-1	CD40, Phosphatidylserine, integrins, selectins, ESCRT machinery proteins (Alix, Vps4)	Annexin A5, phosphatidylserine, caspase 3, histones	
Key functional role	Cell-to-cell communication, cargo	Cell-to-cell communication, cargo	Cell-to-cell communication, cell clearance	

SOD: superoxide dismutase; HSP: heat shock protein; SCP-2: sterol carrier protein 2; TPI-1: triosephosphate isomerase 1; HMGCL: 3-hydroxy-3-methylglutaryl-CoA lyase; ESCRT: endosomal sorting complexes required for transport; ERK: a prototypic mitogen-activated protein kinase; EVs: extracellular vesicles

BASIC CHARACTERISTICS OF EV SUBSETS

Small EVs

Small EVs are also known as exosomes. They are a derivative of the endocytic membrane with an average diameter of 40-100 nm and are released from several types of cells as a result of exocytosis and production of multi vesicular bodies^[18,19]. Multi vesicular bodies move along intracellular tubules, fuse with the plasma membrane and release exosomes into the extracellular space. Small EVs have various cellular components including cytoplasmic and membrane molecules, proteins, hormones (aldosterone), growth factors (vascular endothelial growth factor, transforming growth factor), cytokines [interleukin (IL)-1 β , IL-6, IL-8] and lipids, as well as fragments of chromatin, such as non-coding RNAs and several inactive forms of micro RNAs^[18,19]. There is also a common set of membrane and cytosolic proteins, which are embedded into exosomes that have originated from distinct cell types^[20]. The specific surface markers that ensure recognition of the exosomes are tetraspanins (CD9, CD63, CD81), ESCRT (endosomal sorting complexes required for transport) machinery proteins (Alix, tumor susceptibility gene 10), and flotillin-1^[21].

Medium/large EVs

Medium/large EVs (also known as microvesicles, micro particles, ectosomes) range in diameter from 100 to 1000 nm and result from budding of the cell membrane^[22]. Medium/large EVs are heavily enriched in phospholipids, such as phosphatidylserine and phosphatidylcholine, and numerous membrane-dependent structures (receptors, CD markers) that originated from the parent cells^[23]. Proteomics and lipidomics

structure of microvesicles is extremely variable and includes membrane regulatory (Rab, Sterol Carrier Protein 2) and structure (β -actin, α -actin-4) proteins, heat shock proteins HSP90AB1, adhesive molecules (ICAMs, PECAM-1, MCAM), lipids (SpL, PL, LPS, LPS) and receptors (tetraspanin's receptors, LAIR-1, EGFR), enzymes (superoxide dismutase, Rab GTPase, cytochrome complex, Akt/ERK, triosephosphate isomerase -1, 3-Hydroxy-3-Methylglutaryl-CoA Lyase), immune system proteins (CD14, CD276, MiC-11), and apo-lipoproteins (apo-A-II)^[24-26]. Therefore, microvesicles may yield several non-coding RNAs and chromatin fragments coupled with the complexity of other components^[27].

Apoptotic cell-derived EVs

Apoptotic cell-derived EVs include two types of apoptotic bodies: large membrane-bound vesicles [large apoptotic bodies (ABs) with diameter ≥ 1000 nm] and small apoptotic microvesicles (small ABs with diameter < 1000 nm)^[28]. ABs are particles that are generally larger in size in comparison to both exosomes and microvesicles but have a variable diameter that fluctuates around 1000 nm (from 1000 nm to 2000 nm)^[29]. Both types of ABs result from blebbing of the surface of apoptotic cells and contain proteins, numerous cell organelles and chromatin fractions, such as non-coding RNAs from the nucleus or nucleoli^[30]. The process of AB generation is controlled by several distinct morphological steps (i.e., membrane permeability and blebs, membrane protrusion, and cell fragmentation), which are, in turn, regulated by several molecular factors including the Rho-associated protein kinase and the plasma membrane channel pannexin-1.

ABs contain mitochondria, MHC II molecules, ICAM-3, phosphatidylserine, sialylated and glycosylated ligands, fragments of chromatin, DNAs, and non-coding RNAs. It has been noted that the packaging of chromatin content (DNAs and non-coding RNAs) into the structure of ABs is regulated by apoptosis and there are indeed, ABs with no fragments of chromatin or very low amounts of DNAs^[31]. ABs are also classified depending on their origin from the mother cells including antigen-presenting cells, mononuclear cells, endothelial cells, fibroblasts, cardiac myocytes, and epithelial cells^[32]. The clearance of ABs has been ensured by phagocytes^[33]. To accurately differentiate ABs from other particles including cells and debris, there are several specific surface markers such as Annexin A5/phosphatidylserine^[34].

Biological role of EVs

The key biological functions of EVs that originate from various cells are cell-to-cell communication and the transfer of materials called the secretome. Acting as cargo for numerous molecules [heat shock proteins (HSP-90, HSP-70), ILs, tumor necrosis factor-alpha, active molecules, enzymes, peptides, growth factors], EVs are recognized by target cells through specific antigens, bind and fuse with them to supply the packaged materials within to the cells. Therefore, small and medium/large EVs have wide range of biological functions including immune response, antigen presentation, and the transfer of RNA and DNA^[29,35]. The full spectrum of pleiotropic effects of circulating EVs is shown in [Figure 1](#).

Recent studies have revealed that EVs may contain inactive forms of non-coding RNAs, which can be transferred to another cell and become functional in that new microenvironment^[36,37]. Indeed, there is strong evidence that hypoxia and ischemia are triggers for monocyte-dependent production of pro-inflammatory cytokines including IL-2 and TNF-alpha, and the supply of these cytokines to target cells are mediated through package as cargo into EVs^[38]. On the other hand, HSPs, growth factors, non-coding RNAs, and active molecules, which are all transferred by EVs, are involved in the regulation of reparative response, immune reactions and cytoprotection^[39,40]. The wide spectrum of biologically active molecules that are transported by EVs from the mother cells to target cells are able to regulate the endogenous repair system activity including proliferation, differentiation and migration of endothelial progenitor cells and angiogenesis^[41,42]. Through appropriate receptor-ligand (integrin $\alpha\beta 3$, CD40 ligand, neuregulin-1, VE-cadherin and beta-catenin) interactions and the cargo content of EVs, the intracellular signaling pathways

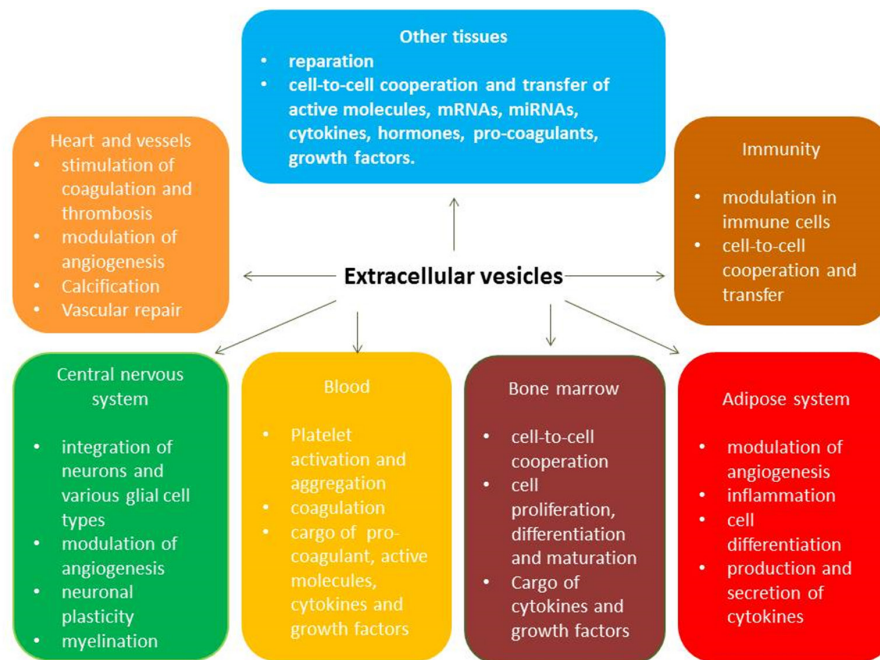


Figure 1. Pleiotropic effects of extracellular vesicles

can be regulated to ensure activation of endothelial cells as well as the attraction and internalization of various types of circulating blood cells (platelets, mononuclear cells, macrophages, lymphocytes) by the endothelial cell surface^[42]. Moreover, vascular growth, restoration of vascular integrity and function, as well as the recruitment of inflammatory cells, may all be directly related to up-regulated expression of neuregulin-1 in endothelial cells as a result of EV-dependent stimulation, because circulating EVs can be a source of a variety of pro-angiogenic mRNAs including neuregulin-1 mRNA^[43]. Additionally, EVs may induce a cytoskeleton-junction response from endothelial cells that is characterized by myosin light chain phosphorylation, contractile fiber reorganization, VE-cadherin phosphorylation and adherent junction dissociation. This process is a key mechanism of increasing permeability of the vascular wall, releasing neutrophil extracellular traps containing citrullinated histones and myeloperoxidase, and in developing senescence and acceleration of atherosclerosis^[44-46]. The proteome of EVs consists of pro-coagulant components such as tissue factor and phospholipids, which play a pivotal role in coagulation and the triggering of vasoocclusion in CAD^[47,48].

EVS IN VARIOUS STAGES OF ATHEROSCLEROSIS DEVELOPMENT

Modification of macrophages' phenotype and function

Macrophages are the primary antigen-presenting cells in atherosclerotic lesions and provide the fundamental link between microvascular inflammation and atherosclerotic plaque development and progression. It has been suggested that endothelial cell-derived EVs export microRNA-92a from mother cells to macrophages in response to atheroprone stimuli to change macrophage phenotype, regulate their functions and enhance atherosclerotic plaque shaping^[49]. Indeed, over-expression of microRNA-92a in endothelial cells in atherosclerosis enhances the pro-inflammatory response in the vasculature, supports low-density lipoprotein (LDL) uptake, and impairs the migration of macrophages through changes in their phenotype from atheroprotected to atheroprone^[50]. Interestingly, the expression of microRNA-92a in mature endothelial cells is up-regulated by the combination of several factors such as low shear stress, atherogenic oxidized LDL, IL-6, atheroprotective Kruppel-like factor (KLF)-2 and KLF-4, and suppressor of cytokine signaling 5^[51,52]. The expression of pro-inflammatory cytokine-induced markers such as

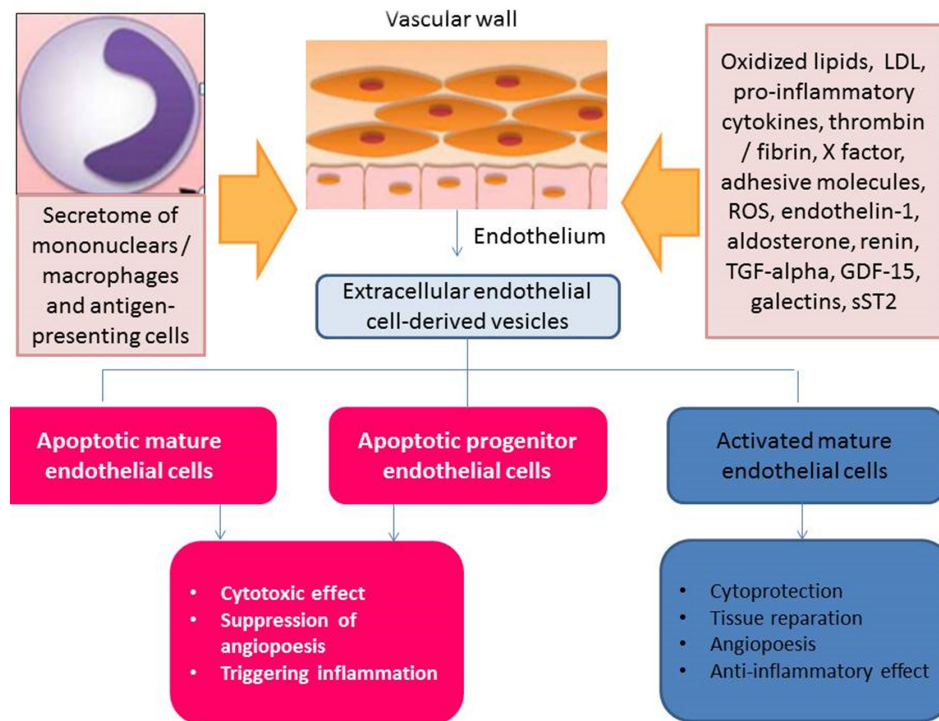


Figure 2. The controversial roles of apoptotic endothelial cell-derived EVs and activated endothelial cell-derived EVs in vascular homeostasis. EVs: extracellular vesicles; LDL: low-density lipoproteins; ROS: reactive oxide species; TGF: transforming growth factor; GDF-15: growth-differential factor-15; sST2: soluble suppressor tumorigenicity-2

monocyte chemotactic protein 1, vascular cell adhesion molecule-1 (VCAM-1), E-selectin, VE-cadherin, and endothelial nitric oxide synthase, as well as KLF-2 and KLF-4, all strongly correspond to down-regulated microRNA-92a in the endothelium in animals^[53]. Therefore, atherosclerosis induces oxidized LDL, and KLF-2 regulates the expression of inflammation-associated microRNA-155 in endothelial cells^[53]. Moreover, it has been found that endothelial cell-derived EVs enriched in oxidized LDL and microRNA-155 influenced monocyte activation by shifting the monocytes/macrophages balance in the vasculature from the anti-inflammatory M2 phenotype of macrophages to the pro-inflammatory M1 phenotype of macrophages^[52,53]. Accumulation of macrophages with the M1 phenotype in the vascular wall also ensured a link between microvascular inflammation and impaired vasodilatory responses to flow via the regulation of microRNA-92-dependent presentation of KLF-2 and oxidative stress stimulation^[54,55]. Additionally, endothelial cell-derived EVs that were packaged with pyruvate kinase muscle isozyme 2 triggered re-programming of B cells and the activation of T cells via its cargo of interferon-gamma^[56]. This mechanism was found to be an important element for the suppression of mononuclear transformation into macrophages with the inflammatory phenotype. The total number of endothelial cell-derived EVs was significantly and positively correlated with oxidative stress and systemic inflammation in healthy younger individuals. While the ability of activated endothelial cells to release EVs packed with pro-angiogenic molecules progressively decreases in patients with established CAD, apoptotic endothelial cell-derived EVs appear to be detected in higher concentrations^[57,58]. This phenomenon probably reflects maladaptive responses of the endothelium in advanced atherosclerosis and decreased control of local vascular inflammation is associated with an altered, intra-plaque immune phenotype of the cells including macrophages and endothelial cells [Figure 2]. It is not clear whether local vascular injury appears first or the alteration in gene regulation of pro-inflammatory genes emerges initially as a microvascular response, thereby triggering acceleration of atherosclerosis.

Mediating angiogenesis and neovascularization

Endothelial cell-derived EVs are involved in the regulation of vascular function, integrity and angiogenesis through transportation of a wide spectrum of micro-RNA (microRNA-126-3p, microRNA-222-3p, microRNA-let-7d-5p, microRNA-21-5p, microRNA-26a-5p, microRNA-92a-3p, microRNA-139-5p, microRNA-30b-5p, microRNA-150 and microRNA-199a-5p) that have been implicated in MAPK/ERK1/2, c-Jun N-terminal kinases/stress-activated kinases signaling cascade, and nuclear factor- κ B signaling pathway to target cells [progenitor and mature endothelial cells, macrophages, smooth muscle cells (SMC), fibroblasts] in arterial endothelium^[51,52,59,60]. There is evidence that the expression of thrombospondin 1 (THBS1), an inhibitor of angiogenesis and a target for microRNA-92a-3p, was significantly up-regulated in the endothelial cells in atherosclerosis and that endothelial cell-derived EV transferred microRNA-92a-3p to reduce THBS1 expression^[52]. Interestingly, the number of activated endothelial cell-derived EVs decreased, whereas the number of apoptotic endothelial cell-derived EVs increased in patients with atherosclerosis when compared with healthy individuals. This discrepancy was found to be closely associated with impaired repair in atherosclerotic injury of the vasculature^[61].

Plaque formation and vascular calcification

Endothelial cell-derived EVs contain a wide spectrum of intercellular signaling molecules, enzymes, regulatory proteins, and growth factors, all of which are involved in the regulation of plaque formation and vascular calcification^[61,62]. Transforming growth factor-beta (TGF- β), which induces the expression of von Willebrand Factor, proliferation of SMCs and progenitor endothelial cells, and recruitment of monocytes/macrophages, is transferred by activated endothelial cell-derived EVs^[63,64]. There is a wide range of evidence that pro-inflammatory cytokines including tumor necrosis factor-alpha and some adipocytokines (adiponectin) induce VCAM-1 production in mature endothelial cells, which is then accompanied by enhanced circulating leukocyte attachment and a weakened ability to release EVs after activation^[65,66]. Therefore, the cellularity of the plaque can be modified by signaling molecules transferred by EVs. Indeed, deficiencies in endothelial microRNA-126 and microRNA-92a transmitted as cargo with endothelial cell-derived EVs has demonstrated acceleration of neointimal lesion formation of carotid arteries, increased smooth muscle cell turnover, and its release was reduced by atheroprotective laminar shear stress^[67-69]. It has been established that autophagy of endothelial cells, fibroblasts, and CD45⁺ hematopoietic cells that accumulates into an atheroma plays a certain role in shaping vulnerable plaques and act as a trigger for plaque rupture^[70]. There were intriguing findings that clarify a role of down-regulated microRNA-92a-3p in endothelial cell autophagy through de-repressed autophagy-related gene 4a and increasing activity of luciferase in autophagy-related gene 4a containing 3'UTR^[71,72]. Moreover, EV-derived microRNA-92a-3p upregulates the expression of cell cycle and mitosis-related genes including claudin-11, and downregulates the adhesion-related gene expression in endothelial and foam cells^[73]. Thus, the packaging and transfer of microRNA-92a-3p by endothelial cell-derived EVs ensures both a protective effect and induces repair of the endothelium, thereby preventing plaque rupture and intravascular thrombosis^[74].

The mediation of osteogenic trans-differentiation of vascular SMCs by inflammation, endothelial dysfunction and reactive oxygen species by EVs may be considered as the key regulators of vascular and plaque calcification. EVs also participate in the formation of microvascular calcifications that are implicated in atherosclerotic plaque formation and rupture^[75,76]. Interestingly, the release of exosomes by EVs may promote microvascular calcification in response to environmental calcium stress^[75]. Sortilin, which is a key regulator of SMC calcification through its recruitment to EVs^[76], has been found to regulate loading of the calcification protein - tissue nonspecific alkaline phosphatase - into EVs and thereby confer calcification potency. SMC calcification also requires Rab11-dependent trafficking and FAM20C/casein kinase 2-dependent C-terminal phosphorylation of sortilin; the deficiency of sortilin was thus found to suppress ectopic vascular calcification^[75,76]. Although it is not clear how EVs influence vascular calcification, previous clinical studies have shown that cell plaque composition, volume of the plaque, a lipid core and

the Agatston coronary calcium score were all inversely correlated with the number of endothelial cell-derived EVs^[77]. However, a recent clinical study based on positron emission tomography/computed tomography imaging has shown that early microvascular calcifications can be identified frequently, even in high-risk patients with plaques^[78]. Early microvascular calcification was found to be associated with atherosclerotic plaque instability and rupture, whereas advanced macrovascular calcification can potentially contribute to plaque stability^[79,80]. There is a suggestion that M1 macrophages that have accumulated in a plaque may release EVs enriched in S100A9 and annexin A5 as a result of weakly activated endothelial cell-derived EVs' stimulation. This contributes to accelerated trans-differentiation of SMCs into osteogenic cells, and the potentiation of microvascular calcification^[79]. Notably, endothelial cell-derived EVs contain bone-related extracellular matrix proteins (osteopontin, osteonectin, osteoprotegerin) and the deficiency of these EVs in the circulation may impair the migration of and cell fusion required for osteoclast formation in the vasculature^[81,82]. Moreover, the immune phenotype and the number of cells accumulating into an atheroma, as well as the extracellular environment are all under the control of endothelial cell-derived EVs^[83]. In fact, EVs originating from progenitor and mature endothelial cells have the unique ability of regulating the osteogenic transformation of SMCs and the activation of fibroblasts in the vasculature. The endothelial cell-derived EVs support microvascular calcification in the collagen-poor fibrous cap, and promote plaque rupture by acting through the TGF- β /SMAD signaling and platelet-derived growth factor-BB pathway^[84]. Interestingly, endothelial cell senescence may increase the release of EVs as carriers of molecular information, which then contributes to the development and calcification of atherosclerotic plaques. The role of senescent EVs in microvascular calcification is not certain however, and requires further investigation^[85]. Whether the altered balance between EVs produced by activated and apoptotic EVs is a cause of microvascular calcification, or if the adaptive reaction prevents inflammatory-related injury of the vasculature and thereby reduce the risk of calcification, is not fully understood.

Endothelial dysfunction and EVs

Endothelial cell-derived EVs are established biomarkers of endothelial dysfunction^[86]. The interplays between cell components that are embedded into the pathogenesis of vascular tone impairment and vascular remodeling in atherosclerosis are mutually activated and sophisticated. There is a wide range of evidence that foam cells and both progenitor and mature endothelial cells may work with each other by releasing exosomes and ABs. Foam cells may secrete exosomes that suppress endogenous activity of endothelial cells and their precursors to modulate endothelial-dependent vasodilatation and prevent intravascular blood cell adhesion and thrombosis^[87]. Moreover, the foam cell-derived EVs promote migration and proliferation of SMCs by regulating the actin cytoskeleton and focal adhesion via ERK and Akt pathways, thereby acting as a trigger of atherosclerosis^[87].

There are findings that demonstrate a causative impact of EV-packaged microRNA-145, microRNA-150 and microRNA-126 on the progression of endothelial dysfunction and atherosclerosis *in vivo*^[88]. These microRNAs appear to respect tissue specificity and are both expressed in and released from endothelial cells due to several stimuli including shear stress, inflammatory cytokines, cell adhesion and thrombosis. Down-regulated microRNA-145, which plays a key role in the control of SMC differentiation, promotes lesion formation. The endothelial cell-specific microRNA-126 is a powerful signal transducer, which is essential for endothelial repair through its transfer from apoptotic endothelial cells derived EVs. Interestingly, splicing of the X-box binding protein 1 (XBP1) in vascular SMCs may control endothelial cell migration via EVs-mediated transfer of microRNA-150 and microRNA-150-driven vascular endothelial growth factor-dependent PI3K/Akt pathway activation, thereby supporting homeostasis of the vasculature^[89]. In fact, XBP1 deficiency in vascular SMCs and endothelial progenitor cells significantly attenuate angiogenesis and neovascularization, as well as maintain endothelial integrity and resistance to apoptosis^[90,91]. Foam cell shaping driven by CD36 mediated internalization of oxidized LDL activates mononuclear cells and endothelial cells, and the subsequent release of EVs embedded with pro-inflammatory leukotriene B₄,

which promotes endothelial dysfunction and accelerates atherosclerosis through the high-affinity receptor BLTR1^[92]. In fact, the presence of dysregulated inflammatory molecules on the surface of the endothelial cell layer was associated with increased coagulation due to over-expression of the glycoprotein (GP) IIb/IIIa (integrin α IIb β 3) receptor, anomalous clot formation or shaping amyloid fibrin^[93,94]. Importantly, the GPIIb/IIIa receptors were additionally shed into EVs and transferred as cargo to the target cells for translation of activation signals remotely. Another finding has demonstrated that heat shock protein 27 (HSP27) packaged into EVs via activating TLR-4/NF- κ B in the target cells can attenuate endothelial function, reduce vascular and plaque inflammation, lower cholesterol levels and suppress atherogenesis in animal models^[95]. However, the angiopoietic role of activated endothelial cell-derived EVs is not always considered as having a positive impact on the endothelium. For instance, neovascularization of the shoulder region of the plaque's cap was associated with instability of the atheroma due to the increased risk of rupture^[94,96]. There are several excellent reviews that are dedicated the role of EVs in vascular homeostasis and its relation to CV disease development^[97,98]. Thus, EVs promote the function of target cells through the transfer of surface integrins and receptors, cellular fusion and the delivery of various active molecules.

EVs as diagnostic and predictive biomarkers of atherosclerosis

The diagnostic and predictive roles of endothelial cell-derived EVs in atherosclerosis and MACEs are uncertain^[99]. However, there has been progress in the diagnosis, prognostication and treatment of CV diseases with EVs^[100]. For instance, HSP27 packaged in endothelial cell-derived EVs was found to be a predictor of a lower CV risk among patients having a heart attack, stroke, or death from CV disease^[95]. The imbalance between activated and apoptotic endothelial cell-derived EVs has provided additional prognostic information for patients with established CV disease, including acute myocardial infarction, acute coronary syndrome, ischemic heart failure, MACEs, and arrhythmias, as well as individuals with metabolic diseases having a higher risk of CV events and disease^[101-104].

Future directions toward the role of EVs in atherosclerosis

EVs have demonstrated a pivotal role in transferring numerous bioactive molecules, supporting cell-to-cell cooperation, and regulate gene expression in target cells. EVs-based therapeutic regenerative strategies may thus be used to attenuate tissue injury and promote vascular regeneration and repair^[105,106]. Accumulating evidence implicates EVs in the development and progression of atherosclerosis, and creates the possibility of using EVs for personalized therapeutic strategies. Therefore, single-EV analysis may identify signatures of exosome-derived DNA/non-coding RNAs including microRNA, regulator proteins, and other components both as diagnostic and predictive biomarkers in atherosclerosis^[107]. Large clinical studies are required for further elucidation of whether EVs can be excellent options for point-of-care diagnosis and individual treatment.

CONCLUSION

EVs could be promising biomarkers with both diagnostic and predictive values, while their number, content, immune phenotype and origin may provide more useful information about the pathophysiology of atherosclerosis and help stratify patients at risk of MACEs. The emergence of endothelial cell-derived EVs provide favorable and promising strategies, not only for CV risk stratification in vulnerable populations but for individualized treatment of atherosclerosis and other CV diseases.

DECLARATIONS

Authors' contributions

Participated in drafting the article, revised it critically for important intellectual content: Berezin AE

Gave final approval of the version to be submitted: Berezin AA

Both authors make equal contributions to conception and design, searchig data, analysis, interpretation of data and writting of the manuscript.

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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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Transcatheter aortic valve implantation combined with other heart interventions: current status and future perspectives

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Abstract

Transcatheter aortic valve implantation is a well-proven effective treatment option for patients with severe symptomatic aortic valve stenosis requiring valve replacement in all risk classes. Frequently, however, other concomitant cardiovascular conditions demanding intervention are present, and a combined approach is required. Here, we discuss some of the most frequent combined settings. The decision about which approach (staged or simultaneous) is more suitable for each patient needs to be based on individual characteristics and clinical, anatomical, and procedure-related factors.

Keywords: Transcatheter aortic valve implantation, combined procedure, heart valve disease, percutaneous coronary intervention, outcomes

INTRODUCTION

In the last years, transcatheter aortic valve implantation (TAVI) has become a well-proven effective treatment option for patients with severe symptomatic aortic valve stenosis (AS) requiring valve replacement at high or intermediate surgical risk. Nowadays, with the expansion of TAVI for low-risk populations, the number of individuals undergoing TAVI will increase even more. Frequently, however,



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patients with AS have other concomitant cardiovascular conditions demanding a combined approach. In this scenario, the ability to perform TAVI combined with other transcatheter heart interventions becomes crucial.

Here, we discuss some of the most frequent settings that have been considered for combined intervention with TAVI, either concomitantly or staged.

PERCUTANEOUS CORONARY INTERVENTION

As the majority of patients with AS scheduled for TAVI are older than 75 years^[1-3], it is not a surprise that up to 50% of preoperative cardiac catheterizations reveal coexisting coronary artery disease (CAD)^[4]. In high- and intermediate-risk TAVI trials, for instance, concomitant CAD was present in about two-thirds of patients^[2,5-7], and even in the low-risk cohorts, concomitant coronary revascularization was indicated in about 7%^[8,9].

According to current European guidelines, percutaneous coronary intervention (PCI) should be considered in patients with primary indication to undergo TAVI presenting with coronary artery diameter stenosis > 70% in proximal segments (Class IIa, Level C)^[10]. It has not been addressed, however, as to when PCI should be performed.

Simultaneous procedures have the advantage of decreasing repeated puncture or incision of vessels, reducing patients' suffering and length of hospital stay, and saving medical resources^[11]. However, radiation exposure and the amount of contrast used are usually higher^[12].

PCI before TAVI, on the other hand, has the potential to minimize ischemic risk during TAVI, particularly during rapid ventricular pacing, and to overcome difficulties associated with coronary access post-TAVI. Conversely, the patient remains at risk for valvular decompensation and needs to be put on dual antiplatelet therapy, which may increase the risk of bleeding during TAVI^[11-13].

PCI after TAVI is relatively rare because prosthetic valve commissures, or stent frame, may be positioned close to coronary ostia, interfering with coronary diagnostic or guiding catheters^[14]. Trying to facilitate future coronary reaccess, Tang *et al.*^[15] have suggested some landmarks to predict coronary overlap severity based on initial TAVI deployment orientation. These findings have significant implications as TAVI moves to younger and low-risk patients, where valve durability and CAD progression are notable concerns. Neo-commissure alignment thus becomes a new trend to be pursued during TAVI intervention.

Regarding scientific evidence supporting simultaneous or staged procedures, a systematic review of 4 studies, comprising 209 patients, showed no difference in 30-day mortality (OR = 1.47, 95%CI: 0.47-4.62), renal failure (OR = 3.22, 95%CI: 0.61-17.12), periprocedural myocardial infarction (OR = 1.44, 95%CI: 0.12-16.94), life-threatening bleeding (OR = 0.45, 95%CI: 0.11-1.87), and major stroke (OR = 3.41; 95%CI: 0.16-74.2) when PCI was performed concomitant or staged with TAVI^[11].

Similarly, a recent paper demonstrated no significant difference in life-threatening or major bleeding, and acute kidney injury among patients who underwent planned pre-TAVI ($n = 156$), post-TAVI ($n = 40$), or concomitant ($n = 77$) PCI. Cumulative 2-year mortality was also similar across the groups (29.7% vs. 14.8% vs. 10.3% in pre-TAVI, concomitant, and post-TAVI, respectively; $P = 0.11$)^[16].

In current practice, PCI has been carried out at the time of TAVI in the presence of significant coronary lesions provided that procedural risk does not outweigh the potential benefit [Figure 1]. The final decision

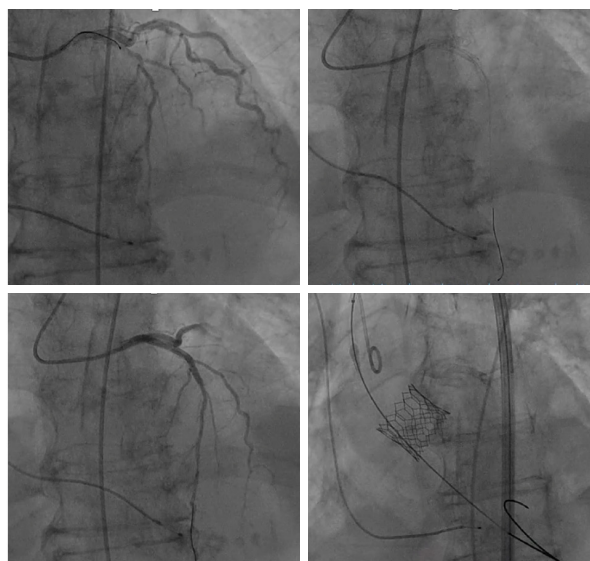


Figure 1. Transcatheter aortic valve implantation simultaneous to percutaneous coronary intervention in an 81-year-old female patient with severe symptomatic aortic stenosis and severe proximal left anterior descending coronary artery stenosis. In this patient, percutaneous coronary intervention using 2 drug-eluting stents was performed first, followed by transfemoral implantation of a 26-mm balloon-expandable Edwards SAPIEN 3 valve

should take into account patient clinical conditions, CAD burden, and the amount of myocardium at risk^[10]. Penkalla *et al.*^[12] suggested some additional anatomical criteria, as follows: (1) left main coronary artery stenosis > 50%; (2) coronary stenosis of 90% or more in the proximal or mid-left anterior descending coronary artery; or (3) coronary stenosis of 90% or more in the proximal or mid-right coronary artery (if dominant artery); or (4) coronary stenosis of 90% or more in the proximal or mid-left circumflex artery (if dominant).

MITRAL VALVE INTERVENTIONS

Moderate or severe mitral regurgitation (MR) is present in 22% to 48% of patients with severe AS undergoing TAVI^[17-20], particularly in those inoperable or at high-risk^[1,2]. These individuals constitute a particular subgroup that could benefit from combined transcatheter interventions.

Although few studies have suggested that MR is not an independent predictor of mortality after TAVI^[20,21], the majority of authors point out a significant increase in mortality risk if moderate to severe MR is present at the time of TAVI^[22-24].

Among the factors that should tailor indication of combining TAVI with an MR intervention are the following: individual patient's characteristics such as age, comorbidities, life expectancy, and frailty; valve characteristics such as MR severity and etiology; together with some technical aspects and procedural risks^[10,25].

MR etiology is a particularly important issue since a less aggressive management could be indicated in the setting of functional MR, assuming that some improvement in MR severity is expected to occur after TAVI^[26,27]. Thus, a staged approach may be reasonable, with the aortic valve being addressed first, and the mitral valve treated only in those who remain symptomatic in spite of a successful TAVI. On the contrary, in the setting of a predominantly primary MR, as structural valve alterations are not expected to improve, bivalvular interventions should be advocated^[25,28].

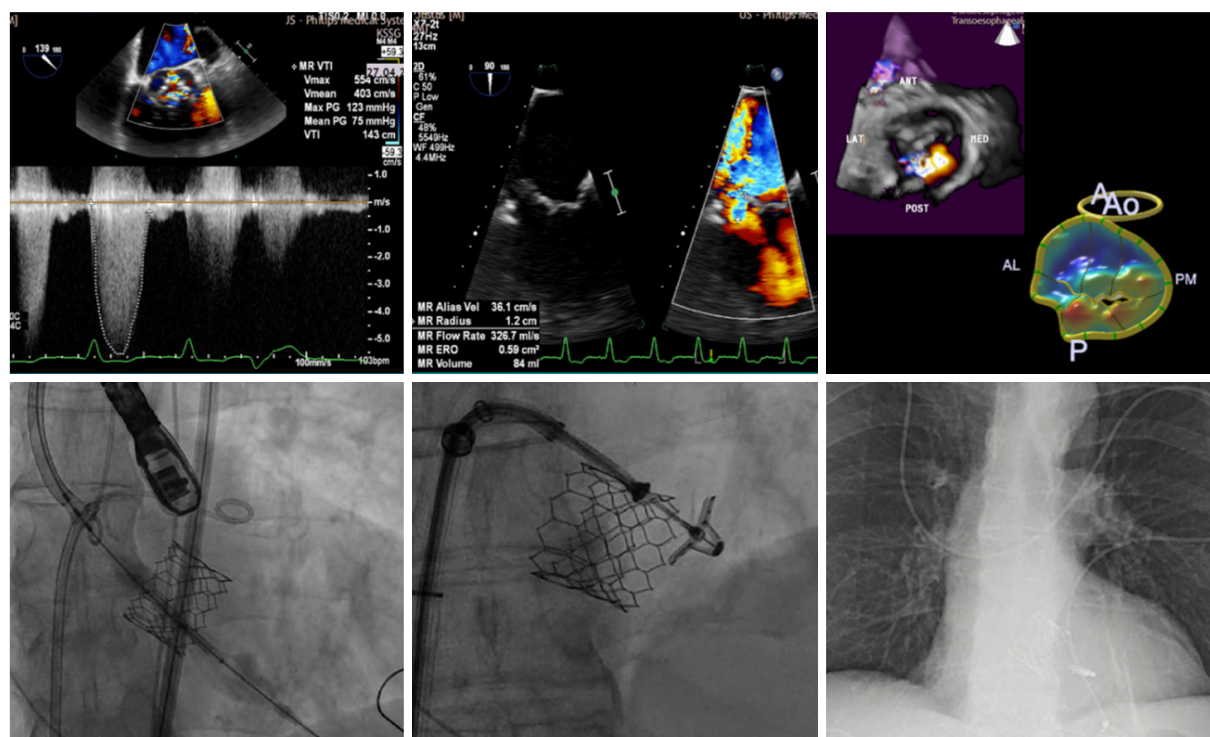


Figure 2. Transcatheter aortic valve implantation concomitant with percutaneous edge-to-edge mitral valve repair in a 70-year-old male patient with severe symptomatic aortic stenosis and severe degenerative mitral regurgitation due to posterior leaflet prolapse (the first 3 images). The procedure was started by the transseptal puncture, followed by transfemoral implantation of a 29-mm balloon-expandable Edwards SAPIEN 3 valve. The last step was MitraClip implantation under transesophageal echocardiogram and angiographic control

If a double-valve transcatheter replacement is indicated since the majority of the mitral devices are delivered transapically, a simultaneous approach through the same access can be used^[29]. Regarding the best order, usually the aortic valve is performed first. The rationale for this strategy is that, since the aortic and mitral annuli are contiguous, bridged by the aorto-mitral fibrous curtain^[30], some degree of obstruction for the new aortic valve deployment can happen if the mitral is treated first^[29]. This same order has been adopted during transapical TAVI simultaneous with NeoChord implant^[31].

In the case of TAVI combined with percutaneous edge-to-edge mitral valve repair, a common strategy is to start with the transseptal puncture under partial anticoagulation, followed by TAVI delivery under full anticoagulation, finalizing with the percutaneous mitral valve repair [Figure 2]. To minimize the time with a large device in the iliofemoral system, the arterial sheath can be removed before starting the mitral valve procedure^[25].

Last but not least, some reports have also supported TAVI combined with valve-in MAC (mitral annular calcification), either by transfemoral/transseptal or transapical access^[32,33].

Summarizing current data, a systematic review of combined aortic [TAVI or transcatheter aortic valve-in-valve (TAViV)] and mitral valve interventions [transcatheter mitral valve replacement (TMVR), transcatheter mitral valve-in-valve/valve-in-ring (TMViV/ViR), or percutaneous mitral valve repair (PMVR)], involving 60 patients from 37 studies, showed that the main reason for combined approach was severe AS (92%) associated with moderate/severe MR (65%). In the majority of the cases, the aortic valve intervention was performed before the mitral valve. Mortality rates were 25% for TAVI plus TMVR, 17% for

TAVI plus TMViV/ViR, 0% for TAViV plus TMViV/ViR, and 15% for TAVI/ViV plus PMVR. Significant post-procedure paravalvular leak was rare in all combinations^[29].

It is also important to remember some caveats regarding MR evaluation in the presence of AS. MR jet velocity can be increased due to high left ventricular pressures^[34], while AS transvalvular gradient and flow can be hampered by the presence of MR^[35].

TRICUSPID VALVE INTERVENTIONS

Significant tricuspid regurgitation (TR) is diagnosed in about 10% of patients with severe AS, and usually indicates right ventricular dysfunction^[36,37].

Although severe TR has been associated with high mortality in patients with concomitant severe AS conservatively treated, some authors have suggested that moderate or severe TR can improve after TAVI^[17,38]. Contradicting this idea, others have argued that TR will persist and that it is independently associated with increased mortality following TAVI^[17,39,40].

To clarify the true impact of TR in patients with severe AS undergoing TAVI, a recent meta-analysis evaluated 12 studies enrolling 41,485 TAVIs. Early and mid-term mortality were 1.80- and 1.96-fold increased, respectively, in the presence of significant TR (OR = 1.80, 95%CI: 1.01-3.19; OR = 1.96, 95%CI: 1.35-2.85)^[40]. These findings were similar to those reported by Pavasini *et al.*^[41], whose study demonstrated a 2.0-fold increase in all-cause mortality if moderate to severe TR was present after TAVI (95%CI: 1.52-2.91). The authors' conclusion was that a more detailed and shared TR severity evaluation would be necessary to understand its impact and the need for a combined approach in patients undergoing TAVI.

Opposing the above-mentioned statements, Barbanti *et al.*^[42] showed that, in patients with moderate to severe TR submitted to TAVI, all-cause mortality risk was higher only in the presence of left ventricular ejection fraction > 40%, suggesting that in patients with severely depressed ejection fraction, severe TR is rather a surrogate marker of advanced disease than a real cause of worse outcomes.

Regarding the feasibility of combined approaches, in 2017, Reichart *et al.*^[43] described a successful case of concomitant TAViV and tricuspid valve-in-ring using a 23-mm self-expanding transcatheter bioprosthesis in aortic position and a 29-mm balloon-expandable transcatheter bioprosthesis in tricuspid position. The authors highlighted that, for the rapid ventricular pacing maneuver, external pacemaker patches were used to avoid the lead crossing through the tricuspid valve. Another option could be the use of a temporary lead inserted through the coronary sinus^[44] or pacing over the left ventricular guidewire^[45].

Another appealing case was reported by Abdi *et al.*^[46], who combined TAVI with tricuspid valve-in-valve in a patient with rheumatic heart disease. The balloon-expandable valve implanted in tricuspid position was oversized by about 20% relative to the internal diameter of the previous bioprosthesis. The authors stressed, however, that an excessive oversizing may impair leaflet opening, leading to early valve degeneration.

Considering the above evidence, it is clear that the tricuspid valve is a relatively non-explored territory, but a potential target for combined transcatheter approaches.

AORTA INTERVENTIONS

Abdominal aortic aneurysm occurs in around 6% of patients undergoing TAVI, a number that is increasing due to population aging, especially because both conditions share similar risk factors^[47].

Despite no clear recommendation available, the idea of combining TAVI with abdominal endovascular aneurysm repair (EVAR) seems appealing. Some potential benefits of simultaneous approaches are as follows: to prevent a potential aortic rupture related to the abrupt rise in blood pressure after relieving aortic valve gradient; to reduce vascular complications by accessing the artery a single time; and to shorten length of hospital stay by combining the two strategies in a single procedure^[48,49].

Regarding which specific procedure should be done first, Sato *et al.*^[49] and Mauri *et al.*^[50] prefer to perform TAVI before EVAR, while Natour *et al.*^[51] advocate starting with EVAR and then to use the same sheaths and wires for the subsequent TAVI.

Performing EVAR first has the advantage of reducing the risk of vascular dissection caused by crossing the aorta with the valve delivery system and preventing aneurysmal sac lesion due to sudden blood pressure rise following TAVI. Furthermore, the increased clot burden within the aneurysmal sac and the consequent risk of distal embolization may be an additional reason to prefer doing EVAR first. On the other hand, performing TAVI first would avoid the risk of crossing the aortoiliac prosthesis, as well as reducing the risk of local thrombosis due to a large EVAR sheath prolonged time within peripheral vessels^[50].

Another emerging possibility is combining TAVI with thoracic endovascular aortic repair (TEVAR). In 2014, Komlo *et al.*^[52] reported the first case of a single-stage TAVI and TEVAR in a patient with critical AS and descending thoracic aorta aneurysm. In this case, direct transaortic access via minimally invasive partial sternotomy was chosen.

Furthermore, a relatively new concept is to treat the aortic valve and the ascending aorta together, using a special proximal transcatheter aortic valve connected to an uncovered stent-graft portion, called the “endo Bentall” concept. Unquestionably, this is an exceptional procedure, indicated only in highly selected cases, but it shows that even the ascending aorta could be suitable for totally endovascular interventions^[53,54].

LEFT ATRIAL APPENDAGE OCCLUSION

Atrial fibrillation (AF) occurs in more than 10% of octogenarians, and it is the most common arrhythmia in the TAVI population^[55]. In the first PARTNER cohorts, chronic AF was present in 32.9% of inoperable ones^[1] and in 40.8% of high-risk patients who underwent TAVI^[2]. Compared to sinus rhythm, AF was associated with double 1-year mortality (26.2% vs. 12.9%)^[56].

Regardless of the type (paroxysmal, persistent, or permanent), AF is a strong predictor of stroke^[57], and it is independently associated with late cardiovascular morbidity and mortality after TAVI^[58]. The explanation for these risks goes beyond its thromboembolic potential; it is also linked to the risk of major bleeding due to oral anticoagulation (AF treatment) plus dual antiaggregation (indicated after TAVI)^[59].

Although there is a lack of evidence supporting the best strategy for stroke prevention in TAVI patients with pre-existing AF, the left atrial appendage occlusion (LAAO) strategy, especially in patients with high risk of bleeding, could be an attractive option. The main advantage of combining LAAO with TAVI is to provide a treatment that has proven to prevent stroke with efficacy similar, or even superior, to warfarin^[60,61].

It is important to remember, however, that LAAO mitigates only the risk of embolization of thrombi formed inside the left atrial appendage, having no effect on clots formed in the valve, or on calcium embolization due to TAVI advancement or positioning. To try to cover these other factors, Gafoor *et al.*^[62] suggest that the use of smaller sheaths, better delivery systems, and easier-to-position devices, besides carotid protection systems, may provide more complete stroke prevention during TAVI.

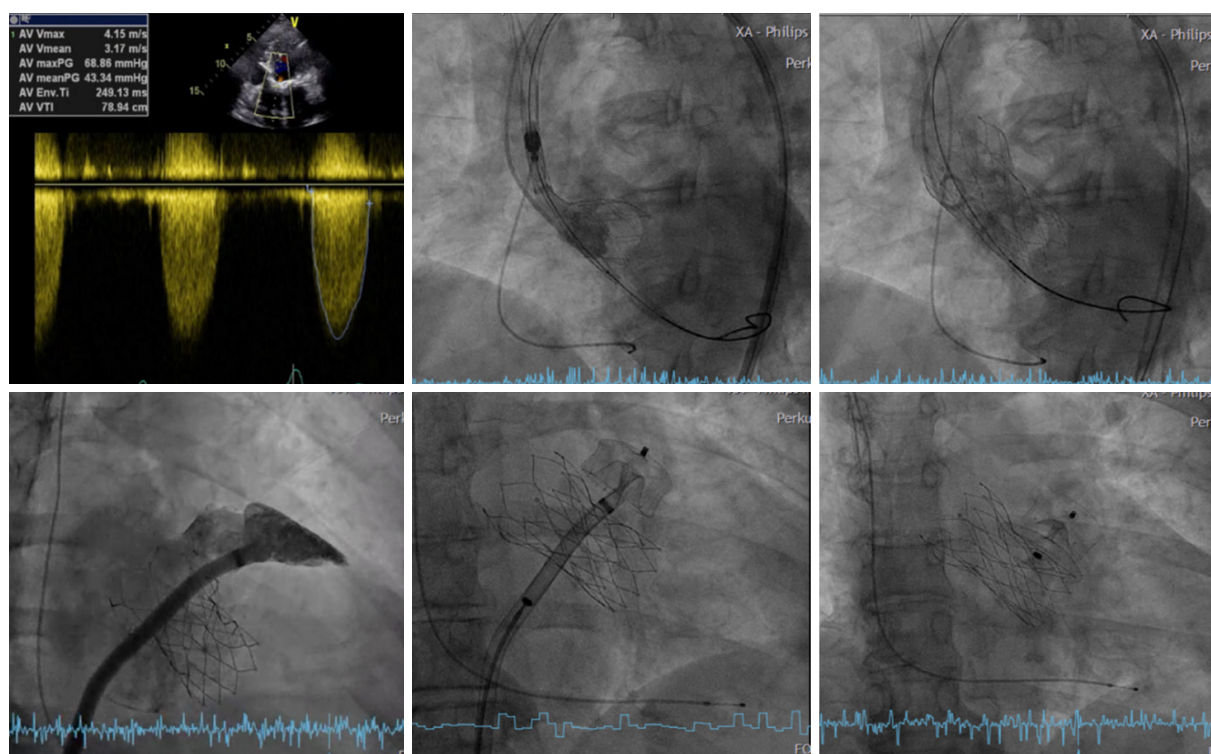


Figure 3. Transcatheter aortic valve implantation simultaneous with left atrial appendage occlusion in a 73-year-old female patient with severe symptomatic aortic stenosis (the first image) and persistent atrial fibrillation. The patient had a previous history of anemia and lower gastrointestinal bleeding associated with anticoagulation use. The procedure was started by a transfemoral implantation of a 27-mm self-expanding Portico valve. After transcatheter aortic valve implantation, the left atrial appendage was angiographically measured, and an left atrial appendage occlusion occluder was implanted (Amplatzer Amulet Occluder 28 mm)

In terms of potential risks, combined procedures require an additional venous access and a transseptal puncture, increasing procedural time and the contrast volume used. Despite this, no impact on periprocedural morbidity and mortality of LAAO combined with TAVI has been observed^[55,63].

Based on these data, the best candidates for LAAO concomitant with TAVI seem to be those with chronic AF and established contraindications to anticoagulation, or those with high risk of bleeding. When a concomitant approach is chosen, usually LAAO is addressed after TAVI [Figure 3].

ATRIAL SEPTAL DEFECT AND PATENT FORAMEN OVALE CLOSURE

Patent foramen ovale (PFO) and atrial septal defect (ASD) are defects of the interatrial septum. Normally, after birth, the elevation in left atrial pressure forces the septum primum and septum secundum together, collapsing the space between them. In approximately 20% of the population, however, a PFO persists^[64], and it becomes a possible conduit for thrombi, air, or vasoactive peptides.

Percutaneous PFO and ASD closure are feasible and effective in reducing paradoxical embolism and preventing secondary stroke, as demonstrated by the extended follow-up of the RESPECT trial (Randomized Evaluation of Recurrent Stroke Comparing PFO Closure to Established Current Standard of Care Treatment) and the CLOSE trial (PFO Closure or Anticoagulants Versus Antiplatelet Therapy to Prevent Stroke Recurrence). In the former, PFO closure significantly reduced ischemic recurrence when compared to medical therapy, with a number needed to treat (NNT) of 45 (HR = 0.55, 95%CI: 0.31-0.999; NNT 45), while in the CLOSE trial, NNT was only 17 (HR = 0.03, 95%CI: 0-0.26)^[65,66].

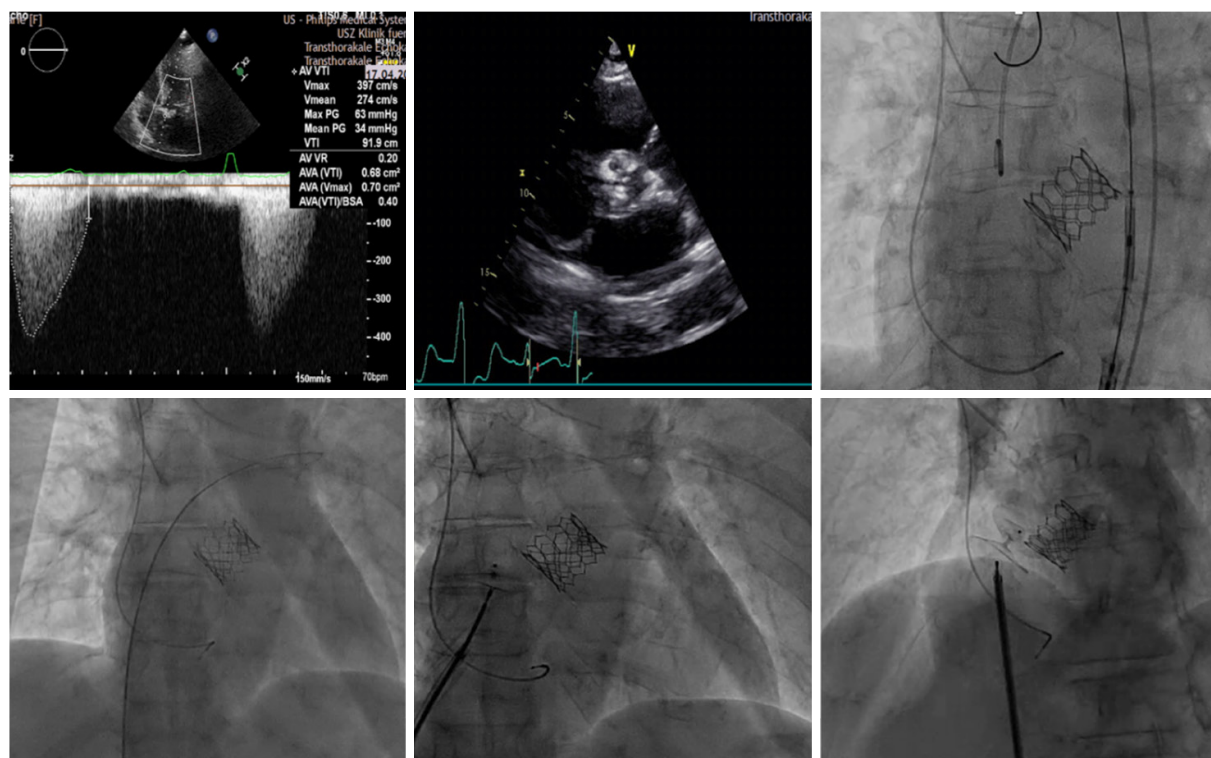


Figure 4. Transcatheter aortic valve implantation concomitant with patent foramen ovale closure in a 70-year-old female patient with severe symptomatic aortic stenosis (the first 2 images) and patent foramen ovale with atrial septal aneurysm. The patient had a previous history of stroke and Heyde syndrome (induced Von Willebrand disease, lower gastrointestinal bleeding, and anemia). A transfemoral 26-mm balloon-expandable Edwards SAPIEN 3 valve was implanted, followed by patent foramen ovale closure using a 30-mm patent foramen ovale occluder

Considering that PFO closure is safe, technically simple, effective, and can be carried out by a single surgeon in less than 15 min, Taramasso *et al.*^[67] believe that this intervention may be justified, especially in the presence of anatomical characteristics such as atrial septal aneurysm, Eustachian valve and Chiari network, or in the presence of a large spontaneous shunt.

In terms of a combined PFO/ASD and TAVI procedure, Pasic *et al.*^[68] reported, in 2011, the first case of TAVI simultaneous with an ASD transcatheter closure. Later, in 2014, Khattab *et al.*^[63] reported the results of 10 TAVI procedures combined with other structural heart interventions. PFO closure was performed using a 25-mm Amplatzer PFO Occluder in two patients, while ASD closure was performed using a 24-mm Amplatzer Septal Occluder in one patient. No residual shunts or thrombi were seen after the procedure. The authors pointed out the feasibility of the combined approach, but also stressed that high-volume centers, with experienced surgeons, are needed to obtain proper results.

When a concomitant approach is performed, the strategy is similar as with LAAO, starting with TAVI [Figure 4].

COST-EFFECTIVENESS ANALYSES

Despite the fact that a precise definition of the best timing for treating concurrent comorbidities in patients undergoing TAVI remains unknown, a common additional factor to take into account in the decision-making process is the local reimbursement policies. Although TAVI procedural costs exceed those of surgical aortic valve replacement, many cost-effectiveness analyses have shown that a shorter length of

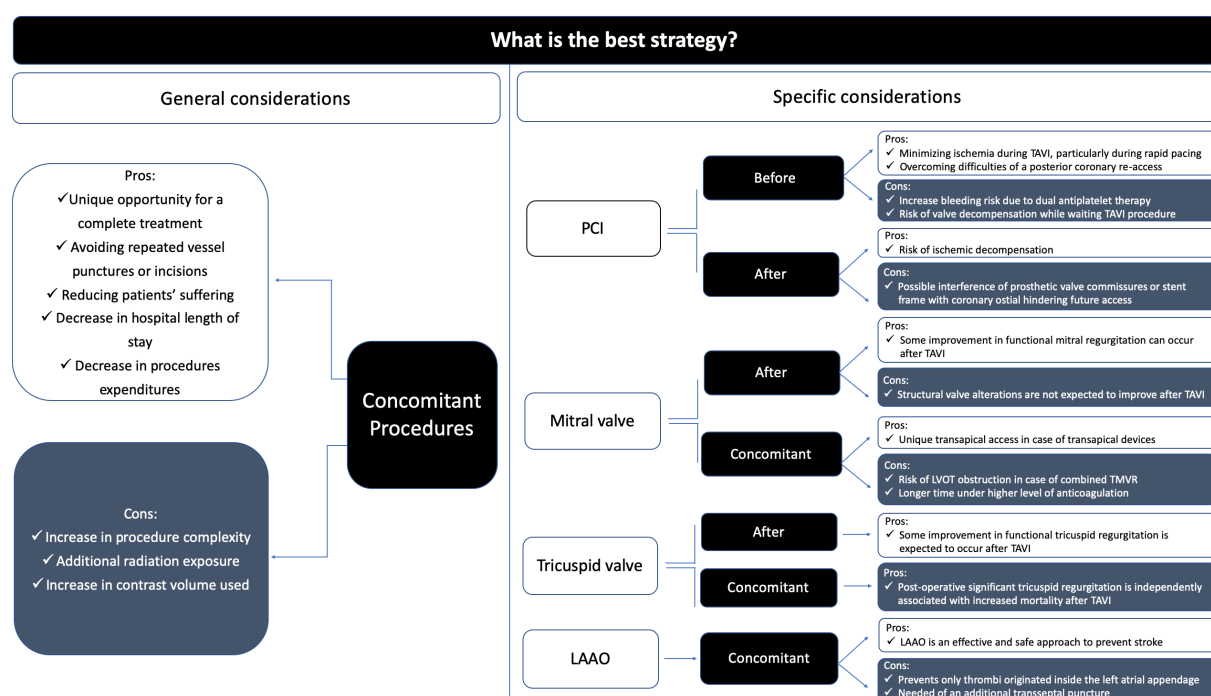


Figure 5. Pros and cons of TAVI combined with other transcatheter interventions. TAVI: transcatheter aortic valve implantation; PCI: percutaneous coronary intervention; LAAO: left atrial appendage occlusion

hospital stay and a reduced need for post-acute rehabilitation services make TAVI a cost-effective approach, particularly when transfemoral access is suitable. In the setting of concomitant functional MR and AS, a cost-effective approach would be to perform TAVI first, followed by the mitral intervention in those who do not experience improvement in MR, reducing, therefore, the number of mitral valve interventions in comparison to routine simultaneous procedures^[28]. On the other hand, concomitant PCI and TAVI have the potential to reduce operational and re-hospitalization costs, and has been considered^[69]. Regarding the other possible combinations, many health insurance companies have the policy of not paying for a combined intervention, which may have an impact on final reimbursement value and somehow discourage concomitant approaches^[70].

CONCLUSION

In this paper, we present an update on the most frequent indications for TAVI combined with other transcatheter procedures, whose pros and cons are summarized in [Figure 5](#).

In patients with concomitant heart disease, such as CAD, mitral or tricuspid valve disease, atrial fibrillation, PFO or ASD, or aorta aneurysm, especially in those not candidates for complex conventional surgery, TAVI combined with other transcatheter interventions offers a unique opportunity for a complete treatment, either by staged or simultaneous interventions.

The decision about which approach (staged *vs.* single session) is more suitable for each patient needs to be based on individual characteristics and clinical, anatomical, and procedure-related factors.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception, design and review of this article: Tagliari AP, Taramasso M

Availability of data and materials

Not applicable.

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Conflicts of interest

Dr. Taramasso is a consultant for Abbott Vascular, Boston Scientific, 4TECH, and CoreMedic; and has received speaker honoraria from Edwards Lifesciences.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Review

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Extracellular vesicles, from pathogenesis to biomarkers: the case for cerebral malaria

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Abstract

Malaria infections due to the *Plasmodium* parasite remains a major global health problem. *Plasmodium falciparum* is responsible for majority of the severe cases, resulting in more than 400,000 deaths *per annum*. Extracellular vesicles (EVs) released by vascular cells, including parasitised erythrocytes, have been detected with increased levels in patients with malaria. EVs are thought to be involved in the pathogenesis of severe malaria, particularly cerebral malaria, and represent a unique molecular signature for different forms of the infection. In this review, we will cover the known effects of EVs on the vasculature and discuss their potential use as a biomarker of disease severity.

Keywords: Cerebral malaria, extracellular vesicles, biomarker, pathogenesis, microvesicles, exosomes

INTRODUCTION TO MALARIA

Malaria can be a life-threatening disease and remains a global health problem with an estimated incidence of 228 million cases and 405,000 deaths in 2018^[1]. While its incidence has decreased significantly in the last 15 years, progress has stalled and case numbers are starting to increase again in some countries with drug resistance a major threat^[1]. *Plasmodium falciparum* (*P. falciparum*) is one of six *Plasmodium* species, all of which can cause disease in humans, and is associated with the development of severe disease.



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Clinically, malaria can be either uncomplicated or severe. Uncomplicated malaria presents as a non-specific, flu-like syndrome and diagnosis is based only on clinical features, which is often unreliable. Approximately 1% of diagnosed cases will progress to severe malaria for reasons that are not fully understood^[2] and amongst these, up to 30% will be at risk of developing life-threatening or debilitating complications^[3]. Severe malaria is defined by precise diagnostic criteria related to specific signs and symptoms, with cerebral malaria (CM) and severe malarial anaemia (SMA) being two of the most serious life-threatening complications associated with *P. falciparum* infection. Both target children under the age of five and although not yet fully understood, the pathogenesis of CM and SMA is likely to be different. The clinical hallmark of CM is a diffuse, symmetrical encephalopathy with coma and a general absence of focal neurological signs. CM is characterised by the sequestration (binding) of infected red blood cells (iRBCs) in the vasculature of most organs, including the brain, coupled with an uncontrolled inflammatory response^[4]. This sequestration of iRBCs during CM is associated with endothelial dysfunction leading to coma, respiratory distress syndrome and placental malaria when it occurs in the brain, lungs or during pregnancy, respectively. SMA is defined by a haemoglobin (Hb) concentration < 5g/dL and a packed cell volume (PCV) < 15% in children, and by Hb < 7g/dL and PCV < 20% in adults^[5]. SMA is also associated with increased clearance of both iRBCs and non-infected red blood cells (nRBCs), as well as altered haematopoiesis^[6-8]. In both CM and SMA cases however, iRBCs remain within the vasculature, adhere to and activate endothelial cells that are then likely to release pathogenic factors into the surrounding tissues such as the brain parenchyma. This review will focus mainly on CM and its association with extracellular vesicles.

PATHOGENESIS OF CM: FROM HOST CELLS TO EXTRACELLULAR VESICLES

As mentioned above, CM is characterised by sequestration of iRBCs within the cerebral vasculature although the neurological lesion extends beyond blood vessel alteration to damage to the brain parenchyma, with clear involvement of the blood-brain barrier (BBB). There is a fine and complex interplay between the cells on each side of the BBB, with vascular cells, (i.e., endothelial cells, platelets, T cell lymphocytes, macrophages and to lesser extent neutrophils), microglial cells, neurones, and astrocytes, all having either target or effector roles (and sometimes both) at some point in disease development^[9]. In addition, extracellular vesicles (EVs) are potentially released by all these cells adding another level of complexity to this intercellular crosstalk. A combination of *ex vivo* studies using patient samples (biological fluids or *post-mortem* tissues), *in vitro* assays mimicking the intravascular lesion, and *in vivo* experiments using mostly murine models allows for a better understanding of the cellular interactions and pathogenesis of the disease.

How much of CM is attributable to the sequestration of iRBCs is still unknown. *Post-mortem* studies have shown various levels of iRBC accumulation within the microvasculature of the brain in patients with diagnosed CM, but this was similarly observed in patients who died of non-CM causes^[10,11]. Of note, this observation is correlated with the severity of the disease in both children and adults^[10,12]. *Post-mortem* histopathology in Malawian children with clinically defined CM (coma and *P. falciparum* parasitaemia) identified different disease patterns: (1) iRBCs sequestration only; (2) iRBCs sequestration with associated peri-vascular changes such as haemorrhages or micro-thrombi; and (3) little to no sequestration^[11]. In the latter, the real cause of death was only identified after autopsy, adding to the complexity of CM and the difficulty in establishing a precise diagnosis. In this study^[11], only fundus examination allowed discrimination between malarial and non-malaria coma. In Vietnamese adults, iRBC sequestration was more frequent in patients with CM than in those without, and was correlated with coma and time of death^[12]. Consequently, vascular congestion was proposed as a cause for coma since sequestration leads to decreased cerebral blood flow, impaired brain function and cerebral hypoxia.

Sequestration occurs via the binding of parasite-related ligands, expressed on the surface of iRBCs, to receptors on the surface of vascular endothelial cells. *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is one such molecule expressed on the surface of iRBCs that then binds to a series of endothelial receptors such as CD36, intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, P-selectin, E-selectin, endothelial protein C receptor or thrombospondin. The expression of these receptors is further modulated by pro-inflammatory cytokines such as tumour necrosis factor (TNF) or interferon-gamma (IFN- γ), thereby supporting inflammation as a critical player in the regulation of sequestration^[13,14].

Together with iRBCs and nRBCs, platelets also play an important role in CM^[15]. Thrombocytopenia is a hallmark of CM but whether platelet counts can be predictive of lethality in CM is still controversial^[16,17]. Platelets were found in high numbers in vascular lesions of the brain of Malawian children who succumbed to CM^[18] and are thought to have contributed to the severity of the disease through clumping^[19], activation of endothelial cells^[20] or increased sequestration via the transfer of CD36 to brain endothelial cells that are otherwise devoid of it^[21]. On the other hand, platelets also are thought to have a protective role during CM by killing intra-erythrocytic parasites^[22,23]. Therefore, platelets could have different roles at different stages of the disease, i.e., a protective role during the early phase of disease and a pathogenic role when severe^[24].

The BBB is at the centre of the neurovascular lesion occurring in CM although iRBCs do not actively cross this barrier as seen in other pathogens with brain tropism^[25,9]. *Post-mortem* histopathological brain studies have demonstrated impairment of the BBB which suggests that the localised sequestration of iRBCs increases the pressure within microvessels to act on cellular tight junctions, thereby altering the permeability of the BBB which results in micro-haemorrhages when these junctions rupture. Neurological sequelae observed in children who have recovered from CM are also suggestive of neuronal damage^[26]. Neuronal dysfunction is likely an indirect consequence of the sequestration of iRBCs, activation of the endothelium, alteration of junctional permeability and passage of cytokines, chemokines and other inflammatory mediators into the perivascular space^[27,28]. However, in most cases, it is likely that these alterations are localised, as symptoms are quickly reversed once parasites have been eliminated. More recently, Magnetic Resonance Imaging has been successfully used as a non-invasive way to predict fatal outcomes in paediatric CM, notably in Malawi^[29,30].

As mentioned earlier, crosstalk between vascular cells, including immune and brain parenchyma cells, via direct contact, soluble mediators and molecules leaking through the BBB, can all contribute to the neurological syndrome. In addition, subjecting all these cells to various stimuli can lead to the release of EVs that in turn, target other cells distant from their site of production. Long considered as inert cellular debris, EVs are now accepted as biological effectors in many infectious and inflammatory diseases including malaria^[31,32].

EVs represent an ensemble of membrane-bound structures grouped into three main categories: exosomes, microvesicles, and apoptotic bodies. This nomenclature can vary and the term EVs usually encompasses subpopulations of vesicles ranging in size from 30 nm to 4 μ m, i.e., exosomes produced by membrane invagination of multivesicular bodies, microvesicles (MVs) released after budding of the plasma membrane, or apoptotic bodies that result from blebbing of the plasma membrane of apoptotic cells^[33]. It is now clear that the role of EVs goes far beyond simple structural function to active mediators of important biological processes for parasitic infections such as immunomodulation, parasite virulence, target cell invasion and parasite-parasite communication^[34,35].

In malaria infection, two categories of EVs (i.e., exosomes and microvesicles) have been studied the most. While known in other illnesses for several decades, MVs in malaria patients were first described by our

group in 2004 in Malawian children with CM^[36], where an elevation in the number of MVs of endothelial origin was described. MVs released by RBCs were later found to be increased in both *P. falciparum* and *P. vivax* malaria^[37,38]. EVs have been shown to be involved throughout the entire life-cycle of malaria infection and at different stages, the parasite can affect various immune and vascular cell types in different ways, ultimately altering the endothelium and BBB function^[32].

A pan-vascular, cell-derived MV release was also observed in children with CM in Cameroon. Of these MVs, an increase in platelet MVs was most significantly correlated with disease severity^[39]. Exosomes were first explored in 2011 in a murine model of malaria^[40] and will be discussed in a later section. While mostly descriptive, these clinical studies were essential for suggesting a role for these EVs as either markers of CM severity, or as players in the pathogenesis of CM infection, and paved the way for subsequent work on the composition and functional potential of EVs during malaria infection.

IN VITRO MODELS OF MALARIA - INTERACTIONS BETWEEN HOST CELLS AND EXTRACELLULAR VESICLES

Most *in vitro* models of CM simulate the interactions between microvascular endothelial cells and circulating vascular cells (e.g., iRBCs, nRBCs, platelets, and leucocytes) in either static or shear stress environments^[41,21]. The brain endothelial cells used can be of human, simian or murine origin (primary or immortalised), and co-cultured with one or more other cell types in two-dimensional systems^[42-45]. The recent introduction of more complex three-dimensional models will help to examine and understand the pathogenesis of this disease better^[46-48].

Very much like their cells of origin, EVs interact with their target cells and modulate their responses. *In vitro*, platelet MVs behave in a similar fashion as platelets by increasing the adherence of iRBCs to human brain endothelial cells (HBECs) by providing iRBCs with surface receptors such as CD31 and CD36^[49] such that platelet MVs act as a bridge between HBECs and iRBCs. The internalisation of platelet MVs by vascular endothelial cells is also associated with an alteration of their phenotype such that ultimately, their inflammatory effects and subsequent activation can be potentiated^[50].

RBCs release increased levels of EVs when infected with a *Plasmodium* parasite and late-stage infections are associated with even greater release of EVs^[38]. This is mainly due to membrane changes occurring within iRBCs during parasite maturation. The composition of EVs derived from iRBCs is also dependent on the parasite's stage of development. Indeed, specific parasite proteins, considered as virulence factors, were present in EVs only at specific developmental stages and PfEMP1 was only detected in EVs from iRBCs with parasites at early stages. Potentially, such developments would allow EVs to bind and prime endothelial cells for later adherence and sequestration of late-stage iRBCs^[51].

EVs from iRBCs have also been shown to contain a functional microRNA-argonaute 2 complex that can modulate gene expression and alter barrier function^[52,53] when transferred to endothelial cells after vesicle uptake. Such EVs do not only affect endothelial cells but are also able to induce pro-inflammatory responses, particularly the activation of macrophages, monocytes as well as other immune cells through the upregulation of cytokines^[54,55]. Interestingly, when these EVs were compared to their mother cells, they were able to activate inflammation and immune activation to a greater degree^[54,55]. EVs from iRBCs also contain small RNAs and genomic DNA. After internalisation by monocytes, they can induce the innate immune cytosolic adaptor-dependent DNA sensing pathway (STING), leading to downstream alterations of DNA sensing pathways in target cells^[56]. Activation of these pathways has been shown to correlate with parasite survival^[57]. Thus, this could possibly be used as a decoy method for immune escape by the parasites^[57]. Similarly, the release of PfEMP1-containing EVs as previously mentioned, has also been suggested as a

decoy strategy as it is capable of inducing both the production of inflammatory cytokines (IL-12, CCL2, and CCL4) by monocytes after internalisation and transcriptomic changes^[51]. Furthermore, as the majority of the body's natural immune response to *P. falciparum* targets PfEMP1, the secretion of PfEMP1-containing EVs could possibly work as a smokescreen by attracting neutralising antibodies that protect the parasite from the immune system^[13,51]. EVs can also mediate immunosuppression in mice infected with malaria with EVs from *P. berghei*-iRBCs able to inhibit CD4⁺ T cell proliferation in response to antigen presentation. This process seems to be mediated by two potential virulence factors, histamine-releasing factor and elongation factor 1 α (EF-1 α). Importantly, this work also showed that mice immunisation with EVs from *P. berghei*-iRBCs or recombinant *P. berghei*-EF-1 α resulted in resistance to infection, further suggesting the role of EVs in immune-modulation and potential for vaccine development^[58].

Exosome-like vesicles derived from iRBCs have been reported to facilitate communication between iRBCs and therefore, promoting gametocytogenesis between parasites *in vitro* via the transfer of a *P. falciparum* protein^[59]. This communication is also used to improve parasite survival within the host as well as transmission to mosquitoes.

Although not specifically studied in an *in vitro* model of CM, endothelial MVs interacting with T lymphocytes have been found to assist cell proliferation by inducing cell activation and antigen presentation by immune cells^[60]. In addition, when MVs from lipopolysaccharide-stimulated monocytes are internalised by HBECs, they release high levels of MVs (usually a sign of cell activation) and at the same time, display an increase in trans-endothelial resistance (i.e., tightening of endothelial junctions) which could have a protective effect on the BBB if occurring *in vivo*. This suggests that MVs from monocytes, as was shown for MVs from neutrophils, could trigger contrasting protective and pathogenic responses^[61-63].

In vitro models of malaria are limited in their ability to mirror the pathogenesis of CM and more complex systems are needed to understand the fine interplay between host cells and EVs during malaria infection.

IN VIVO MODELS OF MALARIA: WHAT DO EXTRACELLULAR VESICLES BRING TO PATHOGENESIS?

Although there is still debate regarding the usefulness of murine models for studying the pathogenesis of CM, human studies are limited and often, *post-mortem* analyses are the only way to explore some parameters. However, the number of studies that find parallels between human and experimental CM (ECM) continues to grow. Most recently a study^[64] observed that in *post-mortem* cases of paediatric CM, CD8⁺ T cells were found within both the vascular lumen as well as the juxtavascular space as was previously shown in murine CM studies^[65,66]. Therefore, animal models can still provide relevant basic scientific knowledge and allow testing of important hypotheses related to the pathogenesis of the disease^[67-71]. For instance, whole-animal imaging using transgenic fluorescent parasites has demonstrated that sequestration, and not only accumulation, of iRBCs, does occur in all organs similar to humans^[72-74]. In addition, recent quantitative mapping of mice brains during ECM showed similar numbers compared to human CM despite the distinct aetiology^[75]. However, as for any model, it is not perfect and should be used with caution and one should be aware of its limitations before drawing direct conclusions with human disease.

Two different *Plasmodium* species are commonly used in CM models, *P. yoelii*^[40] and *P. berghei*^[76], notably *P. berghei* ANKA (PbA). During the acute phase of infection, mouse strains that are susceptible to CM (e.g., CBA/J, C57BL/6, DBA1) display increased levels of plasma MVs similar to that observed in humans^[76-78]. We examined the ATP-binding cassette transporter A1, which modulates the distribution of phosphatidylserine to the outer leaflet of the cell plasma membrane at the time of MV production^[76]. We found that mice lacking this ATP-binding cassette had resistance to the malaria-associated neurological

syndrome in C57BL/6 mice^[76]. These animals displayed basal levels of plasma MVs but numbers failed to increase following infection (as observed in wild type counterparts), had lower levels of plasma TNF, reduced expression of endothelial cell adhesion molecules and had increased survival of leukocytes and platelets. Another study examining the blocking of phosphatidylserine using low-molecular-weight thiol pantethine found a similar reduction in MV production, which correlated with reduction in inflammation and resistance to the disease^[78]. When passively transferred into the circulation of mice, plasma MVs from infected animals localised to the inflamed vessels of infected animals, notably in the brain, which suggests that they could potentiate the neurovascular lesions by interacting with other vascular cells. In addition, healthy mice injected with TNF-generated endothelial MVs developed CM-like pathology with cerebral and pulmonary oedema and haemorrhage, the two main histopathological features of human and murine CM^[77,79,80].

Interaction with and internalisation of MVs derived from iRBCs by astrocytes and microglial cells leads to increased production of IFN- γ -inducible protein 10 (IP-10), which coincided with increased levels of inflammatory cytokines within both plasma and brain tissue of PbA-infected mice^[81]. Plasma MVs from PbA-infected mice were also able to activate immune cells, in particular macrophages, leading to the up-regulation of CD40 as well as TNF production^[54]. Knock-out mice that lack pro-inflammatory cytokines (TNF^{-/-}, IFN- γ ^{-/-}, IL-12^{-/-} and RAG-1^{-/-}) displayed levels of plasma MVs similar to those of their wild-type counterparts^[54], suggesting that their production is not solely dependent on the presence of inflammation.

When exosomes, purified from the blood of *P. yoelii* (nonlethal strain, 17XNL)-infected mice, were injected into mice infected with *P. yoelii* (lethal strain, 17XL), these mice were protected against the lethal syndrome, showing that exosomes could also modulate the immune response^[82,40]. We propose a model in [Figure 1](#) that summarises the role EVs play in the pathogenesis of CM.

[Figure 1](#) was designed by modifying free images provided by Smart Servier Medical Art (<https://smart.servier.com/>) and available under the creative commons license.

EXTRACELLULAR VESICLE CARGO: EFFECTOR, BIOMARKER OR BOTH?

Biomarkers have long been used as diagnostic and prognostic tools to determine the presence of disease as well as the regression, progression or outcome after treatment^[85]. The field of malaria, especially severe malaria, currently lacks reliable markers for the prediction of morbidities such as neurocognitive impairment and/or mortality that can be widely applicable regardless of country or endemicity. Such biomarkers would allow for the prediction of severe complications and allow early implementation of adjunctive therapies. Current adjunctive therapies used to aid anti-malarial drugs have so far been ineffective^[86], possibly due to late implementation due to the lack of predictive biomarkers. Therefore, early identification of patients at risk of severe malaria complications (lethal or not) would allow for prompt treatment and potentially decrease the risk of long-term disabilities.

Currently, only a handful of candidate markers have been identified for severe malaria, though they are not fully reliable. The proposed molecular markers include erythropoietin, angiopoietin 2, von Willebrand factor, *P. falciparum* histidine-rich protein 2 and ICAM-1 which, although indicative of severe disease when elevated, still has limited clinical utility. This is mainly due to the highly variable sensitivity and specificity for the detection of severe CM^[41,87-90]. It is now clear that EVs and their cargo have potential as biomarkers. Their elevated numbers, notably endothelial-, platelet- and RBC-derived MVs, in the circulation of human patients with CM has already been proven in multiple studies^[39,84,91]. The role of EVs as a biomarker in severe malaria is still in its infancy and in-depth multi-centre studies are still needed to ascertain their predictive value to improve rapid detection in bodily fluids. In addition, although blood, urine, and saliva have all been used for diagnostics^[92,93], urine and saliva have not been investigated in malaria but we

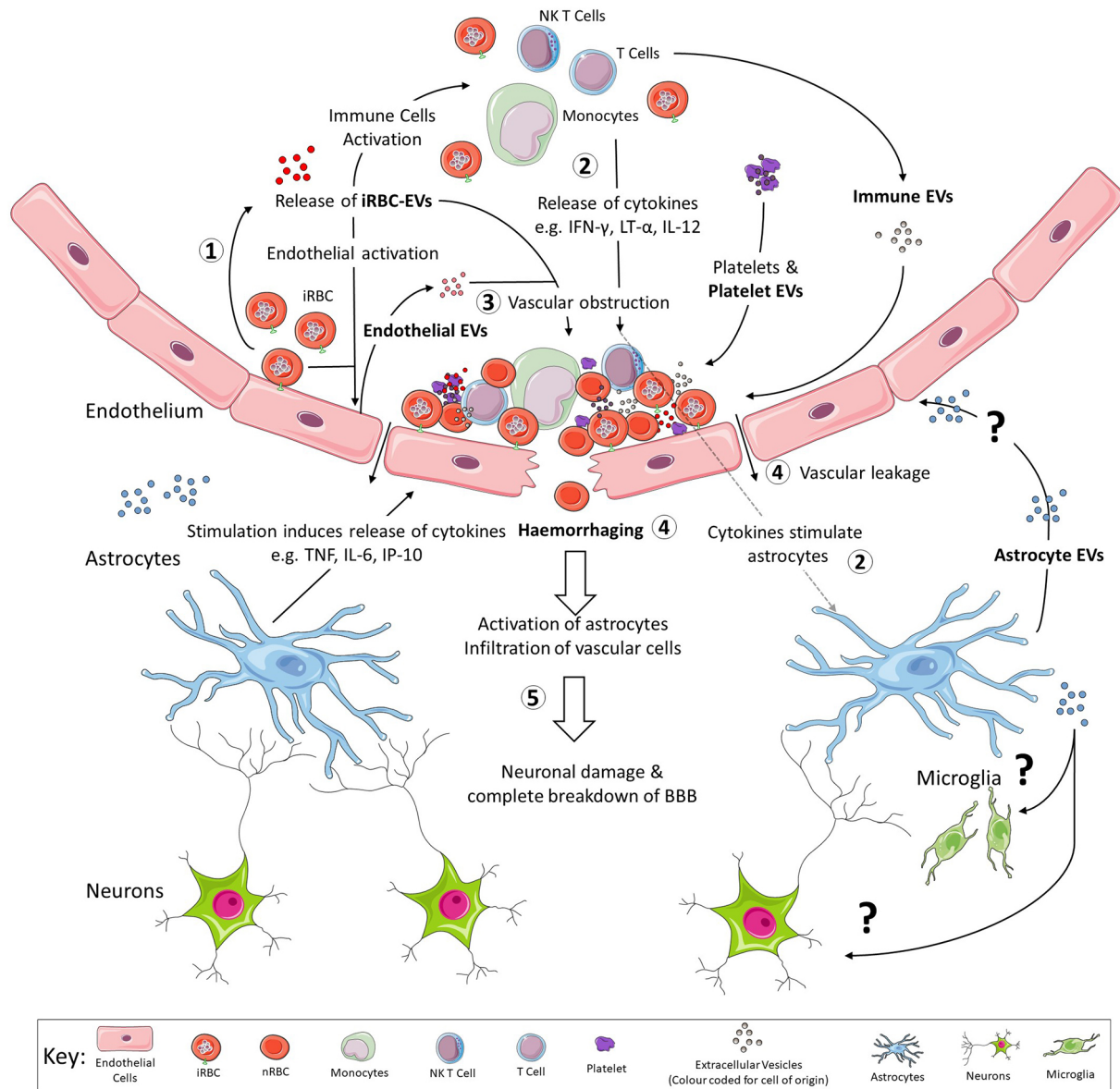


Figure 1. Outline of the role that EVs play in the pathogenesis of cerebral malaria. (1) As iRBCs adhere to vascular ECs, they are also releasing EVs into the blood. These EVs from iRBCs stimulate all vascular cells including immune cells, ECs and platelets to release EVs of their own^[83] as reviewed by Babatunde *et al.*^[84]. Both ECs and platelet EVs have been shown to assist with the formation of neurovascular lesions by providing mechanisms of binding for iRBCs and other vascular cells as reviewed by El-Assaad *et al.*^[77], 2014 and Faille *et al.*^[49]. (2) EVs from iRBCs have been suggested to act not only as a decoy, by providing alternative targets expressing PfEMP-1 for immune cells to attack^[51], but also promote secretion of increased levels of pro-inflammatory cytokines, notably IFN- γ , LT- α and IL-12 once internalised by immune cells^[54,55]. These cytokines have also been shown to stimulate astrocytes, which then respond by secreting additional cytokines and chemokines of their own^[13]. The effect of astrocyte EVs have not been studied in humans but these EVs could have effects on both endothelial cells and cells of the brain parenchyma. (3) The release of these pro-inflammatory cytokines and chemokines further activates the already stimulated ECs, leading to greater adherence of vascular cells and ultimately, formation of the neurovascular lesion. (4) Once the neurovascular lesion has formed, increased intravascular pressure on the endothelium leads to vascular leakage and ultimately results in haemorrhage. (5) The infiltration of vascular cells and cytokines or chemokines causes neuronal damage and subsequently, a localised breakdown of the blood brain barrier^[32]. EVs: extracellular vesicles; ECs: endothelial cells; iRBCs: infected red blood cells; nRBC: non-infected red blood cell; BBB: blood-brain barrier; TNF: tumour necrosis factor; IFN- γ : interferon-gamma; NK: natural killer; LT- α : lymphotoxin alpha; IL: Interleukin; IP-10: IFN- γ -inducible protein 10

cannot exclude the possibility of them becoming a source of biomarkers to assess disease severity in the future. As mentioned above, all current markers have their limitations and one could hypothesise that the

combination of these existing biomarkers with newly discovered EV-associated markers could significantly improve both the specificity and sensitivity of testing.

As a consequence of their biogenesis, EVs harbour a peculiar set of proteins, nucleic acids, and lipids that can be transferred from a parent to recipient cells, rendering these sub-micron structures unique sources and vehicles of biomarkers. Various analytical approaches including proteomics, transcriptomics, and metabolomics, although mostly focused on cancer-related conditions, are currently employed to study the content of EVs derived from different cell types and bodily fluids^[94]. Nonetheless, the cargo of EVs is now becoming an important research topic in severe malaria allowing us to both understand disease pathogenesis and identify novel biomarkers, with proteins and microRNA (miRNA) being the most studied components of this cargo.

EV-associated proteins can typically be studied using either untargeted proteomics, to characterise the whole protein content, or through a hypothesis-driven targeted approach, to investigate individual proteins or a selected set of proteins based on previous evidence. Compared to other parasitic diseases, high-throughput untargeted proteomics - the leading technique for the discovery of new protein markers - has not been widely applied to investigate malaria-associated EVs yet, but has been explored in the last couple of years. The first report dates back to 2011 when exosomes from *P. yoelii*-infected BALB/c mice were analysed and revealed to contain both classical exosomal markers as well as parasite proteins^[40]. Interestingly, 30 parasite proteins belonging to two major classes, proteins associated with RBCs membrane and proteins involved in parasite invasion into RBCs, were identified within iRBCs exosomes. Then, the presence of *Plasmodium* proteins within EVs from human and mice malaria infection was confirmed by a number of proteomics-based studies^[40,51,58,82,95]. Although the majority of these studies did not have as their main objective the identification of biomarkers, they all contributed to prove the presence of parasite-derived proteins with antigenic and immunomodulatory properties, or as potential virulence factors within EVs that, in the future, might be found useful for the development of novel diagnostic and prognostic tests.

Only a few studies have focused on EVs as a novel source of markers for severe malaria. In our group, we used high-throughput proteomics to characterise the protein cargo of MVs released during ECM in *P. berghei* infected mice^[96]. The vast majority of identified proteins were host-derived and only a couple were from *P. berghei*. The protein content of MVs released during severe disease was significantly altered compared to that released upon early infection or in uninfected mice. Network analysis showed that proteins with altered abundance during ECM were associated with CM pathogenesis. Two of these proteins, carbonic anhydrase I and S100A8 were verified to be associated with CM MV in both murine and clinical samples, highlighting the importance of MV protein content to understand the role of EVs both in severe malaria and as a source of protein markers^[96]. The protein cargo of MVs obtained from *P. falciparum*-infected individuals was later investigated by Antwi-Baffour and colleagues^[97], although cases with severe malaria were not investigated. The study identified several different host-derived proteins in infected and non-infected human subjects, as well as parasite-derived proteins in infected samples. Nonetheless, the results remained primarily descriptive and no diagnostic marker was actually proposed. More recently, proteomics was applied to identify novel potential biomarkers of *P. vivax* liver stage infection^[98]. By taking advantage of a human liver-chimeric mouse model, plasma EVs obtained after *P. vivax* infection were studied to identify potential liver-stage expressed parasite proteins that could be indicative of infection. Among mouse and human proteins, they also identified parasite proteins showing variable distribution in abundance over different time points post-infection, indicating that parasite proteins contained within EVs vary with parasite developmental stages, supporting their potential role as a source of biomarkers^[98]. In mice and human studies, there has been a consistent indication of EVs' importance in the role of malaria pathogenesis and their potential as markers for disease severity; however, more research is required to confirm the potential of these EVs derived protein as biomarkers for severe malaria.

Within the groups of non-coding RNAs, miRNA are now considered promising biomarkers in many pathological conditions^[99,100] due to their stability in various bodily fluids such as saliva, serum, plasma and CSF, and their role in gene expression regulation^[101-106]. One of the advantages of studying EV-associated miRNAs is their particular stability as they are protected within a plasma membrane^[107]. These short, non-coding RNA molecules display critical regulatory functions as they are involved in nearly all physiological processes such as cellular differentiation, proliferation, metabolism, development, and homeostasis^[108,109].

As previously mentioned, a large portion of EV miRNA studies are focused on cancers, which have shown the significance of identifying cargo miRNA. In a 2019 clinical cancer study, miRNA from whole plasma, EVs and EV-free plasma from lung adenocarcinoma and granuloma patients were evaluated. The study determined that whole plasma, EVs and EV-free plasma had differing miRNA expression profiles and the prediction performance of EVs was better than EV-free plasma. Plasma was the best predictor however, due to the lack of knowledge in storage and processing techniques of EVs^[110]. Elevated levels of plasma EVs have since been observed in patients affected by various forms of cancer compared to healthy subjects and, interestingly, these levels decreased upon removal of the tumour, simultaneously decreasing tumour specific miRNA profiles within the plasma EVs^[111,112], which provides a further link between cancer and increased EV production. Similar results were demonstrated in patients with autoimmune, infectious and cardiovascular diseases, and neurological disorders^[113-116].

Next-generation sequencing technology is the recommended, standard approach when investigating the miRNA content of EVs for novel biomarker identification^[117]. Accumulated sequencing data suggest the potential for miRNAs as diagnostic and prognostic markers, as well as for parasitic diseases caused by platyhelminths, arthropods, and protozoa, including *Plasmodium spp.*^[118]. When analysing EVs derived from helminth parasites (*Trichuris muris*), the content was sequenced using the HiSeq 500 system, identifying 56 miRNA, 22 of which were novel^[119]. A similar study looking at hookworms using the NextSeq 500 system identified 52 miRNA, many of which were found to be involved in inflammation regulation when mapped to mouse genes^[120].

One of the first studies on miRNA in *Plasmodium* infection suggested that this parasite did not have specific miRNAs but rather, takes advantage of the transcriptional machinery within RBCs for the activation and suppression of gene expression^[121]. This study also identified miR-451 as highly expressed in iRBCs, although its accumulation was not associated with malaria infection^[121].

A study from Thailand observed, for the first time, lower expression of miR-451 and miR-16 in the plasma of adults infected with malaria, suggesting their role as biomarkers for malaria infection, especially in *Plasmodium vivax* infected individuals^[122]. However, a large portion of transcriptomic studies have been performed using murine models focusing on the host's response to infection^[123-125], or using *in vitro* systems to target a specific cell-type. For instance, let-7i, miR-27a, and miR-150 were found to be over-expressed in the brain of CM-mice but not in non-CM animals^[124]. Overexpression of these miRNA during infection may be essential for the instigation of neurological syndromes by regulating their downstream targets, thus having a potential regulatory role in the pathogenesis of severe malaria, as well as being targets for therapeutic intervention^[124].

Similar to iRBCs, EVs from iRBCs display higher levels of miR-451a and let-7b when compared to nRBCs, and once miR-451a within EVs is engulfed by endothelial cells, their gene expression and barrier properties are affected, which may then lead to vascular dysfunction, making the miRNA a possible target for therapeutic intervention^[52]. Using a more complex model, our group analysed plasma EVs from mice with CM and found that the miRNAs from malaria EVs played a regulatory role in severe malaria pathogenesis. miR-146a levels were higher and miR-193b levels lower in plasma-derived EVs while miR-205, miR-215,

and miR-467a were all elevated in brain tissue from CM mice when compared to non-infected or non-CM infected mice^[126]. This difference in miRNA profiles suggests that miRNA present in circulation could have different functions from those present in tissues. Further investigation to verify the potential of these EVs derived miRNA as biomarkers for the cerebral syndrome using both experimental models and clinical samples will be necessary.

CONCLUSION

In vivo and *ex vivo* studies point towards a role for EVs in the modulation of disease and the host response. No study has looked at the behaviour of EVs *in situ* however. Rather than passively transferred EVs, animal models utilising both transgenic parasites and transgenic host cells expressing tags that can be traced, combined with high-resolution imaging in the animal, will allow us to truly understand the complex involvement of EVs with their target cells. For instance, recent work used high-resolution microscopy to visualise circulating EVs in zebrafish embryos using a tissue-specific expression of genetically encoded markers of EVs. This approach will allow us to not only decipher the role of EVs in physiology including cargo delivery but also, to assess the effects of disease or treatment on EVs release and function^[127].

In addition, although evidence confirming the importance of EVs as a source of biomarkers are scattered, they also highlight a number of questions and unsolved problems. Indeed, most of the work performed so far still lack validation steps and clinical studies remain scarce. These limitations are such that most studies remain mainly descriptive and hamper the process of biomarker validation and implementation into clinical practice. In-depth investigations should also be carried out to understand the mechanisms of protein and miRNA packaging into EVs, as well as the signals involved in cell targeting. Deciphering these processes will contribute to the selection of highly specific biomarkers for larger validation studies. While biomarker studies applied to severe malaria and EVs are still in their infancy, there is hope for this field to provide novel strategies to fight severe malaria in the future.

DECLARATIONS

Authors' contributions

Conceived and designed the review: Cheng IS, Sealy BC, Tiberti N, Combes V

Made equal contribution to the writing of the sections: Cheng IS, Sealy BC

Provided feedback for manuscript revision: Cheng IS, Sealy BC

Read and approved the final manuscript: Cheng IS, Sealy BC, Tiberti N, Combes V

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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Editorial

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Atherogenesis, atherosclerosis and related diseases: unresolved issues

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Atherosclerosis is highly prevalent and affects most of the elderly population. It often develops in parallel with type 2 diabetes mellitus, metabolic syndrome, obesity and an excessive body mass, all of which worsen a patient's comorbidities. The clustering of these pathologies, clinical manifestations of atherosclerosis and conventional cardiovascular risk factors is often observed in tandem in epidemiological and clinical studies. While isolated risk factors can account for risk variability, the combination of several risk factors translates to a highly unfavorable risk ratio. This concept of the accrual of several risk factors supports the association between atherosclerotic disease and cardiometabolic abnormalities. On the other hand, this concept also suggests that underlying genetic and metabolic mechanisms may either be similar or different, and can therefore be subdivided into common and disease-specific risk factors. Common risk factors may be partially explained by one's genetic background, but the role of genetic factors in the clustering of risk factors in individuals remains unclear. Uncertainty over the common pathogenetic mechanisms, complexity of phenotypes, and the biases from lifestyle factors and therapeutic intervention give rise to the need for further discussion on unresolved issues in atherogenesis, atherosclerosis and related diseases.

Inspired by the opportunity to review and consolidate results from recent findings in the field of vascular remodeling, we have launched this special issue of "Vessel Plus". We aimed to gather the latest research from both basic science and clinical investigations on the pathogenesis of atherosclerosis-related diseases and metabolic pathologies, and molecular pathways for further development of targeted therapy.

It is notable that atherosclerosis is the common denominator and characteristic of the above mentioned pathologies. Atherosclerosis is also either the cause of, or sequelae from these pathologies. Therefore, studies on the molecular and cellular mechanisms of atherogenesis are of utmost importance. Over



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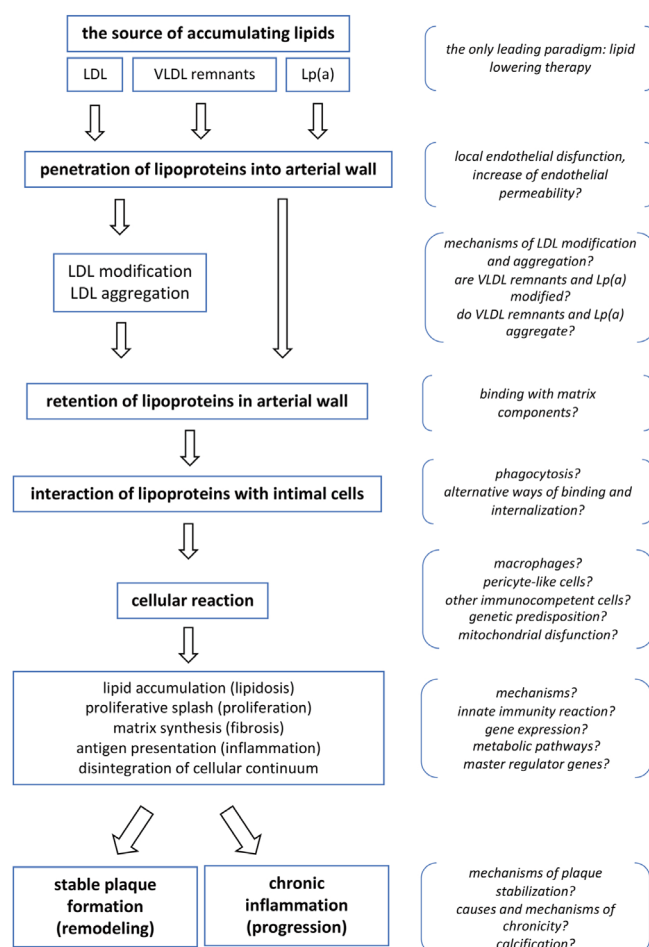


Figure 1. Current concepts of atherogenesis: schematic outline of key events and unresolved issues. LDL: low density lipoprotein; VLDL: very low density lipoprotein; Lp(a): lipoprotein (a)

the last few decades, progress in the reduction of cardiovascular mortality has been achieved mainly due to successful cardiovascular surgery and healthier lifestyles, which suggests that the battle against atherosclerotic disease has been won. However, the reduced role of cardiovascular disease in overall mortality is observed only in several developed countries. Globally however, atherosclerosis and related diseases still remain as one of the leading causes of mortality. It is true that patients have gained additional years of life from surgery and lipid-lowering medications, but did the underlying atherosclerosis resolve? Have the cellular and molecular mechanisms of atherogenesis been altered? Was there progress in the development of new strategies in anti-atherosclerotic treatment? No. Thus, it is time to discuss both the novel and debatable aspects of atherosclerosis and atherogenesis, with due consideration for the latest developments in molecular and cellular biology in vascular medicine.

Atherosclerosis can generally be described as an excessive fibro-fatty, proliferative and inflammatory response to arterial wall damage and involves several cell types such as monocyte-derived macrophages, smooth muscle cells, dendritic cells, lymphocytes and platelets^[1,2]. It is well known that at the level of the arterial wall, the deposition of intracellular cholesterol and foam cell formation are the typical features of early atherosclerosis^[3,4]. Current understanding of the development of early atherosclerotic lesions is shown in Figure 1. In brief, circulating lipoproteins - low density lipoprotein (LDL), the remnants of very low density lipoproteins (VLDL remnants) and lipoprotein(a) - serve as sources of lipids which can accumulate further in the arterial wall^[4-8]. After penetrating the subendothelial intima, lipoproteins may induce lipid

accumulation in cells as the initial and key step in the formation of atherosclerosis lesion^[9,10]. However, LDL must first undergo proatherogenic modification, rendering it atherogenic i.e. capable of inducing intracellular lipid deposition^[11-14] and this requires interactions between lipoproteins and connective tissue matrix components of the intima. In turn, this leads to retention of lipoproteins in the arterial intima, which increases the possibility of further interaction with intimal cells^[15,16] to undergo complex cellular reactions such as the binding and internalization of modified lipoproteins, and abnormal intracellular processing, culminating in foam cell formation^[4,10,17,18]. In parallel, intimal cells also exhibit a proliferative burst, increased synthesis of proteins and the components of connective tissue matrix, a pro-inflammatory response with the synthesis and secretion of cytokines, and the presentation of bound lipoproteins as autoantigens^[4,19]. Thus, all the major characteristics of early atherosclerosis (lipidosis, fibrosis, proliferation and inflammation) are demonstrated at the cellular level at this stage of atherogenesis. In cases of relatively successful resolution of the cellular reaction to pathogenic lipoproteins, early lesions may either undergo spontaneous regression, or transform into stable atherosclerotic plaques. Such a result should be considered as compensatory arterial remodeling. On the other hand, an adverse outcome is characterized by a chronic inflammatory response, recruitment of immunocompetent cells from the circulation, and a vicious cycle of lipid accumulation in the arterial wall^[20-22]. This unfavorable result will lead to growth of the atherosclerotic plaque, development of new lesions and plaque instability that will in turn, manifest as a clinical event.

Certainly, such schema is rather straightforward and oversimplified. It does not account for some mechanisms, such as the effectiveness of reverse cholesterol transport, high density lipoprotein (HDL) functioning, immunogenicity of modified LDL, the formation of atherogenic LDL-containing immune complexes, thrombotic events, *etc.*, all of which have a role in atherogenesis. However, this scheme allows to demonstrate the unresolved issues with our knowledge on the molecular and cellular mechanisms of atherogenesis, i.e. those “white spots”, which obviously need further in-depth investigations.

The only existing paradigm in the prevention and treatment of atherosclerosis is extensive lipid lowering. This idea is based on the role of circulating lipoproteins as the source of cholesterol and as a key player in initializing atherogenesis. Thus, all current medical approaches are based on improving the lipid profile of blood plasma (lowering LDL cholesterol and triglycerides and increasing HDL cholesterol) and aims to eliminate major lipid risk factors for atherosclerosis. The development of alternative approaches for anti-atherosclerotic therapy through the targeting of other key pathogenetic mechanisms is extremely difficult due to the lack of fundamental knowledge on potential molecular and cellular targets for therapy and prevention. Among the latter are endothelial dysfunction and local violation of the permeability of the endothelial barrier, atherogenic modification of lipoproteins, retention of lipoproteins in the subendothelial intima of the arteries, alternative pathways of lipoprotein uptake by intimal cells, fibrotic and proliferative responses of intimal cells to modified lipoproteins, specificity of the reaction of different cells populating the intimal layer (smooth muscle cells, pericyte-like cells, macrophages, lymphocytes, other cells that migrated from circulation), the development of a local inflammatory reaction, ineffective resolution of local inflammation, local reaction of innate immunity, mitochondrial dysfunction, and the mechanisms for stabilization of atherosclerotic plaque and remodeling, *etc.* [Figure 1].

One of my own research interests is the mitochondrial genetics of atherosclerosis. There are several reasons for considering mutations occurring in mitochondrial DNA (mtDNA) as the mechanistic factor involved in atherogenesis. Atherosclerosis may be considered as an age-related degenerative pathology, accompanied by cell senescence which is generally characterized by reduced cell proliferation, irreversible growth arrest and apoptosis, epigenetic modifications, shortening of telomere length, increased mtDNA damage, and mitochondrial dysfunction^[23]. The structural alterations of mitochondria and mtDNA damage are the most evident signs of mitochondrial aging^[24]. Mutations of the mitochondrial genome can lead to structural defects in some energy-generating enzymes and transfer RNAs (tRNAs) synthesized directly in the

mitochondria, thus playing a pathogenic role in the formation of atherosclerotic lesions. The decrease in concentration of these enzymes and tRNAs in mitochondria, and the resulting mitochondrial dysfunction contributes to oxidative stress, deterioration of ATP production and acceleration of atherogenesis^[25].

Recently we have performed a series of studies on the relationships between (1) mtDNA variability and the changes in cellular composition of arterial atherosclerotic intima and the expression of apoptosis- and inflammation-related genes; (2) mtDNA variants, carotid atherosclerosis and conventional cardiovascular risk factors; and (3) individual mtDNA mutation burden and functional activity of cells in cell culture studies. The results of these studies strongly support the hypothesis on the atherogenic role of mtDNA mutations^[26,27]. In further studies, we aimed to create cell models that reproduce the pathological cellular atherosclerotic phenotype with the use of promising approaches such as cytoplasmic hybrids (cybrids). At this stage of our studies, we have demonstrated that cybrid cells obtained from the homogeneous THP-1 line acquire completely different functional properties, and these changes were due solely to the functional activity of the donor mitochondria and the properties and mutational load of the introduced donor's mtDNA^[28,29]. However, the effects observed in cybrid cell lines are dependent not on some specific mtDNA variant, but on a unique combination of variants due to extremely high individual variation. Therefore, we needed more precise cellular models to investigate the intrinsic molecular mechanisms, which may be involved in the formation of the atherosclerotic phenotype due to mtDNA mutations; the use of direct mtDNA editing seemed plausible. We have launched a new research project, which would consistently carry out the design of liposomal delivery of nucleic acids and antisense RNA into cells and further into mitochondria, the delivery of CAS9 nuclease, sgRNA and ssODN recombination matrix, for the implementation of point mutations in mitochondrial genes^[30]. This approach was approved and supported by the Russian Science Foundation. Currently, we have developed cationic liposomes with different addressable modules, evaluated the ability to deliver DNA and to transfect a vector expressing GFP into cells, developed the specific vector for introducing double-stranded breaks in mitochondrial genes, and for visualization of CAS9 localization in mitochondria^[30]. These studies will help to evaluate the pathogenic role of deleterious mtDNA mutations in the formation of atherosclerotic phenotypes at the cellular level, and to find novel molecular targets for the prevention and treatment of atherosclerotic pathology.

In conclusion, I would like to thank the scientists and researchers who have contributed to this special issue of “*Vessel Plus*” and shared their own thoughts on recent fundamental, generalized and clinical findings, all of which are aimed at evaluating atherosclerosis-related and metabolic pathologies^[31-34].

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

Availability of data and materials

Not applicable.

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Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Review

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Obesity and atherosclerosis: the exosome link

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Abstract

Obesity is a global public health issue with serious health consequences and rising prevalence. It is a risk factor for a broad range of diseases, particularly atherosclerosis and cardiovascular disease. Long-term weight loss is difficult to achieve, even with diet, life-style changes and anti-obesity drugs. The causes of the association between obesity and atherosclerotic cardiovascular disease are the subject of ongoing investigation. It is known that a chronic surplus in nutritional intake results in expansion and remodeling of adipose tissue, leading to chronic inflammation. Lipid overloaded adipocytes secrete pro-inflammatory adipokines and other mediators that produce this inflammatory state that may in turn, promote atherosclerosis, which is considered an inflammatory disorder. This review discusses the potential role of exosomes from adipose tissue in accelerating atherosclerosis in the setting of obesity. Exosomes are small membrane-bound vesicles that circulate in body fluids and are important participants in intercellular communication both locally and at a distance. They can transfer their cargo of protein, DNA, RNA and microRNA between cells, thus impacting cellular function and signaling. Adipose tissue-derived exosomes may be involved in heightening of the atherogenic environment and, if so, suggests a therapeutic target for the treatment and prevention of cardiovascular complications of obesity.

Keywords: Obesity, atherosclerosis, adipocyte, macrophage, exosome

INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of morbidity and mortality worldwide^[1-3]. Obesity increases the risk of ASCVD and death even after accounting for other known risk factors such as dyslipidemia, smoking, and hypertension^[4]. The underlying mechanisms that produce the added harmful effects of obesity are poorly understood. Elucidating the mechanisms behind differences between obese individuals with and without atherosclerosis^[5,6] could reveal therapeutic targets for treating



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the harmful cardiovascular consequences of obesity as an alternative or adjunct to weight-loss programs, which are known to have limited long-term success.

Adipose tissue acts as an active metabolic endocrine organ that releases not only hormone-like adipokines and inflammatory cytokines, but also cargo-carrying vesicles such as exosomes that may be considered a form of adipokine that contributes to the development of atherosclerosis^[7-10].

Adipose tissue in obese subjects is inflamed as compared to lean subjects and displays greater macrophage infiltration^[11]. In the obese state, adipose tissue can no longer accommodate excess energy stores and among the maladaptive changes that occur are infiltration by a variety of inflammatory immune cells that interact with adipocytes to promote chronic inflammation^[12]. Atherosclerosis progression is driven by inflammation and the pro-inflammatory environment fostered by excess adiposity is thought to be a critical link between obesity and ASCVD^[13,14]. Sequential steps in atherosclerosis are: circulating monocyte adhesion to endothelium, penetration through the compromised barrier, differentiation into macrophages and excessive uptake of lipids^[15]. Each of these steps may be vulnerable to interference by exosomes. This review will discuss the connection between adipose tissue and atherosclerosis and the potential role of exosomes in communicating atherogenic signals from fat depots to the arterial wall. Understanding these relationships may be invaluable in the understanding, prevention and treatment of ASCVD.

ATHEROSCLEROSIS, INFLAMMATION AND LIPIDS

Atherosclerosis is a process that takes place in the arterial wall and its earliest stage involves a breach of the vascular endothelium by monocytes, which settle in the subendothelial space and become macrophages^[16]. In an inflammatory environment, these macrophages in the subendothelial intima may exhibit impairment of cholesterol efflux, which leads to intracellular accumulation of modified low-density lipoprotein (LDL) and subsequent formation of plaque-forming lipid-rich foam cells^[17,18]. Macrophages may become classically or alternatively activated to the M1 or M2 phenotype, respectively. During atherogenesis, monocytes enter the atheroma and differentiate into the M1 macrophage subtype and it is these M1 macrophages that play a crucial role in the initiation and progression of atherosclerosis^[19]. M1 macrophages are considered pro-atherogenic because they easily transform into cholesterol-overloaded foam cells while the M2 subtype is less atherogenic and has a lesser propensity to form foam cells. M2 macrophages are associated with tissue repair and are enriched in regressing plaques^[20].

Macrophage cholesterol homeostasis is a delicate balance among influx, endogenous synthesis, esterification/hydrolysis and efflux^[21]. The low grade chronic inflammation associated with obesity is a likely driver of dysregulated macrophage cholesterol homeostasis. It has also been shown to adversely affect expression of the proteins responsible for cholesterol influx and efflux by our group and others^[22-29].

A variety of cytokines may stimulate the atherosclerotic process, including interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and interleukin (IL)-1 β ^[30,31]. TNF- α and IL-1 β induce cytokine and adhesion molecule expression and also encourage the migration of vascular smooth muscle and endothelial cells^[32,33]. IFN- γ promotes foam cell formation^[25,34].

One of the most compelling clinical challenges of our time is the increasing prevalence of obesity and its detrimental effects on the cardiovascular system. Obesity influences inflammation and the pathophysiological processes involved in atherosclerotic disease development^[35]. Obesity and overweight are accompanied by unfavorable blood lipid profile patterns^[36,37]. Dyslipidemia is a major risk factor for coronary artery disease. Among obese patients, the estimated prevalence of hypertriglyceridemia is twice as high as in non-obese individuals^[38]. In addition, the atherogenic combination of hypertriglyceridemia with high LDL and low HDL is more prevalent in obese and overweight patients^[39,40]. Unfortunately, high

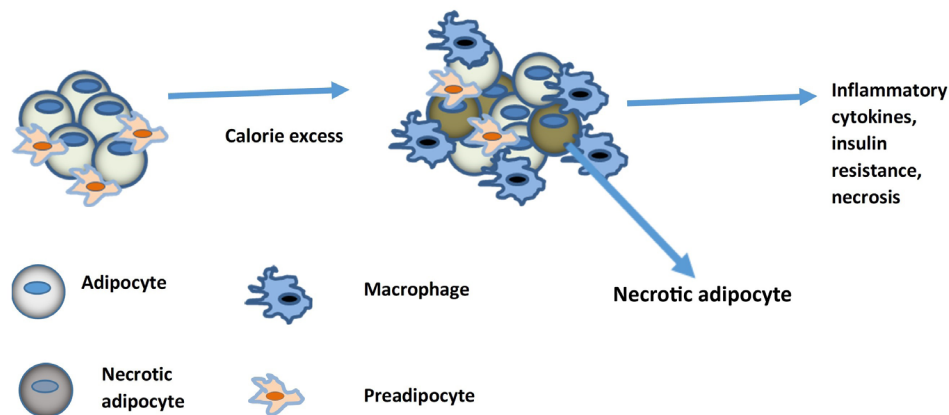


Figure 1. Change in white adipose tissue with unhealthy weight gain. Excess calorie intake results in dysfunctional adipose tissue characterized by a chronic inflammatory state with macrophage infiltration and phenotypic switching, inflammatory cytokine secretion, adipocyte necrosis, reduced insulin sensitivity and hypoxia

residual ASCVD risk remains even when LDL cholesterol is reduced to target levels and comorbidities are optimally treated^[41-44]. Pathological processes within the arterial wall may continue despite statin and other pharmacologic therapies. The standard lipid profile would not be sensitive to this type of regional arterial process because it measures liver metabolism of cholesterol and other systemic effects not localized at sites of atherosclerosis. Lipid dyshomeostasis at the cellular level within the artery is not reflected.

ADIPOSE TISSUE

Adipose tissue is not simply an inert tissue for storing excess energy and a thermal insulator. It is an active endocrine organ at the center of metabolic dysfunctions associated with obesity^[45,46]. Adipose tissue contains a variety of cell types including adipocytes, preadipocytes, pericytes, fibroblasts, endothelial cells and macrophages. The biology of adipose tissue is complex as it can exist in different forms and is classified as white adipose tissue (WAT) or brown adipose tissue (BAT) based on morphology and function^[47]. WAT holds energy in the form of triglycerides as a buffer against starvation and is the largest free cholesterol reservoir in the body, while BAT is more energetically active, with a greater number of mitochondria and higher energy production^[48]. Mature WAT adipocytes each contain a single large lipid droplet. Obesity induces changes in WAT leading to increased lipolysis, insulin resistance, adipocyte hypertrophy and regions of hypoxia [Figure 1]. WAT secretes into the bloodstream many adipokines, which are bioactive molecules that are thought to contribute to the inflammatory milieu, thus promoting atherosclerosis^[49-52]. However, anti-inflammatory treatments have failed to reduce ASCVD, indicating that factors other than inflammatory mediators are involved in the interplay between adipose tissue and blood vessels^[53,54]. Exosomes may be one of the links that contribute towards development of ASCVD in obesity^[55].

Over the last few years, BAT has also been recognized as a potential therapeutic target in the prevention of atherosclerosis^[56-58]. BAT consumes energy and generates heat through the action of uncoupling protein 1, which disconnects the electron transport chain from ATP synthesis^[59]. The distribution of brown adipocytes in the body maximizes the cytoplasmic-lipid interface, making their involvement in fatty acid metabolism more effective than white adipocytes. In mice, brown adipocyte-derived endocrine factors significantly diminish body weight via elevation of oxygen consumption and decrease in total body fat mass^[60]. Activation of endogenous brown adipocytes induces intracellular lipolysis of triglycerides and

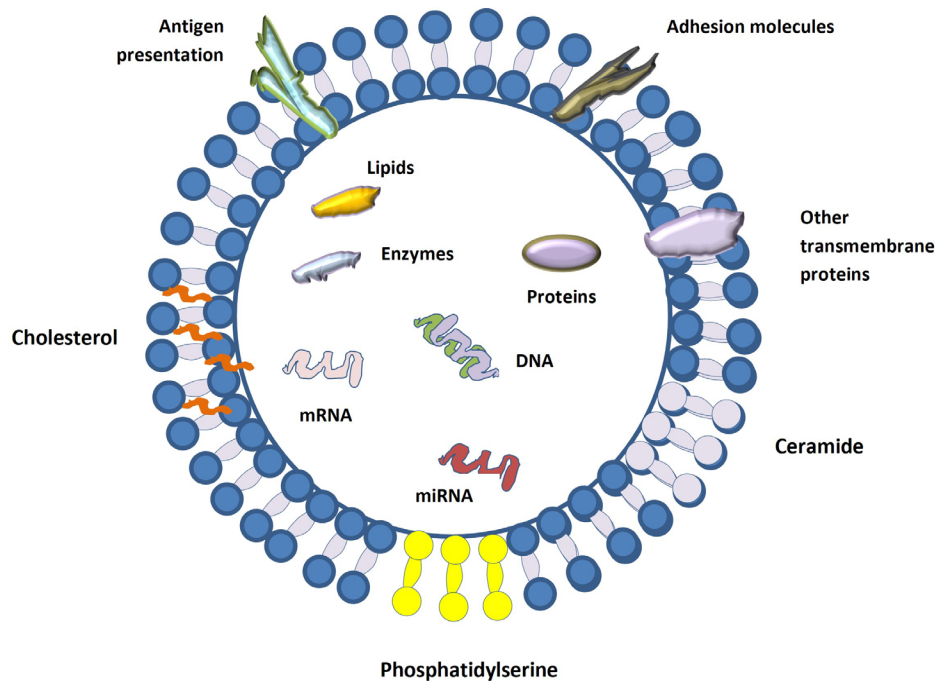


Figure 2. Exosome structure. Exosomes are microvesicles delineated by a membrane enriched in phosphatidylserine and contain DNA, mRNA, miRNA, proteins and lipids

thus, leads to release of fatty acids and glycerol in the cytoplasm with reduced plasma triglyceride levels and obesity^[61,62].

Adipose tissue is a key organ that controls lipid metabolism and energy distribution, as well as regulation of endocrine function related to cardiovascular disease. Endocrine functions of adipose tissue are mostly attributed to their ability to secrete adipokines, hormones and cytokines that regulate energy homeostasis and satiety^[7]. There are over 600 known adipokines but the most well-studied are the anti-inflammatory adiponectin, which is decreased in obesity, and leptin, which is secreted mostly by WAT and is present unbound in the circulation at higher levels in obesity^[63-65]. Adipokines are carried by human adipocyte exosomes and leptin has been detected in mouse serum exosomes while adiponectin has been found in rat adipose tissue exosomes^[66-68].

EXOSOMES AND ADIPOCYTE-DERIVED EXOSOMES

Exosomes are a type of extracellular vesicle with a size of 30-150 nm and a specific density of 1.13-1.21 g/mL. They are found in blood and other biological fluids. Exosomes are released into the extracellular space when multivesicular bodies fuse with the cellular plasma membrane^[69,70]. Exosomes carry nucleic acids such as microRNA (miRNA), messenger RNA (mRNA) and mitochondrial DNA as well as proteins and lipids [Figure 2]. These exosome components are encased in a phospholipid membrane rich in ceramides, cholesterol and sphingomyelin, often with high phosphatidylserine content^[71-73]. Exosomes help mediate signal transduction and provide a means for cell-to-cell communication over a distance and between organ systems^[74]. Signaling pathways can be impacted by exosomes through the miRNAs they carry. miRNAs are small non-coding RNAs that negatively regulate gene expression by impeding translation or inciting instability of complementary mRNA targets, thus inhibiting protein formation^[75]. Exosomes carrying miRNAs can be taken up via endocytosis or pinocytosis into recipient cells^[76].

It should be noted that there are different circulating particles in the blood and bodily fluids, collectively known as extracellular vesicles. These are heterogeneous in size and include not only exosomes, but also

microparticles, which are larger in size but have similar composition and structure^[77]. It is difficult to differentiate between these, but we have tried to confine this discussion as much as possible to exosomes. The appearance of adipose-derived exosomes in the circulation has been documented in humans and mice^[78,79]. Adipocyte-derived exosomes may be considered a form of adipokine^[79]. In mice, adipose tissue is an important source of circulating exosomal miRNAs in the obese state^[79]. The miRNA cargo of adipocyte-derived exosomes may influence pathways involved in obesity and atherosclerosis^[80-82]. Many miRNAs have been shown to be differentially expressed in obese adipocyte exosomes, compared to lean adipocyte exosomes in both mouse and human^[83]. Adipocyte-derived exosomes affect insulin resistance^[84]. Mice with adipose tissue-specific knockout of Dicer, a large multi-domain ribonuclease enzyme responsible for the biogenesis of miRNA, produce exosomes with low miRNA content and exhibit a form of lipodystrophy marked by loss of WAT and whitening of BAT, as well as insulin resistance and dyslipidemia^[79,85]. When WAT from wild type mice is transplanted into Dicer knockouts, circulating miRNAs are restored and glucose tolerance improves. Phenotypic change of cultured Dicer knockout brown preadipocytes to a white adipocyte-like state was modulated by specific miRNAs miR362, miR365 and miR346. Exosomes from adipose tissue macrophages of obese mice confer poor glucose tolerance and insulin resistance when transferred to lean mice^[86]. A comparison of miRNA content of adipose tissue macrophage exosomes of obese versus lean mice showed that miR155 was much more abundant in exosomes from obese mice and this miRNA was shown to inhibit insulin signaling via downregulation of peroxisome proliferator-activated receptor γ , a key regulator of adipocyte differentiation, glucose and lipid metabolism. Mice with knockout of miR155 fed a high fat diet for 12 weeks exhibited less obesity-induced glucose intolerance and insulin resistance, compared to wild type mice on a high fat diet. When wild type bone marrow was transplanted into miR155 knockouts, glucose tolerance and insulin sensitivity were impaired with feeding of high fat diet.

In mice, fibroblast growth factor (FGF)-21, a member of the FGF family with hormone-like actions that regulates glycolipid metabolism, can be downregulated in liver by miRNA29b carried in exosomes^[87,88]. This effect of adipose tissue exosomes on FGF21 may be pro-atherogenic since FGF21 is considered atheroprotective and improves the cardiometabolic profile in obesity and diabetes^[89]. Exosomes released from adipose tissue of obese mice and injected into wild type mice induce activation of monocyte differentiation to macrophages in the latter, causing inflammatory cytokine production through the toll-like receptor (TLR) 4 pathway^[90]. Macrophages in atherosclerotic lesions express TLRs, including TLR4, a type of pattern recognition receptor that is known to mediate inflammatory activation and TLR4-deficient mice are protected from forming atherosclerotic lesions^[91]. Both pro-inflammatory/pro-atherosclerotic (M1) and anti-inflammatory (M2) macrophage phenotypes were induced by adipose tissue exosomes. The obese mouse adipose exosomes also caused insulin resistance in wild type mice. Mouse exosomes derived from visceral adipose tissue cause foam cell formation in a mouse macrophage cell line, likely due to inhibition of cholesterol efflux due to decreased expression of ATP binding cassette transporter (ABC) A1 and ABCG1, reverse cholesterol transport proteins that are needed to prevent lipid overload^[92,93]. Adipocyte exosomes affect macrophage function in humans as well^[94].

Exosomes from adipose tissue may also influence vascular endothelial cells, but this is not as well-studied as in macrophages. Vascular endothelial cells take up adipose tissue exosomes and it is postulated that obese adipose tissue may secrete exosomes with pro-inflammatory cargo that could then activate the endothelium^[95-97]. Confirmation of the interaction of adipocyte exosomes and vascular endothelium awaits further study.

Pericytes are pluripotent contractile cells embedded in the basal membrane surrounding endothelial cells that directly interact with endothelium, and are increasingly recognized for their involvement in atherosclerosis^[98,99]. At this time, there is no data on adipocyte exosome effect on pericytes or pericyte-

endothelial interaction, but it is known that pericytes can affect the endothelium through exosomes and so, adipocyte-to-pericyte communication via exosomes may merit investigation^[100].

Both proteins and miRNAs within exosomes may be involved in their effects. Adipocyte exosomal miRNAs can influence macrophages resident within adipose tissue towards an inflammatory direction and can be delivered to the vasculature where *in vitro* studies have shown that they induce pro-atherogenic changes in macrophages^[101,102]. One example is miR-34a, which is expressed at a higher level in adipose tissue of obese compared to lean mice and also, in obese compared to lean humans^[101]. In mice, miR-34a downregulated Kruppel like factor 4, a transcription factor that drives M2 macrophage polarization, and this resulted in less M2 and more M1 macrophages in adipose tissue. In human studies, the number of circulating adipocyte-derived extracellular vesicles has been found to correlate with insulin resistance in obese subjects and with serum triglyceride levels^[103,104].

CONCLUSION

The link from obesity to adipose tissue dysfunction to adipose-derived exosome influence on atherosclerosis is only being explored now. Many of the experiments cited in this review utilize particles produced *in vitro* and then introduced into *in vivo* animal models. Even though this is a useful initial approach towards understanding the effects of different particles, it does not provide cause-and-effect evidence of what is occurring in humans *in vivo*. Rather, it guides direction for future studies.

Exosomes from adipose tissue are formed by inward budding of the limiting membrane of late endosomes, fuse with the plasma membrane and released into the blood or extracellular fluid^[105]. We now have the technology to isolate exosomes of adipocyte origin directly from the blood for analysis of their content and sequencing of their miRNAs^[105]. As more miRNA sequences are found to affect specific signaling pathways, we can expect further elucidation of how they impact multiple aspects of atheroma formation and maturation. A working hypothesis is that obesity induces chronic low-grade inflammation within adipose tissue leading to specific changes in exosome cargo from both adipocytes and resident macrophages. The miRNA and protein in these exosomes enter the circulation, reach the blood vessels and influence the endothelial monolayer, macrophages and the stability of the plaque. The adipocyte exosomes may also indirectly foster atherosclerosis by playing a role in insulin resistance and type 2 diabetes. Exosomes from adipose stem cells may exert protective, anti-inflammatory effects on macrophages, suggesting a means to develop countermeasures to the pro-inflammatory influence of adipose tissue^[106]. While it is clear that macrophages are integral to the atherosclerotic process, their precise role is still uncertain. Macrophages may be part of the formation of a plaque, or they may be attracted to lipid deposits within the arterial wall and act as phagocytes absorbing these lipids, as has been observed in early atherosclerotic changes, and may participate either way^[107]. Whatever the context, the effect of exosomes on macrophages in atherosclerosis is worthy of further study. Knowledge of processes through which adipose tissue exosomes may accelerate atherosclerosis progression would open up an opportunity to mitigate these negative effects, even in persons who do not lose weight. One such approach would be to design and produce exosomes harboring antagomirs to neutralize undesirable and overexpressed miRNAs^[108].

DECLARATIONS

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Authors' contributions

Researched data for the article, discussed its content, and wrote, reviewed, and edited the manuscript: Reiss AB, Kasselmann LJ, De Leon J

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Editorial

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Endovascular treatment for type A aortic dissection - What are our critical concerns?

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INTRODUCTION

Endovascular graft intervention has brought new therapeutic concepts to conventional cardiovascular surgery. In 1991, an Argentinean team first described aortic endograft placement in the repair of abdominal aortic aneurysms, paving the way for new interventions in the management of aortic disease. Following this, endovascular aneurysm repair (EVAR) and thoracic EVAR (TEVAR) were developed in succession^[1]. In 1999, Dake *et al.*^[2] reported promising results (100% technical success and 16% 30-day mortality) in the endovascular management of patients with acute aortic dissection, including 4 patients with retrograde acute type A dissections and 15 patients with acute type B dissections.

With the advantages of minimally invasive procedures, TEVAR plays an important role, especially in older patients with multiple co-morbidities suffering from acute type A aortic dissection (ATAAD). Complete coverage of the entry tear is essential in the endovascular intervention of ATAAD as it depressurizes the false lumen, redirecting blood flow into the true lumen, resulting in thrombosis of the false lumen, thus allowing for aortic remodeling.

Nienaber *et al.*^[3] reported an international multi-centre experience of 12 type A aortic dissection (TAAD) patients (6 cases of acute and subacute or chronic each) with a mean age of 81 ± 7 years and a Euro SCORE



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II 9.1 ± 4.5 . These patients received endovascular therapy using ZENITH TX2 (Cook, Bloomington, Ind), GORE C-Tag (Gore Ltd., London, United Kingdom), or Relay NBS (Bolton, Barcelona, Spain). The early results revealed a technical success rate of 91.7%, all with proper aortic remodeling and low complication rates (1 transient stroke and 1 death due to wire induced perforation). Follow-up CT scans demonstrated good aortic remodeling. In 2020, Ghoreishi *et al.*^[4] reported a single-centre experience of 13 patients with ascending aorta disease and a mean age of 69 ± 9 years (including 7 patients with ATAAD) using Gore TAG (CTAG) (W. L. Gore & Associates, Flagstaff, Ariz). 100% technical success (2 with location of zone 0a) was achieved and proper aortic remodeling was found on follow-up CT scans. Both studies revealed excellent results in the elderly and patients with comorbidities with TAAD.

In general, the procedure of TEVAR consists of the following steps^[3,4]: (1) a temporary pacing wire is inserted to the right ventricle via venous cut-down; (2) a pigtail catheter to the left ventricle is navigated using a soft guide wire through the true lumen under imaging guidance to confirm the location; (3) a soft guide wire is then exchanged to a stiff guide wire, followed by delivery of the endograft device to the ascending aorta under rapid ventricular pacing to reduce the windsock effect during graft deployment; (4) the location of stent-graft, the patency of coronary arteries and arch vessel, the presence of endoleaks, and the occurrence of aortic regurgitation are evaluated by angiogram; and (5) the pigtail catheter and temporary pacing wire are retracted and access sites closed.

Nonetheless, issues have emerged from the current status of endovascular repair for ATAAD based on unmet requirements.

TECHNICAL ASPECTS OF THE PROCEDURE: A REVIEW OF THE LITERATURE

One issue identified is the immediate impact upon deployment of the stent. The lack of specially designed stents for the ascending aorta not only increases difficulties of the procedure and patient selection, but also raises concerns of the unknown effects that the stent-graft has on the ascending aorta in the multiply comorbid patient with poor cardiovascular reserve. In previous studies, it has been reported that endovascular device made of artificial material was of poor compliance and even Dacron polyester fabric grafts were four times less compliant compared to native arteries^[5,6].

Additionally, elderly patients with multiple comorbidities are pro-inflammatory, which leads to systemic microvascular endothelial inflammation with subsequent myocardial inflammation and fibrosis. This increases oxidative stress and alternation in cardiomyocyte signaling pathways which promotes cardiac remodeling and dysfunction. Therefore, it is crucial to explore an ideal stent-graft, which alleviates the harmful long-term effects and potential pathogenicity to these susceptible patients^[7].

Anatomical complexities are another issue surgeons face. Following ATAAD, ascending aorta dilatation, arch dilatation and aortic valve insufficiency can occur, and the walls of the aorta become fragile. In order to achieve proper fixation and seal of the stent graft, adequate length of landing zone in both proximal and distal site of the stent is essential. Proximally, the serrated edge of the stent may cover the coronary orifices or interfere with the commissures of the aortic valve, resulting in an entry tear in the proximal third of the aorta close to the sinus-tubular junction or even the sinus portion. Roselli *et al.*^[8] proposed a modified classification of landing zones, dividing the ascending aorta into zones 0A, 0B and 0C to address the anatomical complexity and importance^[8]. Diseases extending to zone 0A pathology (from the annulus to the distal margin of the highest coronary) had significantly worse outcomes than others. Distally, Sobocinski *et al.*^[9] analyzed the feasibility of endovascular therapy and revealed that it may be acceptable to expand the margin of the distal landing zone from the additional debranching of the brachiocephalic trunk to the left common carotid artery. Of note, orifices of the innominate artery, left common carotid artery and left subclavian artery are in close proximity, which gives us limited choice of commercial stent-grafts

because these grafts are too long for the ascending aorta to obtain a sufficient landing zone. Thus, it is important to have specially designed stent-grafts and accurate imaging study before the procedure.

To date, there still remains some controversy surrounding patient selection for TEVAR. Conventionally, aortic valve insufficiency is a contraindication for TEVAR^[10], however, more than one-third of ATAAD patients, especially in the severe subgroup, were found to have this. In 2014, Rylski *et al.*^[11] introduced the concept of endovascular treatment of ascending aortic pathologies with valve-carrying conduits associated with an uncovered portion for free diastolic coronary blood flow^[11]. Using an endovascular valve-carrying conduit not only resolves the problem of aortic regurgitation, but also effectively results in sufficient anchorage of the device^[12]. Alternatively, Nienaber *et al.*^[3] proposed a combined TAVR (transcatheter aortic valve replacement)-TEVAR technology, in an attempt to treat variants of aortic dissection including those with compromised aortic valve function.

Fatal complications such as aortic rupture^[2], ventricular perforation and cardiac tamponade^[3], or other early morbidities such as supraventricular tachycardia and cardiovascular ischemia have been reported in the current literature. This also raises the concern of cerebrovascular accidents that may occur as the vascular surgeon passes the guide wire and deploys the stent-graft in a calcified ascending aorta and arch of ATAAD patients with advanced age and multiple morbidities. In addition, unidentified acute coronary involvement (ACI) in ATAAD would be fatal and it is worth noting that up to 30% of patients with ACI disclosed no clinical manifestations of coronary malperfusion preoperatively^[13].

Once stent implantation is successfully achieved, the second issue is its delayed impact on aortic stiffness. A healthy aorta has a cushioning function, limiting arterial pulsatility and protects the microvasculature from potentially harmful fluctuations in pressure and blood flow^[14]. With a complex structure close to the left ventricle (LV), any deviation from the natural physiologic character of the ascending aorta (such as increased aorta stiffness) would give rise to complications. Available stent-grafts made of artificial compounds, such as expanded polytetrafluoroethylene and woven polyester, and metal wire, are poorly compliant and foreign to the human body. Current Dacron polyester fabric grafts exhibit four times reduced compliance compared to native arteries^[5,6]. Hence, it is reasonable to assume that wire-containing stent-grafts are less compliant^[5]. An immobilized segment is created after placement of the stent-graft in the ascending aorta, alternating in diameter and area during every heartbeat, and more aortic stiffness develops consequently. Without enough elasticity, the Windkessel effect vanishes leading to more resistance to LV and rebound force on the aortic valve. It is well established that large-artery stiffness (LAS) impairs the aortic cushioning function and independently predicts cardiovascular risk. LAS also contributes to isolated systolic hypertension, excessive penetration of pulsatile energy into the microvasculature of target organs that operate at low vascular resistance, and abnormal ventricular-arterial interactions that promote left ventricular remodeling, dysfunction, and failure^[14]. In a 4-year follow-up retrospective study using patient-specific fluid-structure interaction analysis and image-based measurements of cardiac remodeling from echocardiography and computed tomography angiography, van Bakel *et al.*^[15] demonstrated that TEVAR-induced acute aortic stiffening caused a 26% increase in LV stroke work and cardiac remodeling^[15]. Moreover, alternated left ventricular hemodynamics may raise concerns of impaired function of the LV induced by ventricular remodeling and impairment of the aortic valve. It has been found that aortic stiffness is associated with LV remodeling and reduced LV systolic and diastolic function by magnetic resonance imaging measurement in a large multi-ethnic population^[16]. Therefore, it is crucial to explore an ideal stent-graft so as to alleviate the harmful long-term effects and potential pathogenicity to these susceptible patients. In addition, a thorough examination of cardiac morphology and function should be performed^[5] in the long-term follow up including echocardiographic parameters of (1) key structural alterations, such as the left atrial volume index or left ventricular mass index and (2) key functional alterations, such as an E/e' ratio of early mitral inflow velocity and an E/A denoting ratio of E wave to A wave^[17].

FINAL REMARKS

Reports from the International Registry of Acute Aortic Dissections show that 10% to 30% of ATAAD patients are considered too high risk to receive open repair and would therefore receive only medical management with up to 60% early mortality rates^[3,18]. From an anatomical aspect, based on valvular condition, landing zone, and coronary involvement, 30% to 50% of patients are technically suitable for TEVAR^[19]. To date, ascending TEVAR strategies appear encouraging in the treatment of various ascending aortic pathologies. Thus, ascending TEVAR is feasible and reveals promising early and short-term results based on our updated literature review [Supplementary Table 1]. Nevertheless, the complexity of the anatomy in the ascending aorta continues to be a major obstacle for the use of current endovascular technologies. In other words, ascending TEVAR is often highly compromised by anatomic limitations with the short length of the ascending aorta, the location of the coronary ostia, the location of the entry tear, and the supra-aortic branches^[13]. Of them, the most common criteria contradictory for ascending TEVAR was the lack of a sufficient landing zone (i.e., the distance between the coronary ostia and the entry tear has been measured to be less than 20 mm^[13,20]). Notably, Roselli *et al.*^[8] pointed out that the greater curve is more than 30% longer than the lesser curve along the length of the ascending aorta^[8]. Thus, the next-generation ascending stent-graft device needs to be highly conformable and elastic with adequate strength of fixation in what is a hostile environment^[8]. In summary, there are no commercially available designs of endovascular devices specifically for the ascending aorta currently. The current iteration of stent-graft technology however, needs to be adapted to the specific anatomic features of the ascending aorta^[3]. It is envisioned that with time, combining new knowledge and technological advancements will further pave the way for broader application of ascending TEVAR for patients with TAAD.

DECLARATIONS

Authors' contributions

Authors made substantial contributions to the conception and design of the study, and participated in drafting the article: Chang TW, Yang TT

Authors provided administrative, technical, and material support: Jhou HJ, Ke LY

The author participated in critical revision for important conceptual and intellectual content and gave final approval of the version to be submitted to the Journal: Chen YF

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Case Report

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Aortopathy and coronary anomaly in bicuspid aortic valve: an uncommon tricky association

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Abstract

Bicuspid aortic valves (BAV) can be associated with aortopathy and coronary anomalies. We report the case of a 60 year-old woman undergoing surgery for severe aortic stenosis due to BAV and an ascending aortic aneurysm. During the procedure, an uncommon anomalous origin of the left main coronary artery from the posterior commissure with intramural takeoff of the left coronary artery was found. Routine pre-operative coronary angiography had failed to identify this anomaly. To avoid ischemic events or left main coronary lesions, we placed the aortic bioprosthesis by respecting the commissures, not to occlude the anomalous coronary ostium. The association of BAV, aortopathy and coronary anomalies is a rare finding. Awareness of the anatomy of the coronary arteries in patients with BAV should be considered mandatory to avoid catastrophic consequences and to select the appropriate surgical procedure.

Keywords: Aortic surgery, aortic valve, bicuspid aortic valve, coronary anomaly

INTRODUCTION

Bicuspid aortic valves (BAV) are the most common congenital heart defect, affecting 1% to 2% of the general population^[1], and are often associated with aortic aneurysms and occasionally, with coronary anomalies.



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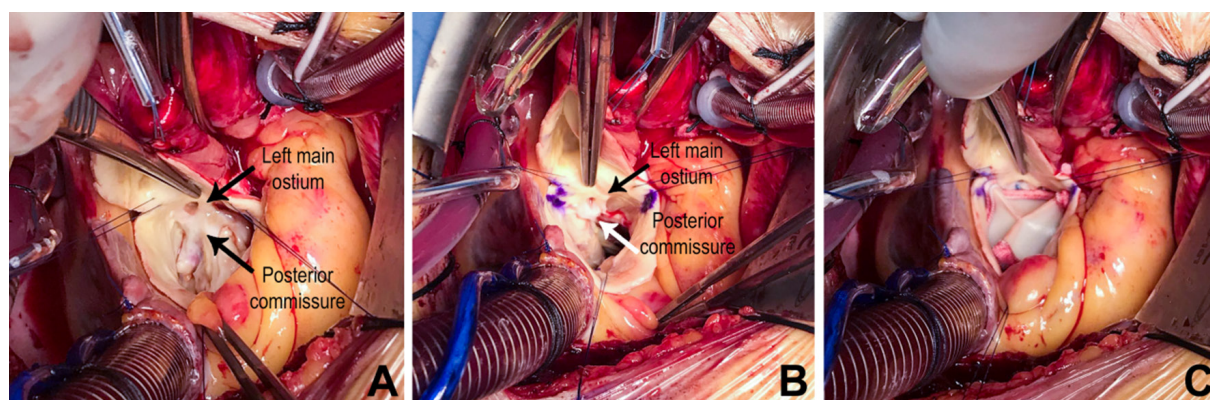


Figure 1. Surgical view of the aortic valve and the left main ostium during the procedure. A: native valve in situ, left main ostium located in proximity to the posterior commissure; B: marked stent location after native valve removal; C: implanted bioprosthetic valve; the valve stents are not in conflict with the left main ostium, which has been left in its original location

Various classifications have been introduced to describe the different morphologies of BAVs. One of the most used in clinical practice is the Sievers classification. Introduced in 2007, it is based on the number of raphe to define the phenotype of the BAV, and includes three classes: types 0 (no raphe), 1 (one raphe) and 2 (two raphe); type 0 valves are divided into anteroposterior (AP) or lateral according to spatial position and orientation between the cusps and the coronary ostia^[2].

BAVs can develop stenosis or regurgitation and be associated with vascular anomalies. Some studies claim that ascending aortic dilatation is the most common vascular anomaly found in patients with BAVs^[3]. Various mechanisms are involved including cystic medial necrosis, fragmentation of elastic fibers or the loss of smooth muscle cells in the ascending aorta wall^[4]. The association between BAV and coronary anomalies has been described as a rare finding in the current literature^[5-7]. We report a case of BAV stenosis associated with an ascending aortic aneurysm and a positional anomaly of the left main coronary ostium.

CASE REPORT

A 60-year-old woman presented with acute respiratory distress and chest pain to the emergency room. Her main cardiovascular risk factors included smoking, recurrent bronchitis, hypercholesterolemia, β -thalassemia trait and a family history of coronary artery disease.

On admission, trans-thoracic echocardiography revealed a dilated ascending aorta (45 mm) with a normal aortic root, severe aortic valve stenosis (mean gradient 83 mmHg) in the presence of bicuspid aortic valves, and a regular left ventricular ejection fraction (50%). Coronary angiography revealed apparently normal coronary anatomy without any critical lesion [Video 1]. Chest x-ray, EKG and Doppler examination of the supra-aortic vessels did not elucidate any anomaly. The patient was therefore transferred to our Division for surgical treatment. We planned for replacement of the aortic valve and ascending aorta through a mini-sternotomy approach, according to current guidelines^[8,9].

In the operating room, a Sievers type 0 AP BAV was recognized. The left main coronary ostium was found to originate in proximity to the posterior commissure, having an intramural take-off and, it was surrounded by thick fibrous tissue, possibly due to acquired jet lesions [Figure 1A]. Interestingly, this anomaly was not detected during the previous routine angiography [Video 1].

Once the native leaflets had been removed and the annulus decalcified, we chose to implant a bioprosthetic valve (Carpentier Edwards Magna Ease size 21), respecting the patient's wishes.

We marked the location of the bioprosthetic valve stent to avoid the left main lesion and/or mechanical obstruction [Figure 1B]. The fibrous tissue around the ostium was deliberately left intact to avoid inadvertent dissection [Figure 1B and C]. After valve implantation, the ascending aorta was replaced with a 26 mm-Dacron Hemashield tube graft (Meadox Medicals, Inc., Oakland, New Jersey, N.J. USA). 2D-echocardiography showed good valve function postoperatively with no leak. The postoperative clinical course was uneventful otherwise and no ischemic events occurred. At 6-month follow-up, the patient is alive, asymptomatic and NYHA class I.

DISCUSSION

Our case included aortopathy and coronary anomaly in a patient with BAV. Coronary anomalies are rare and are found in less than 1% of the general population^[10]. Their association with BAVs has been reported, but there is a lack of focused studies in the literature to draw conclusions on detecting these anomalies and the related operative risks^[11]. Interestingly, routine coronary angiography failed to detect the left main coronary ostium origin anomaly. This is not completely unexpected since a high incidence of false negatives has been reported when the anomaly involves the coronary origin^[12]. The location, orientation, height and number of coronary ostia may necessitate different surgical approaches, not only during valve replacement surgery, but during valve repair and valve sparing surgery too^[13]. During aortic valve replacement, it is crucial to rotate the prosthesis so that the stent does not interfere with the anomalous coronary ostia. The surgeon must be careful not to damage the coronary origin to avoid potentially catastrophic ischemic events. In cases of intramural take off of the left coronary artery, as seen in our patient, the aortic root must be manipulated with caution. If rotating the prosthesis is not sufficient to avoid the risk of ischemia, other treatment options include unroofing the intramural segment, creation of a “neo-ostium” in the appropriate sinus, reimplantation of the ostium, translocation of the pulmonary artery, and pericardial patching of the aorta and proximal anomalous coronary artery^[14,15]. Occasionally, coronary artery bypass grafting is used but it is generally not recommended^[14]. We must be aware of coronary anomalies even during aortic root surgery. Valve sparing surgery is feasible, although it may be necessary to associate it with corrective surgery of the coronary anomaly. A careful surgical plan is mandatory for successful coronary reimplantation and to avoid air embolism^[16].

Despite being used off label with BAV, there are increasing numbers of Transcatheter Aortic Valve Replacement procedures in the last few years^[9]. In view of the asymmetric nature of the BAV orifice and heavy regional calcification, the risk of ischemia related to coronary anomalies during transcatheter procedures is not negligible^[17].

Unfortunately, there is a gap in knowledge such that we are not currently able to identify patients with coronary anomalies and a high risk of ischemia, and to properly stratify the related surgical risk to the correction of coronary anomalies^[15].

Considering the potential issues, an accurate pre-operative diagnosis is crucial to avoid adverse outcomes. Since routine angiography cannot be conclusive, as seen in our case, the use of gated coronary-CT, 3D echocardiography or magnetic resonance imaging may be considered^[18].

In conclusion, coronary anomalies may be associated with BAVs with potential implications for invasive valve procedures. If underestimated or not recognized, coronary anomalies can lead to catastrophic outcomes.

DECLARATIONS

Authors' contributions

Made substantial contribution to the conception and design of the study, performed data analysis and data interpretation: Salsano A, Ricci D, Santini F

Performed data acquisition, as well as provided administrative, technical, and material support: Salsano A, Natali R, Parolari G

Availability of data and materials

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Consent for publication

The individual details used in this manuscript were obtained with the patient's consent for publication.

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Review

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A novel and dominant factor that mediates oxidative stress-induced apoptotic signaling - autocrine/paracrine mechanism of the secreted form of eukaryotic translation initiation factor 5A

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Abstract

Oxidative stress plays a critical role in the pathogenesis of various disorders including cardiovascular diseases, such as ischemia/reperfusion (I/R) injury, atherosclerosis, dyslipidemia, chronic kidney disease (CKD), arrhythmia, and diabetic cardiovascular complications. Although reactive oxygen species (ROS) have been proposed as the key mediator of oxidative stress-induced cell injury, antioxidant therapies have failed in clinical trials, raising the possibility that some unknown mechanism other than ROS may be involved. In 2015, we reported a novel apoptosis-inducing humoral factor in conditioned medium from cardiac myocytes subjected to hypoxia/reoxygenation. This novel 69th tyrosine-sulfated eukaryotic translation initiation factor 5A (eIF5A) was rapidly secreted from cells in response to oxidative stress and then acted as an apoptosis-inducing ligand in an autocrine fashion. We termed the novel secreted form of eIF5A "Oxidative stress-Responsive Apoptosis-Inducing Protein" (ORAIP). Evidence has accumulated that ORAIP may be a common and dominant apoptosis-inducer among various cell types in response to different types of oxidative stress and is involved in a wide spectrum of acute and chronic disorders. Among them, here, I summarize knowledge regarding the possible roles of ORAIP in myocardial and cerebral I/R injury, dyslipidemia in terms of atherosclerosis, cardiovascular complications in diabetes mellitus, and CKD.

Keywords: Atherosclerosis, chronic kidney disease, diabetes mellitus, eukaryotic translation initiation factor 5A,



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ischemia/reperfusion injury, oxidative stress, oxidative stress-responsive apoptosis inducing protein, reactive oxygen species

INTRODUCTION

Oxidative stress has been strongly implicated in the pathogenesis of various disorders including cardiovascular diseases, such as arrhythmia, heart failure, dyslipidemia, atherosclerosis, chronic kidney disease (CKD), diabetic cardiovascular complications, and in particular ischemia/reperfusion (I/R) injury. Oxidative stress induces reactive oxygen species (ROS) production, lipid peroxidation, protein oxidation, and DNA damage in the cells that lead to apoptosis. Until recently, ROS were proposed as the key mediator of oxidative stress-induced cell injury^[1-3]. However, large scale antioxidants (including vitamins, free radical scavengers) clinical trials have been unsuccessful to improve the outcome of cardiovascular and cerebrovascular diseases in humans^[4,5], raising the possibility that there might be some unknown mechanism other than ROS that mediates oxidative stress-induced cell injury.

In 2015, we reported a novel apoptosis-inducing humoral factor in conditioned medium from cardiac myocytes subjected to hypoxia/reoxygenation. We reported that this novel secreted form of eukaryotic translation initiation factor 5A (eIF5A) was sulfated at the 69th tyrosine residue and contained more of the hypusinated isoform than the conventional cytosolic form of eIF5A^[6]. We found that eIF5A undergoes tyrosine-sulfation in the trans-Golgi and is rapidly secreted from cardiac myocytes in response to hypoxia/reoxygenation. It then induces apoptosis by acting as a pro-apoptotic ligand in an autocrine fashion [Figure 1]. We termed this novel tyrosine-sulfated secreted form of eIF5A, Oxidative stress-Responsive Apoptosis-Inducing Protein (ORAIP)^[6]. eIF5A, a member of eIFs regulating the translation initiation step of protein synthesis, is the only known protein to contain the unique amino acid hypusine, which is formed post-translationally via a two-step enzymatic reaction with deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase [Figure 1]^[7]. eIF5A is primarily localized to the cytoplasm, where hypusinated eIF5A facilitates the translation of mRNAs that are involved in cell proliferation. We found that myocardial I/R (but not ischemia alone) rapidly and markedly increased plasma levels of ORAIP, which returned to the control level within 60 min. *In vivo* treatment with an anti-ORAIP neutralizing monoclonal antibody (mAb) significantly reduced myocardial I/R injury^[6]. It seems that secretion of ORAIP is specific to oxidative stresses including I/R, hypoxia/reoxygenation, ultraviolet light, ionizing radiation, cold/warm-stress (heat shock), and blood acidification^[8], then plays a crucial role in inducing apoptosis of target cells such as cardiac and skeletal myocytes, neurons, and cancer cells. Especially, these cells need substantial amounts of oxygen for their activities, making them very sensitive to oxygen concentrations and hence susceptible to oxidative stress-induced apoptosis mediated by ORAIP.

These results strongly suggested that ORAIP may be a specific biomarker and critical therapeutic target for oxidative stress-induced cell injury. We also found that the plasma levels of ORAIP were markedly elevated in patients with chronic disorders such as CKD, atrial fibrillation, heart failure, dyslipidemia, diabetes mellitus (DM), and diabetic retinopathy, in which oxidative stress plays a critical role in the pathogenesis^[9-12]. Thus, evidence has accumulated that ORAIP may be a common and dominant apoptosis-inducing ligand among various cell types in response to different types of oxidative stresses involved in a wide spectrum of acute and chronic disorders, especially cardiovascular diseases.

MYOCARDIAL AND CEREBRAL I/R INJURY

It has been long believed that neutrophils infiltrate into myocardial tissues subjected to I/R and cause myocardial damage, known as reperfusion injury, due to progressive capillary plugging by neutrophils that cause capillary no-reflow as well as ROS formation^[13-15]. Because reperfusion-induced apoptotic cell death

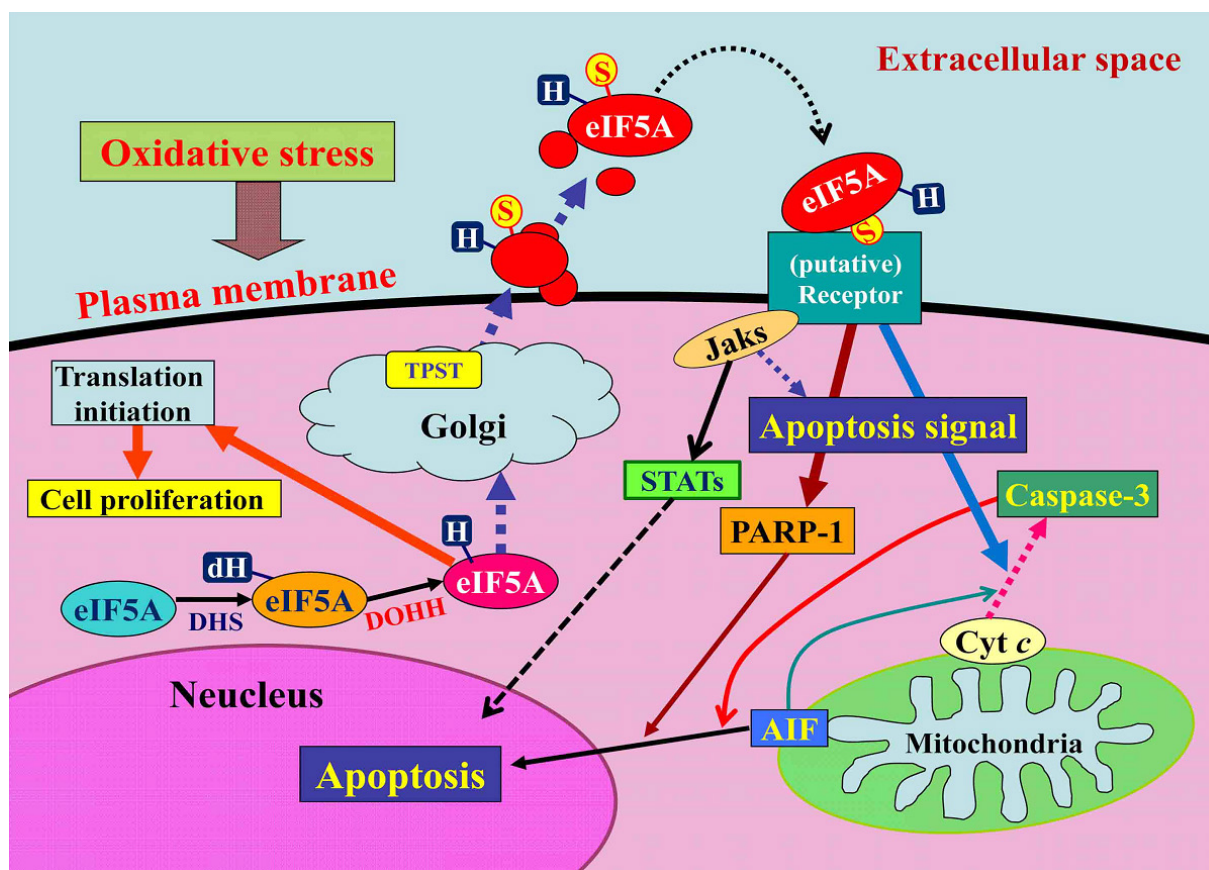


Figure 1. A model for the mechanism by which oxidative stress induces apoptosis via the autocrine secretion of eIF5A (oxidative stress-responsive apoptosis-inducing protein)^[6]. AIF: apoptosis-inducing factor; cyt c: cytochrome c; dH: deoxyhypusine; DHS: deoxyhypusine synthase; DOHH: deoxyhypusine hydroxylase; H: hypusine; Jaks: Janus kinases; S: sulfated; PARP-1: poly (ADP-ribose) polymerase-1; STATs: signal transducers and activators of transcriptions; TPST: tyrosyl protein sulfotransferase; eIF5A: eukaryotic translation initiation factor 5A

cannot be prevented by neutrophil depletion, some mechanism other than neutrophil infiltration triggered by reperfusion may mediate apoptotic signaling before neutrophil infiltration occurs^[16,17]. To exclude the effects of neutrophil infiltration, using an *in vitro* model of myocardial I/R, we identified a novel apoptosis-inducing humoral factor in conditioned medium from cardiac myocytes subjected to hypoxia/reoxygenation, that is ORAIP^[6]. Myocardial or cerebral I/R rapidly and markedly increased ORAIP levels in plasma and cerebrospinal fluid, whereas ischemia alone did not alter ORAIP levels^[6,18]. *In vivo* treatment with the anti-ORAIP neutralizing mAb dominantly reduced myocardial or cerebral I/R injury as compared with conventional therapies^[6,18]. This suggests that ORAIP plays a pivotal role in I/R-induced tissue injury and can be an oxidative stress-specific biomarker.

Muscle cells (especially cardiac myocytes) and cerebral neurons demand a lot of oxygen for their activities, making them very sensitive to oxygen concentrations and hence susceptible to oxidative stress, such as I/R injury, in which ORAIP plays a major role. It is thought that patients with significant stenosis of coronary or cerebral arteries are often subjected to silent myocardial or cerebral I/R, even subacute myocardial or cerebral infarction, which may lead to accumulation of cell injury, resulting in ischemic cardiomyopathy or lacunar infarction. Therefore, anti-ORAIP therapy may be effective in patients with stable ischemic heart or cerebrovascular diseases, as well as those subjected to reperfusion therapy for acute myocardial and cerebral infarction.

DYSLIPIDEMIA AND ATHEROSCLEROSIS

Oxidative stress plays an important role in the pathogenesis of dyslipidemia. Like other risk factors for atherosclerosis, such as DM, hypertension, and smoking, it is believed to play a critical role in atherosclerotic plaque formation and rupture^[19]. According to the low-density lipoprotein cholesterol (LDL-C) modification hypothesis of atherogenesis, normal plasma LDL-C is modified by oxidative stress in the arterial wall into oxidized LDL (oxLDL), then bound and taken up through scavenger receptors by monocytes/macrophages chemoattracted by the oxLDL in the arterial wall. Progressive accumulation of cholesterol in the monocytes/macrophages turns them into foam cells^[20-22]. Then, the accumulation of foam cells in the arterial lesions leads to atherosclerotic plaque formation. Furthermore, it has been postulated that plaque rupture is often associated with thrombosis and plays a major role in acute coronary syndrome and cerebral infarction. However, the precise mechanism of plaque vulnerability leading to rupture has been unclear.

To investigate whether ORAIP has a role in atherosclerosis, especially in the mechanism of plaque rupture, we analyzed plasma levels of ORAIP and oxLDL in patients with dyslipidemia as well as heterozygous familial hypercholesterolemia (HeFH). We also examined the expression of ORAIP and the levels of oxLDL in atherosclerotic coronary arterial tissues obtained from HeFH patients with a coronary artery bypass graft. Plasma levels of LDL-C, oxLDL, and ORAIP in HeFH were significantly elevated as compared with those in dyslipidemia. ORAIP and oxLDL colocalized in the plaque lesion of coronary arteries from a HeFH patient [unpublished observation]. These findings suggested that high levels of plasma LDL-C facilitate oxidative stress in the arterial wall which, in turn, induces oxLDL accumulation, plaque formation, and ORAIP secretion, resulting in arterial cell apoptosis leading to plaque rupture. Although further investigations are needed, our findings suggest that anti-ORAIP therapy could be a way to reduce atherosclerotic plaque rupture and cardiovascular injury in patients with dyslipidemia, especially HeFH.

DIABETIC CARDIOVASCULAR COMPLICATIONS

Diabetic cardiovascular complications include microangiopathy, atherosclerotic macroangiopathy, and muscle injury. There are four main molecular mechanisms implicated in the hyperglycemia-induced cell injury: increased polyol pathway flux, increased advanced glycation end-product formation, activation of protein kinase C isoforms, and increased hexosamine pathway flux. All of these mechanisms have been proposed to reflect the hyperglycemia-induced overproduction of ROS by the mitochondria^[23]. We reported previously^[24] that plasma ORAIP levels in DM model rats were markedly elevated during the diabetic phase as compared to the non-diabetic control phase, and that there was a significant positive correlation between plasma levels of glucose and ORAIP. We also found that high glucose-induced massive apoptosis of cultured cardiac myocytes, which was largely suppressed by neutralizing anti-ORAIP mAbs *in vitro*. Furthermore, recombinant-ORAIP induced the apoptosis of pancreatic β -cells *in vitro*. Hyperglycemia induces pancreatic β -cell apoptosis leading to insulin deficiency^[25]. These findings strongly suggest that ORAIP plays a pivotal role in hyperglycemia-induced myocardial injury as well as pancreatic β -cell injury in DM. ORAIP may be a biomarker and a critical therapeutic target for myocardial injury and progression of insulin deficiency due to pancreatic β -cell injury in patients with DM.

Microangiopathy

Diabetic microangiopathy is often associated with three major complications: nephropathy, retinopathy, and neuropathy. It is proposed that capillary endothelial cells and glomerular mesangial cells are preferentially injured by hyperglycemia because these cells cannot reduce the transport of glucose inside the cell when they are exposed to hyperglycemia^[26]. Although we do not have data on whether these cells secrete ORAIP in response to hyperglycemia, as we reported previously that plasma levels of ORAIP were markedly elevated in patients undergoing dialysis largely due to diabetic nephropathy^[9]. These elevated

levels of ORAIP played a role in other complications such as cardiovascular injury and sarcopenia in these patients.

For diabetic retinopathy, it is thought that vascular endothelial growth factor (VEGF) plays a critical role in retinal neovascularization and diabetic macular edema. VEGF also plays a protective role against retinal apoptosis induced by oxidative stress (perhaps due to retinal I/R)^[26,27]. We reported that vitreous body concentrations of ORAIP were significantly increased in diabetic retinopathy, especially proliferative diabetic retinopathy, suggesting that ORAIP plays a role in oxidative stress-induced retinal cell injury^[12]. Therefore, the combination of anti-VEGF and anti-ORAIP therapies will be complementary against ischemic as well as I/R injury in diabetic retinopathy.

Because neurons (especially cerebral neurons) are susceptible to oxidative stress-induced cell injury, the long-term elevation of plasma ORAIP levels induced by hyperglycemia should injure peripheral neurons as well.

Atherosclerotic macroangiopathy

DM is often associated with hypertriglyceridemia due to insulin resistance. In addition, hyperglycemia in DM induces oxidative stress, which facilitates LDL-C oxidation in arterial tissues leading to plaque formation. Thus, hyperglycemia in DM often causes dyslipidemia, resulting in the development of atherosclerotic macroangiopathy involving coronary, cerebral, and peripheral arteries. Atherosclerosis in these large arteries leads to life-threatening ischemic heart disease, cerebral infarction, and arteriosclerosis obliterans. As mentioned in the “Dyslipidemia and atherosclerosis” section, oxidative stress in arterial tissues induces ORAIP secretion, which may result in apoptosis of arterial cells leading to plaque rupture. Further investigations are needed to prove the usefulness of anti-ORAIP therapy with a neutralizing mAb in protecting from atherosclerotic plaque rupture and cardiovascular injury in patients with DM as well as dyslipidemia.

Muscle injury

Myocardial injury, as well as sarcopenia, often develops later in the course of DM, causing various types of arrhythmias (such as atrial fibrillation and paroxysmal supraventricular tachycardia) and heart failure (such as diabetic cardiomyopathy). Because cardiac and skeletal myocytes are susceptible to oxidative stress-induced apoptosis mediated by ORAIP, and ORAIP has a critical role in hyperglycemia-induced myocardial injury^[24], hyperglycemia-induced high plasma levels of ORAIP may also mediate the development of sarcopenia.

Taken together, these data offer a possible anti-ORAIP therapy against vascular as well as cardiac and skeletal muscle injury involved in DM.

CARDIOVASCULAR COMPLICATIONS IN CKD

Cardiovascular complications critically affect the morbidity and mortality of CKD, especially in end-stage renal disease patients on dialysis. Because conventional cardiovascular risk factors such as DM, hypertension, hypercholesterolemia, and smoking are often associated with CKD, oxidative stress has been implicated in the mechanism of cardiovascular injury in CKD^[28], though the precise mechanism is still unclear. To investigate the roles of ORAIP in oxidative stress-induced cardiovascular injury in CKD, we analyzed the plasma levels of ORAIP in patients with the end-stage renal disease just before and after dialysis^[9]. Plasma ORAIP levels before dialysis were markedly elevated [93.6 ± 5.1 (mean \pm SE) ng/mL] as compared with those of control subjects (6.6 ± 1.5 ng/mL). After dialysis, plasma levels of ORAIP were not decreased, but rather slightly (and significantly) increased to 98.5 ± 5.7 ng/mL ($P = 0.0122$), suggesting that ORAIP may be a little concentrated, but not eliminated by dialysis.

To assess the effects of high concentrations of plasma ORAIP on the cardiovascular injury, we analyzed plasma levels of cardiac troponin T and brain natriuretic peptide (BNP). Plasma levels of cardiac troponin T were considerably elevated and there was a tendency of a positive correlation (but not significant) between plasma levels of ORAIP and cardiac troponin T^[9]. Because many factors may contribute to myocardial injury, the absence of a significant positive correlation between plasma levels of ORAIP and cardiac troponin T does not exclude the possibility that ORAIP may contribute to the myocardial injury. Plasma BNP levels were markedly elevated. There was also a tendency of a positive correlation (but not significant and weaker than that for cardiac troponin T) between plasma levels of ORAIP and BNP. This seems reasonable because cardiac troponin T directly reflects myocardial injury whereas BNP can be affected by several other factors such as overhydration in patients with end-stage renal disease on dialysis as well as myocardial injury. Although the primary mechanism of oxidative stress generation in CKD is unclear, the elevated plasma levels of ORAIP may cause sarcopenia and renal microvascular injury, resulting in the progression of CKD. Although further *in vivo* treatment studies are needed, these data offer support for a possible anti-ORAIP therapy to at least partially protect from cardiovascular injury and sarcopenia in patients with CKD.

CONCLUSION

For life, oxygen is a double-edged sword because it is not only essential for vital activities, but excessive oxygen can be harmful to various cells, known as oxidative stress. Since we discovered a novel secreted form of eIF5A to be ORAIP, we found that plasma levels of ORAIP were elevated in a wide range of acute and chronic disorders (including cardiovascular diseases), in which oxidative stress is known to be involved in the pathogenesis. Using animal models of such disorders, we also demonstrated that anti-ORAIP therapy with neutralizing mAbs could critically reduce oxidative stress-induced cell injury. We speculate that excessive external stresses involved in various pathological conditions including electromagnetic waves (such as ionizing radiation and ultraviolet), physicochemical stimuli (such as heat-shock, acidification, pressure overload, and high osmotic pressure) as well as excessive oxygen (such as ischemia/reperfusion) may trigger the oxidative stress-sensing mechanism of the cells, which results in the secretion of ORAIP. Then, secreted ORAIP induces apoptosis of the cells in an autocrine/paracrine fashion, that contributes to the progression of these disorders. Recently, we identified a cell-surface receptor for ORAIP (that is, ORAIP-receptor; unpublished data), through which ORAIP transduces the apoptotic signal intracellularly. From data of hyperglycemia-induced cell apoptosis^[24], it is strongly suggested that the ORAIP/ORAIP-receptor pathway mediates a great part of oxidative stress-induced cell injury. Therefore, anti-ORAIP therapy may be very efficient and effective in combination with conventional therapies against oxidative stress-mediated cell injury in various disorders, especially cardiovascular diseases.

DECLARATIONS

Authors' contributions

Seko Y contributed solely to the article.

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Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Review

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Management of cardiac manifestations in Takayasu arteritis

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Abstract

Takayasu arteritis (TA) is a chronic vasculitis involving large vessels of unknown aetiology, a disease that is more common among the Asian population and predominant in young women. Cardiac manifestations include hypertension and involvement of the cardiac valves, myocardium and coronary arteries. Surgery on these patients is always a challenge given the tissue quality and the disease activity. They are prone to long-term complications such as restenosis and graft occlusion, hence requiring lifelong surveillance. The prevalence of coronary artery disease (CAD) in TA ranges from 9 to 11%. Coronary artery bypass grafting is preferred to percutaneous coronary intervention, as the latter has a high rate of restenosis and major adverse cardiovascular events. As left subclavian artery is commonly involved, saphenous vein graft is advised as a conduit rather than internal mammary artery. Other surgical procedures described for CAD are surgical angioplasty of the left main coronary artery and transaortic coronary ostial endarterectomy. Aortic regurgitation in TA has an incidence of approximately 20%. These patients tend to have prosthetic valve detachment, paravalvular leak or pseudoaneurysm at the anastomotic site. Further repair of these valves have a high rate of failure. Considering these facts, it is advisable to do an aortic root replacement for TA patients than to consider an aortic valve replacement or David's procedure.

Keywords: Takayasu arteritis, coronary artery, aortic valve, myocarditis, ascending aorta, aortoarteritis, pulmonary arteritis



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INTRODUCTION

Takayasu arteritis (TA) is a chronic vasculitis involving large vessels. It mainly involves the aorta and its branches, pulmonary artery and coronary artery. Though the cause of TA is unclear, there is a strong association with genetic predisposition, environmental factors and role of microbes. The arterial wall hosts the pathogenic activated T lymphocytes and macrophages leading to granulomatous inflammation and ultimately vessel wall damage. Proinflammatory cytokines, namely interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), are elevated in TA, which correlates with disease activity^[1]. It is more common among young women and the Asian population. Recent surveys show that TA is increasing in prevalence among all ethnicities. In this review, we will discuss the management of the cardiac manifestations in TA - myocardial failure, pulmonary arteritis and involvement of coronary artery and aortic valve.

EPIDEMOLOGY

TA is rare, but most commonly seen in the Asian population and immigrant Asian population in Western countries. The prevalence of TA in Scandinavian countries has increased, probably because of the higher rates of immigration from Asia and Africa in the last decade^[2]. The incidence and prevalence of TA in the northwestern part of Turkey is similar to that of Japan. Norway and Sweden has reported higher a prevalence rate of TA than previously observed^[3,4]. The disease has a predilection for females with wide geographic variation ranging from 8:1 in Japan to 1.2:1 in Israel. In a recent Japanese study, females less than 40 years of age constituted a major proportion (83.8%) of the study cohort. The disease is five times more common in females than men, and an interesting feature reported in the study was that there was a larger proportion of elderly compared to a previous study^[5]. TA presents commonly in the second and third decades of life. In women, the disease peaks at 20 years of age^[6]. There are geographic variations of the involvement of the aorta and its branch vessels.

GENETIC PREDISPOSITION

The pathogenesis of TA has been associated with the human leukocyte antigen (HLA) class I (HLA-A, HLA-B, and HLA-C), HLA class II (HLA-DR, HLA-DQ, and HLA-DP) and non-HLA genes^[7]. Though an elaborate list of genes exists in literature, a few are shown in Table 1^[7,8]. There is definite HLA B52 allele association in TA beyond ethnicity^[1]. The correlation with TNF- α 308/G polymorphism has also been reported in TA. FCGR2A/FCGR3A, IL-12B, IL-6, RPS9/LILRB3 are the non-HLA loci related to TA. A report from Italy demonstrated an association with HLA DRB1 *0405 for early onset vasculitis^[8]. DRB1, DR2, DQ 1 are among other alleles related to TA.

DIAGNOSIS AND MANAGEMENT

Clinical presentation

Symptoms such as fever, weight loss, malaise, fatigue and myalgia due to systemic inflammatory reactions constitute the initial presentation of the disease. The inflammation of the carotid artery can cause carotidynia. Patients with vascular compromise may present with features of claudication pain, angina pectoris, transient ischemia attack and stroke. It is important to note that TA patients can be asymptomatic with underlying progressive disease^[9]. The appearance of a new vascular sign in patients such as femoral bruits, absent pulses, or blood pressure differences should alert the physician. Usually, vascular signs are late manifestations of TA, but they can be the presenting symptom of a patient. The specificity and sensitivity of these clinical signs are more than 90% when two abnormal findings are present and not when a pair of examination findings is present^[10]. In a series from Japan, the commonest complication was systemic hypertension followed by aortic regurgitation (AR). The complications were more common in men and patients with late referrals^[5].

Table 1. Associations between Takayasu arteritis and HLA and non-HLA alleles/loci

	Alleles/loci	Observed population	Type of association with TA
HLA class I	A (A10)	Japan	Possible protective role
	B39	Japan, Mexico	Susceptibility, linked to B52
	B5	Japan, India	
	B51	Japan, India, Turkey	Susceptibility (weak)
	B52*01	Japan, China, North America, Turkey, India, Mexico	Susceptibility (strong), severe disease, poor prognosis
	B67	Japan	Susceptibility (weak)
	Cw*07	Europe, America, Turkey	Possible protective role
	Cw*12	Europe, America, Asia	Susceptibility
HLA class II	DRB1*0405	North America, Europe	Susceptibility, early-onset disease
	DRB1*07	Japan, China	Susceptibility
	DPB1*09	China	Susceptibility
	DQw1	Japan	Susceptibility
	DQw2	Korea	Susceptibility (weak)
	DR2	Japan	Susceptibility
	DR7	Korea	Susceptibility (weak)
	DRB1*07	China	Susceptibility
non-HLA	IL6	America, Turkey	Susceptibility
	RSP9/LILRB3	America, Turkey	susceptibility
	IL12B	Japan	susceptibility, possible resistance to therapy
	TNFA 308A/G polymorphism	Japan, China	susceptibility

HLA: human leukocyte antigen; TA: Takayasu arteritis; IL: interleukin; TNF: tumor necrosis factor

Laboratory findings

Pentraxin 3 is produced in the inflammatory region by dendritic cells, vascular smooth muscle cells, fibroblasts and macrophages through the trigger of proinflammatory cytokines, especially TNF- α . The levels are raised in TA and have a better specificity and sensitivity in delineating active and inactive disease^[6,11,12]. Interleukins, such as IL-6 and IL-8, IL-18, BAFF and anti-endothelial and anti-aorta antibodies are correlated with disease activity in TA^[13]. Serum amyloid A is an acute phase protein produced in response to proinflammatory cytokines by activated macrophages. Serum amyloid A levels are significantly raised in patients with active disease^[6,14]. When there is vessel wall inflammation, HLA E is released from the endothelium in the soluble form. sHLA E can also be used as a marker of activity of TA^[15]. The acute phase reactants (ESR and CRP) though used to track disease activity, lack sensitivity and specificity. A study from the Cleveland Clinic showed 23% of patients with normal acute phase reactants in the setting of active disease^[16]. Another analysis from North America showed elevated acute phase reactants in 44% of patients who were considered to be clinically inactive. Furthermore, ESR was elevated in only three-fourths of the patients who had active disease^[17].

Imaging in TA

The majority of Ishikawa diagnostic criteria for TA are dependent on imaging studies. The available imaging modalities lack specificity for disease activity, which emphasizes the complementary role of physical examination and laboratory investigation in assessment of disease activity. The relative advantages and disadvantages of each imaging modality are described in Table 2^[18]. Lack of specificity for disease activity in available imaging tests highlights the complementary role of imaging in clinical assessment of disease activity. Historically, TA diagnosis relied on conventional digital subtraction angiography to identify stenosis, occlusions and aneurysms. The earliest detectable abnormality is usually the thickening of the vessel wall due to inflammation^[10]. Conventional digital subtraction angiography has the least sensitivity for visualizing wall thickness. A systematic review showed the presence of a low attenuation ring in computed tomography (CT) angiogram as 100% specificity for disease activity^[19]. Vessel wall thickening with enhancement had a sensitivity of 88% and specificity of 75%. MRI is highly accurate and sensitive,

Table 2. Merits and limitations of different imaging modalities to assess Takayasu arteritis

	Merits	Limitations
Conventional digital subtraction angiography	Evaluation of severity of stenotic lesions Assment of central blood pressure Concomitant therapeutic intervention as necessary	Invasive Risk of contrast induced nephropathy Radiation Inability to assess the thickness of arterial wall
CT angiography	Ability to evaluate stenotic and aneurysmal lesions Ability to measure arterial wall thickness When used in patients at high suspicion for TA, CTA has a sensitivity of 95% and specificity of 100%, using catheter-based angiography as the gold standard	Risk of contrast induced nephropathy Radiation
Magnetic resonance imaging	Ability to evaluate stenotic and aneurysmal lesions Vessel wall evaluation (thickening, oedema, degeneration) Better assesment of soft tissue when comapred to CTA No radiation exposureSensitivity and specificity of 100% vs catheter-based angiography	Decreased sensitivity for smaller branch involvement Overestimate degrees of severe stenosis or occlusion
18F-fluorodeoxyglucose positron emission tomography (FDG-PET)	Localise active inflammation and intensity of inflammation Sensitivity and specificity 70.1% and 77.2%for evaluation of disease activity	Not an angiographic study modality
Duplex ultrasound	Ability to evaluate localised areas of stenosis and aneurysm Non-invasive No radiation exposure Nocontrast	Unable to provide a "roadmap" of vascular lesions Unable to perform complete imaging of the aortic arch and descending aorta Operator dependent
Ansthoracic and transesophageal echocardiography	Non-invasive Ability for concomitant assessment of aortic root and aortic valve for insufficiency Can be used for surveillance of ascending aorta dilatation, detection of PHT, and possibly aortic wall thickening	Unable to provide a 'roadmap' of vascular lesions Unable to differentiate among pathologies causing hypo echoic aortic wall mural thickening Operator dependent

CT: computed tomography; CTA: CT angiography; TA: Takayasu's arteritis; PHT: pulmonary hypertension

and has the potential to assess disease activity and response to treatment^[10]. FDG-PET is an operator-independent, non-invasive metabolic imaging tool helpful in diagnosis of TA. It has a high sensitivity and specificity, which increases the overall efficacy of the modality in diagnosis.

General consideration in surgery

The disease goes through three phases - pre-pulseless, pulseless and burnt out. This may be an oversimplification of the complex disease process, and not all patients follow this outline. The few general principles that physicians should keep in mind when treating such patients are as follows^[20-23]: (1) usually an emergency surgery is not required as the stenotic lesions are well collateralized; (2) it is preferred to avoid surgery during the active phase. If required, suppress the active disease with medication before considering surgery; (3) TA patients are often on steroid therapy, making them high-risk surgical candidates due to effects of medications - for obesity, immunosuppression, bleeding diathesis; and (4) the disease is progressive, and hence, the patient should be on constant surveillance with medication. It is not uncommon to see complications such as restenosis, graft occlusion, graft site aneurysm and pseudoaneurysm due to the progressive nature of the disease.

The incidence of all the disease is shown in Table 3^[6,24,25].

CORONARY ARTERY DISEASE

Coronary artery disease (CAD) in TA was first described by Frovig and Loken in 1951, and Coronary artery bypass grafting (CABG) was first performed by Young and colleagues in 1973. Coronary angiographic and pathologic studies together have revealed coronary artery lesions in 9 to 11% of cases^[21]. CAD in TA is usually associated with lesions of peripheral branch arteries, and isolated CAD is present in less than 5% of patients^[26-28].

Table 3. Incidence of cardiovascular manifestation in Takasu's arteritis

	Incidence
Hypertension	34%-79.2%
Pulmonary arteritis	7.1%-18.9%
Coronary artery disease	5.20%-20.1%
Ischemic heart disease	10.60%
Myocardial failure	6.60%
Aortic regurgitation	33.20%-38.80%
Aortic aneurysm	15.00%-23.30%

Classification

The coronary lesions are classified into 3 types: (1) Type I: stenotic lesion in the ostial segment of the coronary artery. This is the most commonly encountered type in clinical practice; (2) type II: diffuse involvement of the coronary arteries or any focal segment of the artery - skip lesions; and (3) type III: aneurysm of the coronary artery.

Pathogenesis

Active inflammation leads to intimal proliferation and fibrous contraction in the region around the coronary ostium leading to the narrowing of the coronary artery. Ischemia is one of the major causes of death in TA patients. Though extremely difficult, a high degree of suspicion is necessary to diagnosis these patients in the pre-stenotic phase. In patients with diminished/absent pulse, it may take several months/years, before the coronary artery becomes involved. In a study from Korea, where CT aortogram was performed in 111 patients, there was a high prevalence of coronary artery abnormalities on coronary CT angiography, regardless of disease activity or symptoms. Hence, it was recommended to perform coronary CT angiography in all patients with TA for additional information^[29].

Management

Patients on conservative management have a grave prognosis with many dying of cardiac events^[30]. Revascularisation should be considered as early as possible. It is advisable to avoid surgery in the active phase; however, to avoid any cardiac accidents, revascularisation has to be performed in unstable patients. In such patients, corticosteroid (CS) or immunosuppressive therapy should be administered at the same time to control the inflammatory process^[31]. Biologic therapy, with namely anti-IL-6 (tocilizumab, TCZ) and TNFi (etanercept, infliximab and adalimumab), has been evaluated in TA. Improvement in disease activity was observed, and TA patients were able to taper or discontinue CSs after initiation of TCZ or TNFi. Even though there was clinical efficacy, radiological progression was the concern in TA patients on TCZ. There is evidence favoring the use of TCZ and TNFi in refractory and relapsing TA not responding to csDMARDs (conventional DMARDs). Abatacept (CTLA4-Ig) use in TA did not meet the primary endpoints in randomized controlled trials (RCTs). Rituximab (anti-CD 20) and ustekinumab (anti-IL-12/23) in TA gave variable results in isolated case reports^[32-34]. High-dose prednisolone (1 mg/kg/day) or its equivalent is the initial therapy for active TA with tapering dose for 3 to 6 months. CS dose of 0.5 mg/kg/day together with immunosuppressive agent such as mycophenolate has been recently suggested with equal early response. Tapering of steroids can be started in 4 to 6 weeks^[34]. Doses below 10 mg/day are associated with increased relapse^[34]. Conventional immunosuppressive agents are started simultaneously with CS or while tapering it. There are no RCTs on the use of conventional synthetic disease-modifying antirheumatic drugs for TA. Weekly methotrexate (15-25 mg/week) therapy has been the first option by many physicians because of low cost and easy availability. AZA (azathioprine) at 2 mg/kg/day along with CSs for a period of 12 months showed reduction in inflammatory markers with no radiological progression. Severe TA (retinal vasculitis, severe AR, myocarditis, or pulmonary vasculitis with or without aneurysm) cases were given oral cyclophosphamide at 2 mg/kg/day. Mycophenolate mofetil has been a safe and effective drug at a dose of 2 g/day in treating TA. Both clinical and radiological progression was prevented when given for

at least a period of one year. Leflunomide (20 mg/day), tacrolimus and cyclosporine A have been tried with favourable outcome^[33-36]. Vascular interventional procedures are better to be done during the inactive phase of the disease with perioperative continuation of immunosuppression for desired results^[34]. Preoperative course of steroids might reduce the vasculitic activity^[36].

It has been suggested by some authors that in active TA, endoluminal stenting and rotational atherectomy can be used to postpone surgery^[37]. Though long-term results do not favour percutaneous coronary intervention (PCI) for TA, PCI can be performed in an emergency situation, patients who refuse CABG or in high-risk individuals. With regard to MACE, CABG is superior to PCI despite medical therapy in TA patients with CAD.

A meta-analysis revealed that restenosis occurred more often with PCI than with open surgical intervention for coronary artery involvement^[38]. On a cohort of 75 patients who underwent revascularisation, a recurrent restenosis was demonstrated in more than three-fourths (78%) of angioplasties and 36% of surgical procedures^[16]. Very little exists in the literature with regard to PCI for CAD in TA patients, barring a few anecdotal case reports and small case series. Vasculitis, accelerated atherosclerosis and blood flow alteration due to structural changes in vessel wall lead to acute ischemic events in TA. TXB₂ (thromboxane B₂) level and platelet aggregation is increased in patients with TA. Low doses of aspirin are safe and useful in preventing acute events of ischemia in TA. Antiplatelet therapy in pre- and post-endovascular procedures reduce restenosis occurrence in TA^[33,39].

Surgery in CAD

In contrast to atherosclerotic disease, the use of the internal mammary artery raises concerns even though the TA patients are young. A study of 321 TA patients in Japan showed that the most common involved artery was the left subclavian followed by the carotid artery and right subclavian^[40]. Thus, the long-term benefit of using the internal mammary artery as a conduit rings a bell, while on the other hand, the use of saphenous vein graft has been recommended^[41]. In a review by Amano and Suzuki, saphenous vein grafts were used in 80% of patients^[31]. As TA is a progressive disease, there is intimal proliferation which can cause stenosis of the proximal anastomosis of the vein graft on the ascending aorta. Few authors have suggested replacing a segment of ascending aorta with a Dacron patch, to which the proximal anastomosis of the vein graft can be performed instead of the native aorta^[41]. Though long-term results are unknown, few authors have suggested the use of the free internal mammary artery and radial artery^[42,43]. Late coronary artery bypass grafting was insufficient in 10% of patients undergoing surgery^[6].

Other options for coronary interventions are surgical angioplasty of the left main coronary artery and transaortic coronary ostial endarterectomy^[44]. Surgical angioplasty can be performed using a piece of autologous pericardium, glutaraldehyde-treated pericardium, saphenous vein graft or a patch from the internal mammary artery. Transaortic coronary ostial endarterectomy was indicated in patients with localized lesions at the coronary ostium. The stenotic portion of the coronary artery is held with a piece of thread and resected piecemeal with a scalpel and later punched out with a 4-mm Aorta-Punch. Care has to be taken, as excessive resection may lead to perforation, bleeding, or hematoma at the junction of the aorta and the ostium of the coronary artery.

MYOCARDIUM IN TA

Myocardial failure in TA can be due to systemic arterial involvement, hypertension, acute or chronic AR, and pulmonary vascular involvement in patients with TA^[45]. It was demonstrated that the natural killer cells and T lymphocyte-mediated autoimmune cell injury can happen by releasing the cytotoxic factor and perforin in the vessel wall^[46]. Takeda *et al.*^[47] postulated a similar mechanism of myocardial involvement in certain patients with TA. An immunohistochemical study of the cardiac myocytes was also positive for

HLA classes I and II, and ICAM-1, indicating the involvement of an active inflammatory process^[48]. A study of 204 Korean patients showed elevated NT-proBNP in active TA. The possible reasons for the rise could have been due to elevated filling pressure of the ventricles, pulmonary arterial hypertension, left ventricular failure and/or AR in these patients^[49].

PULMONARY ARTERITIS

Pulmonary arteritis (PA) involvement in TA is not uncommon. The incidence of PA in patients with TA varies greatly in the literature (0-56%)^[50,51]. Pulmonary hypertension occurs in 12% to 13% of patients with TA and in 42.2% of patients with PA^[51,52]. As the patients present with non-specific respiratory symptoms and lack of vessel involvement, the diagnosis is usually delayed in these patients. PA manifests as pulmonary hypertension, which indicates a weak response to treatment and carries a poor prognosis^[52]. The median time from the initial symptoms to definitive diagnosis is 13.5 (1-186) months^[53]. High-dose glucocorticoid remains the standard of care and a combination of glucocorticoid and methotrexate or azathioprine prolongs disease remission. Few case reports of successful pulmonary artery revascularisations exist in the literature, but they are reserved for renal vascular hypertension, extremity claudication or cerebral vascular disease^[52].

AORTIC VALVE AND AORTIC ROOT

A study of 1,069 patients from China showed that more than one-third (34.9%) of the TA patients had cardiac valve involvement^[24]. Regurgitation lesions are more common than stenosis, with AR being the commonest lesion in TA patients. AR in TA was first described by Jervell in 1954^[54]. The incidence of AR ranges from 20.0% to 44.8% in different populations^[17,55,56].

Pathogenesis

It is believed that inflammation rarely involves the aortic valve and that AR develops primarily as a result of annular dilatation. The T lymphocytes and macrophages after infiltration, initiate an immunologic cascade in the aortic wall. This causes release of cytokines and matrix metalloproteinases, destroying the aortic wall^[57-59]. The inflammation involves the aortic wall from the adventitia to intima, damaging the media. This will make the aortic root fragile, which in turn is unable to bear the stress and start to dilate^[24]. The secondary AR jet induces morphological changes in the aortic valve leaflets such as fibrous thickening and enrolling and thereby worsens the AR itself.

Management

Chronic AR leads to heart failure, which is the main cause of death in patients with TA^[24]. Hence, the presence of AR necessitates early surgery before decompensating. However, surgical treatment of AR in TA is difficult because of the need to manipulate fragile and inflamed tissue. The disease actively has to be monitored as mentioned earlier. When examining a patient with AR, the physician should be aware of the common differential diagnosis in valvular heart disease- rheumatic heart disease, degenerative valvular heart. Echocardiogram should identify the pathology of the valve and establish the diagnosis.

Patients who undergo surgery are prone to prosthetic valve detachment, paravalvular leak or pseudoaneurysm at the anastomotic site. The incidence of postoperative anastomotic aneurysm has been reported to be 8.5%-13.8%, while that of the prosthetic valve detachment has been reported as 4%-25% in different series^[60]. Miyata *et al.*^[61] reported that occurrence of anastomotic aneurysm was not related to the presence of inflammation, preoperative use of steroids, or pathological stage. This anastomotic aneurysm could develop any time after the operation, and hence, the patients require lifelong surveillance. There are concerns about the dilatation of the ascending aorta after aortic valve replacement alone. Bougioukas *et al.*^[62] proposed avoiding valve-sparing aortic root replacement in TA patients, as there is a high incidence of

recurrent regurgitation. Matsuura *et al.*^[63] reported that the outcomes of aortic valve replacement (AVR) are comparable to aortic root replacement, though late dilatation of the residual ascending aorta is the major concern in long-term follow-up for patients with AVR. Redo-AVR was done in three-fourths of patients who underwent valve sparing aortic root replacement within 3 years of follow-up after the operation. Considering these facts, it is advisable to do an aortic root replacement for TA patients than to consider an AVR or David's procedure. Aortic root replacement can safely be performed and shows a higher event-free ratio when compared to AVR alone in TA^[47,63,64].

CONCLUSION

TA is a rare with a challenging course of disease process. Since the incidence is low, there are no formal guidelines for treatment of these patients. Considering the facts, the treatment has to be individualised to patients. It is preferred to avoid surgery in the active phase, and if required, disease activity can be controlled with medications and surgery. Long-term surveillance is required for all these patients as they are prone to complications.

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Authors' contributions

Contributed equally to the conception, data collection and writing of the manuscript: Idhrees M, Thilagavathi N, Bashir M, Velayudhan BV

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Review

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Neuroprotective effects of direct activation and transactivation of PDGF β receptors

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Abstract

Platelet-derived growth factor (PDGF) receptors are expressed throughout the body, including the central nervous system (CNS). Although the physiological role of PDGF receptors in the developed CNS is not fully characterized, PDGF signaling appears to provide neuroprotective effects against several neuronal insults. One of the best-characterized neuroprotective effects of PDGF type- β receptors is against human immunodeficiency virus (HIV) protein-induced neurotoxicity, with potential physiological relevance to HAD. PDGF β receptors are also neuroprotective against glutamate excitotoxicity, which is associated with both stroke and neurodegenerative diseases, including Alzheimer's disease. The neuroprotective effects of PDGF β receptors occur both via direct activation by ligand (PDGF-BB), as well as by PDGF β receptors activated downstream of G protein-coupled receptor signaling. In addition to the involvement of PDGF signaling in various pathologies and potential therapies, there is also an emerging body of evidence that PDGF may serve as a biomarker for neurological or psychiatric diseases.

Keywords: Platelet-derived growth factor receptor, serotonin, glutamate, transactivation

PDGF SIGNALING

There are two major platelet-derived growth factor (PDGF) receptor isoforms (α and β) and four ligand isoforms (A-D) that form homo- or hetero-dimers^[1]. PDGF ligands exist as dimers of four polypeptides:



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PDGF-A, -B, -C, and -D that can either homo- or hetero-dimerize into PDGF-AB, -AA, -BB, -CC, and -DD^[2]. The PDGF ligands act through PDGF α and β receptors^[1] and both are necessary for normal physiological development^[2]. Upon binding PDGF, receptors dimerize and the tyrosine kinase domains transautophosphorylate one another on multiple tyrosine residues. Effectors such as phospholipase C (PLC) γ , phosphatidylinositol 3-kinase (PI3-kinase), Src family tyrosine kinases, tyrosine phosphatases such as SHP-2, and a GTPase-activating protein for Ras are activated after phosphorylation of the PDGF receptor^[3]. Thus, by direct activation of effector proteins or via cytoplasmic adaptor proteins, PDGF receptors initiate a wide variety of downstream signaling pathways.

PDGF and its receptors are widely expressed throughout the central nervous system (CNS)^[4] and are involved in neuronal growth and differentiation^[5] although their physiological role in mature neurons is not yet fully characterized. PDGF β receptors are found in pyramidal neurons in the hippocampus^[6,7] whereas the PDGF α receptor and its primary ligand, PDGF-AA, are found in both neuronal^[8] and non-neuronal^[9] cells. Herein, we will briefly review the direct and indirect [i.e., GPCR - receptor tyrosine kinase (RTK) transactivation] neuroprotective effects of PDGF receptor signaling, with a focus on the PDGF β receptors. Sil and colleagues have recently reviewed the PDGF system in the central nervous system and provided a comprehensive and detailed description of PDGF expression and signaling in CNS cell types including neurons, astrocytes, microglia, oligodendrocytes, signaling in the spinal cord, and the role of the PDGF system in the blood-brain-barrier^[4].

THERAPEUTIC APPROACHES TO MODULATING PDGF SIGNALING

Several approaches to target PDGF signaling have been proposed or evaluated, and many hold promise despite technical, logistical, and therapeutic challenges. As large growth factors, PDGF ligands are classified as biologics and would require parenteral administration. Even so, they are too large to cross the blood-brain barrier and enter the CNS, thus would require intrathecal administration directly into the cerebrospinal fluid, or direct injection into the brain, such as intracerebroventricular injection^[10]. An alternative approach, discussed below, could be to indirectly transactivate PDGF receptors after the activation of G protein-coupled receptors (GPCRs). On the other hand, approaches to inhibit PDGF receptor signaling include antibodies that target PDGF ligands or receptors, small molecule inhibitors of PDGF receptor kinase activity, genetic manipulations, or targeting specific downstream signaling pathways at the receptor level^[2]. Papadopolous and Lennartsson recently reviewed the approaches to inhibit PDGF signaling in cancer therapies and provided a detailed description of current and future therapies that target the PDGF system^[11]. Indeed, beyond the neuroprotective roles discussed here, the PDGF system holds the potential to better understand and perhaps treat several non-neuronal disease states including cancer, fibrosis/connective tissue disorders, and vascular disease^[12].

HUMAN IMMUNODEFICIENCY VIRUS - ASSOCIATED NEUROCOGNITIVE DISORDER

Although specific dementias have distinct pathologies, disease courses, and outcomes, all include neuronal dysfunction, and many include disruptions in neurotransmitter homeostasis and signaling. Alterations in glutamate homeostasis in particular, including excitotoxicity, contributes to the pathology of ischemia and several neurodegenerative diseases^[13]. As dementias progress, accumulation of toxic proteins or aggregates, coupled with neuronal dysfunction and inflammation, reduce neuronal cell viability. Thus, there is a significant interest in the role of neuroprotective signaling pathways to treat these conditions.

Human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) are neurocognitive complications resulting from HIV infection and are classified as: asymptomatic neurocognitive impairment, mild cognitive motor disorder and HIV-associated dementia (HAD), in order of severity^[14]. As the efficacy of antiretroviral therapies has improved, the transformation of HIV-infection from a fatal disease to a

chronic one has resulted in an increase in the prevalence of HAND^[15]. Despite their effectiveness in the periphery, limited penetration of antiretroviral therapies into the CNS also contributes to adverse neuronal consequences^[16]. Although the virus does not infect neurons directly, the viral proteins released from infected perivascular macrophages and microglia in the CNS can interact with neurons and cause neuronal damage and loss^[17]. Several growth factors, such as brain-derived growth factor (BDNF) and fibroblast growth factor (FGF), as well as PDGF-BB, have demonstrated neuroprotective effects against HIV protein toxicity^[18-20].

Down-regulation of the PDGF-B chain at both the mRNA and protein level is observed in neuroblastoma SH-SY5Y cell cultures treated with gp120, an HIV envelope glycoprotein, and this protein exerts neurotoxic effects on SH-SY5Y cells, including increased cell death and loss of neurites^[21,22]. These toxic effects are attenuated by pretreating the cells with PDGF-BB prior to incubation with gp120 in both SH-SY5Y cells and rat cortical neurons^[21,22]. The neuroprotective effects appear to be associated with a reduction in apoptosis, as measured by reduced gp120-mediated activation of caspase-3 and increased Bcl-xL/Bax ratio^[21]. Peng and colleagues identified several effectors downstream of PI3K/Akt required for neuroprotective effects^[22]. GSK-3 β inactivation leads to nuclear translocation of β -catenin and NF- κ B, transcription factors that are involved in cell proliferation and differentiation^[22]. Additional potential neuroprotective mechanisms include phosphorylation of Bad, downregulation of the pro-apoptotic protein, Bax, and the inhibition of gp120-induced release of mitochondrial cytochrome C^[22].

PDGF is also neuroprotective against toxicity induced by another HIV-1 protein, Tat^[20,23]. PDGF-BB activation of the PDGF β receptor increases dendrite length and cell viability (via an anti-apoptotic effect) in rat primary midbrain neurons exposed to Tat^[23]. PDGF-BB also reduces reactive oxygen species (ROS) production and caspase-3 activation and these effects involve the regulation of ion channels, specifically, TRPC subtypes TRPC 5 and TRPC 6, and calcium influx^[24]. The PDGF-mediated neuroprotection against Tat via TRPC is also confirmed *in vivo*: PDGF is able to protect dopaminergic neurons in adult mice injected with HIV-1 Tat and TRPC activity contributes to the protection^[23]. Zhu *et al.*^[25] examined the PDGF-mediated neuroprotection against Tat in differentiated SH-SY5Y cells and observed the same neuroprotective effect observed by Yao in rat primary neurons and the neuroprotective effect also involves elevation of intracellular Ca²⁺ and requires Ca²⁺ influx. N-methyl-D-aspartate (NMDA) receptors may also be involved, as the NMDA receptor antagonist MK-801 is able to abolish Tat-induced toxicity in differentiated SH-SY5Y cells^[25].

In addition to direct neuronal toxicity, HIV proteins can impair neurogenesis in the hippocampus^[26]. The PDGF system is important for neurogenesis, both in the developing and mature CNS^[27]. PDGF-BB can increase the number of neural progenitor cells (NPC) in the rat hippocampus in the presence of Tat^[26,28] as well as after cocaine administration^[26,29]. Similar to the previous findings by the Buch group, a role for p38, JNK, MAPK, and GSK3 β and for the TRPC channel and calcium signaling are involved in this neuroprotective pathway^[24]. Knocking-down PDGF β receptor expression reduces neurogenesis after middle cerebral artery occlusion in mice, further suggesting a crucial role for PDGF-associated neurogenesis after neuronal injury^[30].

While many of the neuroprotective effects exerted by the PDGF system appear to be due to PDGF-BB, other ligands also exert protective effects. For example, PDGF-CC is also protective against Tat via similar downstream signaling pathways (PI3K/Akt/TRPC) as PDGF-BB^[31] and PDGF-CC was recently shown to reverse synaptic changes caused by Tat^[31].

STROKE AND ISCHEMIA

One of the major areas of PDGF neuroprotection studies has been focused on the direct inhibitory effect of PDGF on NMDA receptors and the associated neuroprotection against NMDA receptor-induced toxicity.

Valenzuela *et al.*^[32] electrophysiological studies were the first to reveal the inhibitory effect of PDGF on NMDA receptors. Brief activation of PDGF receptors by PDGF-BB can trigger long-lasting inhibition on NMDA receptors in rat hippocampal slices, cultured hippocampal neurons, and *Xenopus* oocytes^[32,33]. The mechanism of this inhibition involves PLC γ -induced elevation of intracellular Ca²⁺ and protein phosphatase activity downstream of Ca²⁺ signaling to modulate the NMDA receptor function^[33]. Follow-up studies determined that PDGF β receptor signaling selectively inhibits NR2B-containing NMDA receptors and decreases surface localization of NR2B subunits^[7]. Abelson tyrosine kinase activated downstream of PDGF β receptors or added directly (intracellularly) to hippocampal neurons similarly inhibits NMDA receptor signaling^[34]. In addition to the inhibition of NMDA receptor signaling, PDGF-BB also reduces oxidative stress and calpain activation induced by hydrogen peroxide via reduction in intracellular calcium and via the PI3 kinase signaling pathway^[35,36].

Excess glutamatergic signaling, and subsequent over-activation of NMDA receptors, is one of the signaling events associated with neurotoxicity after stroke. Tseng and colleagues directly examined the neuroprotective effect of PDGF-BB against glutamate- or NMDA-induced excitotoxicity in cultured hippocampal neurons and found that PDGF-BB pretreatment can protect neurons from these insults in both dose- and time-dependent manners^[37]. Pretreatment with 10 ng/ml of PDGF-BB for 24 h is required for maximal effect^[37], although as little as 10 min of PDGF-BB pretreatment is sufficient to protect neurons from NMDA receptor-induced toxicity, if applied immediately prior to the insult^[7]. Besides the direct inhibition of the NMDA receptor, PDGF-associated neuroprotective effects against excitotoxicity are also attributed to its ability to increase glutamate reuptake by modulating the activity of the glutamate transporter, EAAC1. Sims and colleagues found that PDGF can increase the activity and surface expression of EAAC1 and these effects depend on the activation of PI3 kinase^[38] and the activation of Akt^[39].

In vivo, pretreatment with PDGF-BB, but not AA, two days before forebrain ischemia in rats protects CA1 pyramidal neurons from delayed neuronal death on day 7 after ischemia in a dose-dependent manner^[40]. Continuous infusion of PDGF-BB for 7 days into the cerebral ventricles of gerbils with transient forebrain ischemia improves their performance on a passive avoidance task and subsequent histological examinations revealed that PDGF-BB increases neuronal survival and the number of remaining synapses^[41]. Administration of PDGF-BB into the left neocortex of Sprague-Dawley rats for 7 or 14 days before ischemia decreases the neocortical infarction with the size of infarction the smallest in the 14-day group^[42]. Egawa-Tsuzuki and colleagues found that PDGF-B infusion before and after NMDA injection reduces the size of lesions in young rats when their endogenous expression of PDGF-B in neurons is low^[43].

Clearly, the application of PDGF ligands preceding a controlled insult is neuroprotective, but likely not a feasible therapeutic approach in humans where the insult is not typically predictable. Interestingly, PDGF signaling may be used as an endogenous neuroprotective response by the CNS after the damage has already occurred. For example, focal ischemia in rat brains causes a rapid increase in PDGF-B chain isoform mRNA transcripts that peaks at 24 h^[44] (a similar upregulation of PDGF-B occurs in myocardial tissue after ischemia^[45]). PDGF-BB expression is also increased after ischemic preconditioning in the gerbil hippocampus^[46]. PDGF β receptor expression rises rapidly after ischemia in the rat brain^[47]. Furthermore, the expression of PDGF-A and PDGF-B mRNA and PDGF-BB and PDGF-AB dimer protein expression rises in neurons and supports cells surrounding areas damaged by ischemic events in humans^[48]. In addition, mice without the PDGF β receptor gene are vulnerable to NMDA receptor-induced excitotoxicity in terms of increased cell death and lesion size^[49]. There is a considerable amount of evidence that activation of the PDGF system prior to these neuronal insults is neuroprotective and that these insults also upregulate PDGF system components.

A NOTE ON THE BLOOD-BRAIN BARRIER

Although not a direct neuronal or neuroprotective effect, PDGF signaling has a significant, perhaps much more significant, indirect impact on neuronal health due to its effects on pericytes, angiogenesis, and the blood-brain barrier. As our focus is on the neuroprotective effects of PDGF signaling, we will not review PDGF's vascular effects in detail as these have been reviewed elsewhere^[24,50,51]. While the role of PDGF signaling in the blood-brain barrier is not yet fully understood, several lines of evidence demonstrate that PDGF signaling increases blood-brain barrier permeability, resulting in neuronal damage, while others have demonstrated positive effects of PDGF signaling on blood-brain barrier function and recovery after neuronal insults. Examples of the impact, both positive and negative, of PDGF signaling have been reported for stroke^[52-54], HAND^[24,55,56], Alzheimer's disease (AD)^[57], Parkinson's disease (PD)^[58], epilepsy^[59], and neuroinflammation^[60,61].

ALZHEIMER'S DISEASE

AD is a progressive neurodegenerative disease characterized by cognitive decline^[62]. One of the hallmarks of AD is amyloid- β accumulation which begins decades before clinical diagnosis of the disease^[62]. There is emerging evidence that the PDGF system is involved in AD pathology. For example, PDGF-BB is one of the cytokines that differs between post-mortem patients with high amyloid- β accumulation loads that did not have cognitive symptoms vs. those that did experience cognitive decline^[63]. Amyloid- β is produced by the proteolytic cleavage of the amyloid precursor protein (APP)^[64] and the PDGF system appears to play a role in APP homeostasis. In astrocytes, PDGF treatment increases sAPP α activity two-fold and this effect is blocked by the broad-spectrum tyrosine kinase inhibitor, genistein^[65]. Using a recombinant protein expressing system in Hela cells, Gianni and Zambrano, along with other colleagues, demonstrated that PDGF-BB can induce γ -secretase mediated APP proteolysis by activating PDGF receptors and found Src and Rac1 but not ERKs, PI3K or Abl tyrosine kinase were involved in that signaling pathway^[66,67]. Further studies have identified that the cytodomain of APP (containing YENPTY motif) is required for PDGF-induced APP proteolysis^[66,67].

The mechanism(s) of amyloid- β toxicity in neurons involve both non-specific and receptor-dependent pathways^[68-70], including interactions with receptor tyrosine kinases^[69]. In SH-SY5Y cells, application of PDGF-BB increases cell number and this effect is inhibited in the presence of amyloid- β ^[71]. PDGF-BB-induced phosphorylation of the PDGF β receptor is also blocked by amyloid- β , however, no physical association between amyloid- β -PDGF-BB was detected^[71]. Indirect activation (i.e., transactivation - see the section below) was not affected by amyloid- β ^[71]. We have previously demonstrated that direct or indirect (GPCR-mediated) activation of the PDGF β receptor can protect neurons against NMDA receptor-dependent toxicity^[72]. However in the presence of amyloid- β , PDGF-BB application was no longer able to prevent NMDA-induced toxicity, suggesting that amyloid- β may promote neurodegeneration by blocking a key neuroprotective pathway in the brain.

PARKINSON'S DISEASE

Similar to AD, there is evidence that PDGF and other neurotrophic factors may also be involved in the pathogenesis and treatment of PD due to both its neuroprotective and trophic effects (see two recent reviews^[73,74]). The levels of PDGF-BB and PDGF-AA are correlated with the level of plasma α -synuclein in PD patients, suggesting PDGF may be a potential biomarker for PD^[75]. PDGF-BB provides protective effects against a toxicant associated with PD, rotenone, by countering its effects on mitochondria and ROS production in both cell lines and in astrocytes^[76,77]. With the latter finding among those sparking interest in targeting PDGF signaling in astrocytes in neurologic disease as a therapeutic approach^[78,79]. These and other studies led to human trials with PDGF-BB in phase I clinical trial for PD (a dose-escalation study of intracerebroventricular PDGF-BB delivered via an infusion pump, from 0.2 to 5 μ g/day)^[10]. PDGF-BB

showed good tolerability and resulted in a dose-dependent increase in dopamine transporter expression measure via positron emission tomography (PET) scan, but little to no symptomatic improvement^[10]. Analogous to our own findings demonstrating amyloid- β could impair PDGF receptor signaling^[71], one of the pathogenic processes in PD, α -synuclein, impairs some PDGF-induced processes (chemotaxis) but not others (intracellular signaling to ERK)^[80]. In addition to PDGF-BB, PDGF-CC has demonstrated neuroprotective effects in several neuronal toxicity studies, including 6-OH-dopamine-induced neuronal cell death, in signaling pathways associated with GSK3 β ^[81].

NEUROPROTECTIVE EFFECTS OF PDGF RECEPTOR TRANSACTIVATION

Growth factor receptors such as the PDGF β receptor can be activated directly by their endogenous ligand or be *transactivated* by signals initiated through other receptors in the absence of PDGF ligand^[82,83]. Classic transactivation of growth factor receptors occurs within minutes after activation of GPCRs. For example, the activation of D2-family dopamine receptors initiates an intracellular signaling cascade that increases PDGF β receptor phosphorylation and activity, including an increase in ERK1/2 phosphorylation^[84]. The transactivation of RTK receptors by GPCR initiation may regulate a signaling cascade that differs from those activated by direct ligand binding^[85].

5-HT₇ receptors are G α s-coupled receptors; their activation leads to an increase both in adenylyl cyclase activity and in the intracellular level of cyclic AMP^[86]. 5-HT₇ receptors are expressed in the prefrontal cortex, hippocampus, thalamus, hypothalamus, and the amygdala in both neurons and support cells^[87]. 5-HT₇ receptors increase the number of dendrites and promote synapse formation in neurons^[88]. 5-HT receptors including the 5-HT₇ receptor are also able to transactivate PDGF β receptors (and TrkB receptors)^[89,90].

PDGF β receptors can inhibit NR2B-containing NMDA receptors and this can result in neuroprotective effects. Similarly, transactivating PDGF β receptors, downstream of dopamine receptors, also results in neuroprotection against NMDA-induced toxicity^[91]. 5-HT₇ receptor agonists also lead to an increase in the expression of PDGF β receptors and its phosphorylation at tyrosine 1021, the PLC γ binding site associated with PDGF-induced inhibition of NMDA-evoked currents^[89]. After the application of 5-HT₇ receptor agonists to cultured cells or primary hippocampal or cortical cultures, the cells are resistant to NMDA-induced toxicity due to the upregulation and activation of PDGF β receptors^[72]. In addition, activation of the 5-HT₇ receptor also selectively changes the expression and phosphorylation state of the NR2B subunit of the NMDA receptor, similar to what was observed with dopamine and PDGF-BB-induced changes in NMDA receptor expression^[72]. Interestingly, although long-term agonist treatment with 5-HT₇ ligands inhibits NMDA receptor activity via PDGF β receptors, acute, direct activation of 5-HT₇ receptors increases NMDA-evoked currents^[92].

PDGF β receptor transactivation may also play a role in BBB function. PDGF β receptor signaling protects endothelial cell function and BBB integrity after β -arrestin activation subsequent to protease-activated receptor 1^[93]. Thus, in addition to GPCR-PDGF β receptor transactivating and directly impacting neuronal health, transactivation or indirect activation of PDGF signaling may also impact BBB integrity in the context of stroke or other neurological diseases.

PDGF AS A BIOMARKER

As noted above, the impact of the PDGF system on the vasculature may be more important clinically than the direct effects of this signaling pathway on neurons. Similarly, although there are intriguing possibilities for the development of novel treatments targeting the PDGF system in neuronal health, using PDGF ligands as biomarkers for disease may be more impactful, at least in the short term. For example, the presence of PDGF-AA in cerebrospinal fluid (CSF) of HIV-infected individuals was negatively associated

with HAND^[94]. Stroke patients have higher levels of circulating PDGFβ receptor-positive cells^[95]. PDGF-BB is consistently among the growing number of proteins and molecules identified as biomarkers in the CSF of AD patients^[96-98] and is potentially a serum biomarker for PD^[99]. PDGF-BB levels are also elevated in patients with amyotrophic lateral sclerosis^[100]. PDGF-BB may be a biomarker for schizophrenia^[101] as well as having links to the disease via genetic differences in both PDGF-B and PDGFβ receptor genes^[102]. Last, mutations in PDGF-B and PDGFβ receptor genes are both biomarkers for, and causative agents of, familial brain calcification^[103,104].

CONCLUSION

PDGF signaling is crucial for healthy CNS development. In recent years, its role in the developed CNS, and even in the aging CNS, has been linked to various neuropathologies and neuroprotective pathways. Understanding, and possibly targeting, the PDGF system will be crucial to our understanding and treatment of stroke and neuronal damage, dementias including HAND and Alzheimer's disease, and in Parkinson's disease. In addition to understanding ligand-activated PDGF receptor signaling, indirect, GPCR-mediated transactivation of the PDGFβ receptor, provides a link between GPCR-targeted therapies and PDGF system changes.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study: Vasefi M, Beazely MA

Participated in drafting the article and revising the content: Vasefi M

Contributed in revising the article and approved the revised version: Beazely MA

Availability of data and materials

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Original Article

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Troponin status predicts 30-day in-hospital mortality

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Abstract

Aim: To evaluate the prognostic value of high-sensitivity cardiac troponin (hscTnT) levels in unselected emergency medical admissions.

Methods: We report on all hscTnT tests in emergency medical admissions, performed over an eight year period from 2011-2018. The prognostic significance of hscTnT was related to 30-day in-hospital mortality with multivariable logistic regression, adjusted for Acute Illness Severity Score, Comorbidity Score, Sepsis, and Deprivation Status.

Results: There were 52,214 admissions from 28,982 patients during the study period. HscTnT level was a univariate - odds ratios (OR) 1.67 [95% confidence intervals (CI): 1.60-1.73] and an independent risk predictor in the multivariable logistic regression model - OR = 1.23 (95%CI: 1.16-1.29). 30-day in-hospital mortality increased as a linear function of hscTnT; not performed = 3.6%, ≥ 25 ng/L = 5.3%, > 100 ng/L = 7.4%, > 1000 ng/L = 8.8%. Increasing Comorbidity Score exacerbated risk; 30-day in-hospital mortality at a Score of 6, 10 and 16 points for those with no hscTnT performed or hscTnT < 25 ng/L were 1.8%, 6.5% and 31.3% respectively; for hscTnT ≥ 25 ng/L these increased to 2.2%, 8.8% and 41.3%.

Conclusion: HscTnT is prognostic in acutely ill medical patients; incorporation into hospital mortality predictive algorithms appears warranted.

Keywords: Troponin levels, emergency medical admissions, hospital mortality



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INTRODUCTION

Acute medicine encompasses the rapid specialist management of patients suffering from a wide variety of medical conditions requiring expedited care^[1]. The admitted cohort are a relatively high risk group, although 30-day in-hospital mortality rates in our institution have improved over time from 6.7% in 2002, to 4.7% in 2012, and 3.7% in 2018; the 7% absolute mortality reduction is comparable with the USA National Hospital Discharge Survey data showing a decrease of 8% in inpatient hospital deaths over the years 2000 to 2010^[2]. Nonetheless there has to be a constant emphasis, if outcomes are to be maintained and even improved, on risk factor assessment at admission and identifying prognostic factors amenable to intervention - the two most important of these are illness severity and comorbidities^[3]. As our hospital covers an inner city catchment area with a predominantly aging population and over 50% of patients have a high deprivation index^[4] - we also have a focus on the impact of low socioeconomic status (SES).

Among biomarkers that have become available over the past decade, serum troponin T as a cardio-specific biomarker has achieved widespread use in clinical medicine; with cardiac injury these macromolecules diffuse into the cardiac interstitium with subsequent detection in the peripheral circulation. Cardiac-specific troponins have utility because they combine value as a near ideal biologic marker, and also convey useful prognostic information that can influence therapeutic decision making^[5]. Troponin assays were initially developed for their utility in acute coronary syndromes^[6], however, it was quickly recognized that a range of other medical conditions were also associated with troponin elevation^[7-20]. Although troponin elevation signifies cardiac damage, it may not always imply cardio-specificity with predictive values as low as 56% in the diagnosis of acute coronary syndromes^[21]. Troponin assays are a relatively common laboratory diagnostic request in emergency medical admissions.

There has been little published on the utility of troponin in unselected emergency medical admissions. We have a large database covering all emergency medical patients admitted to our institution (St James' Hospital, Dublin), between 2002 and 2018 inclusive - but high sensitivity troponin data was limited to 2011-18. The purpose of this study was to examine the predictive role of high sensitivity cardiac troponin (hs-cTnT) levels on 30-day in-hospital mortality in acutely ill medical patients admitted via the Emergency Department (ED).

METHODS

Background

Our institution, St James's Hospital provides an emergency admission function covering a population of 270,000 adults. This paper covers all patients admitted under the general/internal medicine service from 2011-2018; patients with acute coronary syndromes (ACS) are by design admitted under cardiology and were not included in this analysis. Medical admissions are by design admitted from the ED to an Acute Medical Admission Unit (AMAU). We have described the details of the operation and performance of the AMAU previously^[22-26]. As a city centre hospital, St James's admits visitors to Dublin city and persons people residing outside of the hospital catchment area, but working in the city, in addition to visitors to the city. 74.5% of emergency admissions are resident in our catchment area.

Data collection

We have established an anonymous patient database within our institution. This database collates information for each hospital admission including details from the patient administration system, national hospital in-patient enquiry (HIPE) scheme, the electronic patient record and laboratory result systems. HIPE is a national database of coded hospital discharge summaries from all public hospitals within Ireland^[27,28]. The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) was used for both diagnosis and procedure coding from 1990 to 2005 with a subsequent transition to

ICD-10-CM. Data recorded in the database include the date of admission and discharge, the unique patient identifier, sex, date of birth, treating physician, address, diagnosis (principal and up to nine secondary), and procedures performed (principal and up to nine secondary). Physiological and laboratory results are automatically cross-linked and added to the database using the hospitals other systems.

Troponin measurements

In January 2011, our institution introduced a hscTnT assay (Roche Diagnostics) to replace the previous 4th-generation cardiac troponin assay. Analysis was performed using a Roche cobas® 8000 analyser. No changes to the analyser or assay were made during the study period. The performance characteristics of the hscTnT assay were such that upper limit of normal was represented by the 99th centile of 14 ng/L. However, as non-ischaemic myocardial strain could result in an elevated hscTnT, a value of ≥ 53 ng/L was deemed to be a strong predictor of significant myocardial injury. In the current study, we have defined troponin-negative as hscTnT < 25 ng/L and troponin-positive as hscTnT ≥ 25 ng/L. We further divided patients into six groups based on hscTnT results: (1) no troponin assay requested; (2) < 25 ng/L; (3) 25-49 ng/L; (4) 50-99 ng/L; (5) 100-1000 ng/L; and (6) > 1000 ng/L. These cut-offs were chosen on the previously defined rounded hscTnT value of 50 ng/L being predictive of true myocardial injury.

Risk predictors

We have previously derived and applied an Acute Illness Severity Score (AISS)^[29], predicting 30-day in-hospital mortality from laboratory parameters recorded in the ED^[30]. This weighted age adjusted score defines six risk groups (I-VI) with cut-points for 30-day in-hospital mortality set at 1, 2, 4, 8 and 16%. We further adjusted for Comorbidity as described below. In addition Sepsis categories of (1) no blood culture request (2) negative blood culture and (3) positive blood culture were identified and used as an adjustor in the multivariable logistic regression model^[31].

Comorbidity score

In this study, comorbidity was assessed by a Comorbidity Score which we have derived^[32]. The first incarnation of this score was published in 2014 and an updated version has subsequently been published^[3]. The Comorbidity Score was derived from searching the hospital system for ICD codes that captured functionally limiting chronic physical or mental health disorders. These ICD codes were then grouped into the following ten systems: (1) cardiovascular; (2) respiratory; (3) neurological; (4) gastrointestinal; (5) diabetes; (6) renal; (7) neoplastic disease; (8) others (including rheumatological disabilities); (9) ventilatory assistance required; and (10) transfusion requirement. We additionally searched other existing databases in our institution for evidence of diabetes (Diamond database)^[33], impaired respiratory function ($FEV_1 < 2$ L), hscTnT ≥ 25 ng/L^[34], albumin < 35 G/dL, haemoglobin < 10 G/dL, and chronic kidney disease - MDRD < 60 mL/min \times 1.73 m²^[35]. The components of the score were then appropriately weighted according to 30-day in-hospital mortality.

Deprivation index

The smallest unit for which the Republic of Ireland Census reports results is the Electoral Division. There are approximately 3,440 of these small administrative areas; data from sparsely populated Electoral Divisions are merged where required to maintain confidentiality. This process resulted in a final total of 3,409 Electoral Divisions having available statistics. The SAHRU investigators employed principle components analysis to generate a Deprivation Index using a weighted combination of four indicators; unemployment, social class, type of housing tenure and car ownership^[36]. Deprivation Index scores were then divided into quintiles according to their ranked raw scores from Q1 (least deprived) to Q5 (most deprived). This data was then joined to the small area polygon geometries based upon their relative geographic positions, using the ArcGIS Geographic Information System software implementation of the Point-in-Polygon algorithm^[37].

Statistical methods

We generated descriptive statistics for background demographic data, using mean and standard deviation, median and inter-quartile range (IQR), or percentage where appropriate. We used chi-square tests for comparisons between categorical variables and 30-day in-hospital mortality. Over our prolonged 8 year study period, many patients were admitted more than once; 62%, 42% and 30.4% were admitted more than once, twice, or three times respectively, with 7.9% admitted > 10 times. There will be a difference in mortality rate if calculated by admission or by patient (only last admission considered if > 1); in this study calculated mortality is therefore explicitly stated as per admission or as per patient. In order to allow for clustering we utilized a logistic regression model with robust estimate^[29]. This logistic regression analysis was used to identify potential predictors of mortality in our dataset. Identified significant univariate predictors, as defined by $P < 0.1$ by Wald test, were then examined in the multivariate model to achieve optimized prediction. We adjusted 30-day in-hospital mortality for other known predictor variables including AISS^[29,38], Comorbidity Score^[39,40] and Sepsis status^[31]. We used computations of average marginal effects to estimate and interpret adjusted predictions for sub-groups while controlling for other variables. The model parameters were stored; post-estimation intra-model and cross-model hypotheses could thereby be tested. We calculated adjusted OR and 95%CI for significant predictors. Statistical significance at $P < 0.05$ was assumed throughout. Stata v.15 (Stata Corporation, College Station, Texas) statistical software was used for analysis.

RESULTS

Patient demographics

There were a total of 52,214 emergency medical admissions in 28,982 patients over the 8 year study period (2011-2018). 48.6% of admissions were male. The median (IQR) length of stay (LOS) was 5.0 (2.1, 9.5) days. The median (IQR) age was 64.7 (45.2, 78.9) years, with the upper 10% boundary at 86.2 years. Between 2011 and 2018, there was a linear decline in 30-day in-hospital mortality. Calculated per admission episode, the 30-day in-hospital mortality averaged 3.9% (95%CI: 3.8%-4.1%) with no statistical change over time ($P = 0.07$). Calculated on a per patient basis (last admission if > 1), the 30-day in-hospital mortality averaged 7.1% (95%CI: 6.8%-7.4%) with a relative risk reduction of 39.7% from 8.1 % to 4.9% ($P = 0.001$) and calculated NNT of 31.1.

The baseline characteristic of admissions stratified by hscTnT level are outlined in [Table 1](#). Admissions with a positive hscTnT result were older at median (IQR) 75 years (60.2, 83.6) vs. 61 years (42.4, 76.4). Gender balance appeared similar at 49.8% vs. 50.2%. Admissions with a positive hscTnT had a longer median (IQR) LOS at 6.6 days (3.1, 12.3) vs. 4.6 days (2.0, 8.7). Admissions with a positive hscTnT were much more likely to have high AISS (Group 5/6 81.6% vs. 54.5%), Sepsis status (Culture positive 3.7% vs. 2.8%) and Comorbidity Score (> 10 points - 65% vs. 41.3%). They had more Major Disease Categories of respiratory (MDC 4) and cardiac (MDC 5), but less neurology (MDC 1) in primary diagnoses.

HscTnT level and 30-day in-hospital mortality

HscTnT was a univariate linear predictor of 30-day in-hospital mortality [OR 1.67 (95%CI: 1.60-1.73)]. In the multivariable model adjusted for other significant risk predictors of AISS [OR 2.59 (95%CI: 2.25-2.98)], Comorbidity Score [OR 1.28 (95%CI: 1.25-1.30)] and Sepsis Status [OR 1.19 (95%CI: 1.06-1.33)], the OR was 1.23 (95%CI: 1.16-1.29). Irrespective of whether 30-day in-hospital mortality was calculated either by patient or by all admissions, mortality increased as a linear function of hscTnT result [[Figure 1](#)]. Per admission 30-day in-hospital mortality with no troponin performed was 3.6 % (95%CI: 3.4-3.9), but for hscTnT ≥ 25 ng/L this rose to 5.3 % (95%CI: 4.9-5.6); and there were further mortality elevations at troponin levels ≥ 100 ng/L and 1000 ng/L to 7.4 % (95%CI: 6.6-8.3) and 8.8 % (95%CI: 7.5-10.0) respectively.

Table 1. Demographics of emergency admissions (2011-2018) by hscTnT status

Factor	Level	hscTnT -	hscTnT +	P-value
N		40,484	11,730	
Gender	Male	19,652 (48.5%)	5,836 (49.8%)	0.02
	Female	20,832 (51.5%)	5,894 (50.2%)	
Age, median (IQR)		61.0 (42.2, 76.4)	75.0 (60.2, 83.6)	< 0.001
Length of stay (days)		4.6 (2.0, 8.7)	6.6 (3.1, 12.3)	< 0.001
Outcome	Alive	39,368 (97.2%)	10,788 (92.0%)	< 0.001
	Died	1,116 (2.8%)	942 (8.0%)	
AISS	1	1,426 (4.2%)	160 (1.5%)	< 0.001
	2	2,868 (8.4%)	321 (3.0%)	
	3	4,852 (14.3%)	559 (5.2%)	
	4	6,302 (18.6%)	935 (8.7%)	
	5	7,035 (20.7%)	1,518 (14.2%)	
	6	11,470 (33.8%)	7,225 (67.4%)	
Sepsis status	1	32,332 (79.9%)	8,591 (73.2%)	< 0.001
	2	7,011 (17.3%)	2,701 (23.0%)	
	3	1,141 (2.8%)	438 (3.7%)	
Co-morbidity score	< 6	23,765 (58.7%)	4,090 (34.9%)	< 0.001
	< 10	13,037 (32.2%)	5,121 (43.7%)	
	< 13	2,793 (6.9%)	1,781 (15.2%)	
	< 16	648 (1.6%)	485 (4.1%)	
	< 20	231 (0.6%)	231 (2.0%)	
MDC	Neuro	7,724 (18.0%)	876 (11.9%)	< 0.001
	Resp.	10,417 (24.3%)	2,327 (31.7%)	
	Cardiac	6,378 (14.9%)	1,796 (24.5%)	

hscTnT: high-sensitivity cardiac troponin; AISS: acute illness severity score; LOS: length of stay; IQR: inter-quartile range; MDC: major disease category

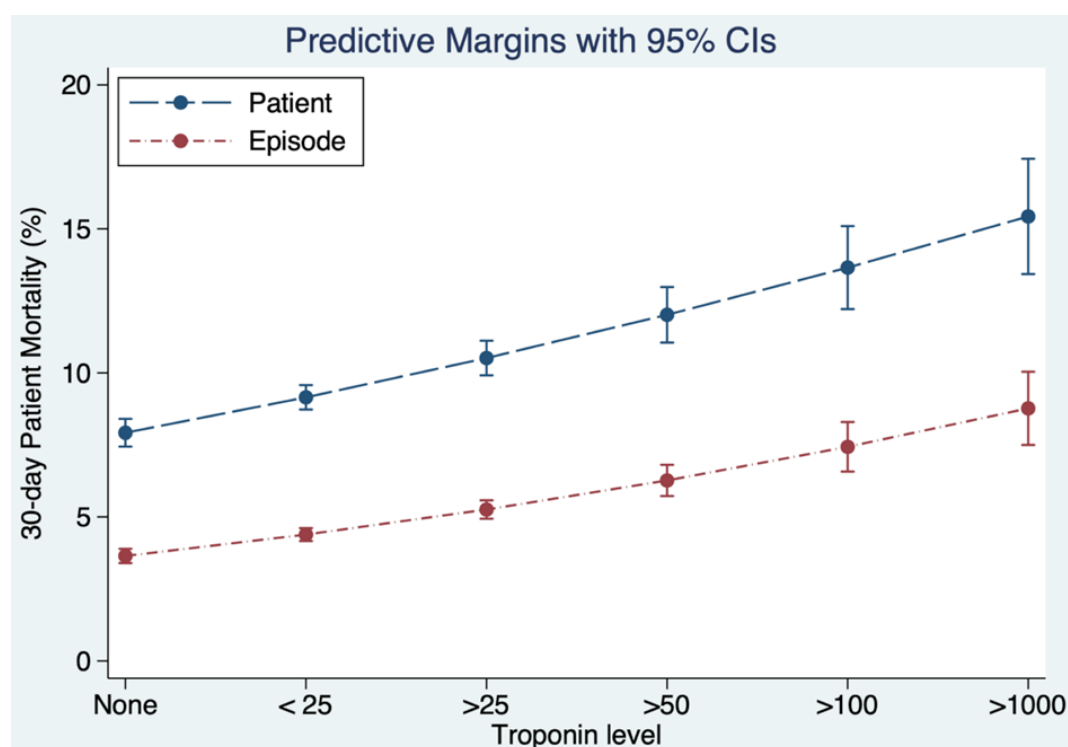


Figure 1. hscTnT level (ng/L) linearly predicted outcome. The multivariable logistic regression model was adjusted for Acute Illness Severity Score, Comorbidity Score, and Sepsis status. Increasing hscTnT level predicted 30-day in-hospital mortality (calculated on per admission episode or on unique patient basis) derived from and plotted based on the model prediction. hscTnT: high-sensitivity cardiac troponin

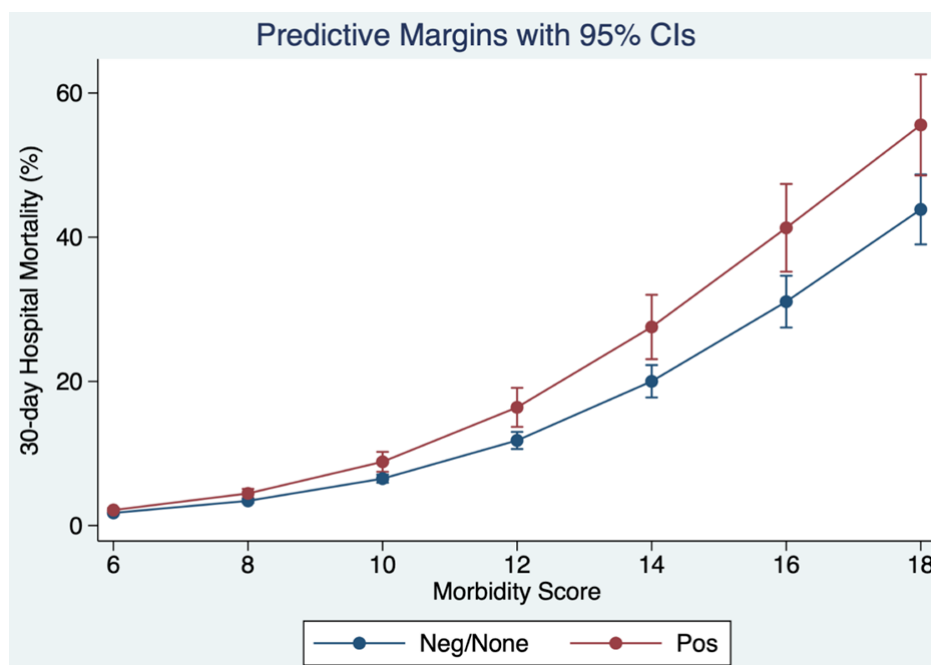


Figure 2. 30-day in-hospital mortality (per admission) related to Comorbidity Score and by hscTnT category from the multivariable logistic regression model. The predicted probabilities were derived from and plotted based on the model prediction. Data adjusted for Acute Illness Severity Score, Sepsis status and hscTnT category [negative (< 25 ng/L) or not performed *vs.* positive (\geq 25ng/L)]. hscTnT: high-sensitivity cardiac troponin

Conditional Dependence of hscTnT on Comorbidity Score

It is important to appreciate the relationship between hscTnT level, the underlying Comorbidity Score, and 30-day in-hospital mortality. Increasing Comorbidity Score was associated with a marked deterioration in 30-day in-hospital mortality [Figure 2]. We considered a Comorbidity Score of 10 points the inflexion point between lower and higher risk with only 11.3% of admitted patients being in this category. The model predicted 30-day in-hospital mortality per admission at a Comorbidity Score of 6, 10 and 16 points for those with no hscTnT performed, or a hscTnT < 25 ng/L were 1.8%, 6.5% and 31.3%, but in the presence of hscTnT \geq 25 ng/L, this increased to 2.2%, 8.8% and 41.3% respectively.

30-day in-hospital mortality for no hscTnT performed *vs.* hscTnT < 25 ng/L

The result of a test clearly conveys information, however, information may also be implied in the performance of a test, even if the result is negative. In our study, 30-day in-hospital mortality for patients with no hscTnT performed and hscTnT < 25 ng/L were quite different, but this observation applied mainly to high risk individuals. Overall, the mortality outcomes for the groups of no test, hscTnT < 25 ng/L, and hscTnT \geq 25 ng/L were 2.7%, 3.9%, and 10.3% ($P < 0.001$: one-way ANOVA with Scheffé's multiple comparison). At the lower end of Comorbidity Score, the difference appeared very small, but at Comorbidity scores of 10, 14 and 16 points, the difference in 30-day in-hospital mortality for no test *vs.* hscTnT < 25 ng/L were (10 points) 3.3% *vs.* 5.0% (14 points) 8.0% *vs.* 13.3% and (16 points) 12.1% *vs.* 20.0% [Figure 3].

Do those with lower SES have greater risk?

We have previously reported that in general our patients with lower SES have worse outcomes^[39,41]. We analysed, based on lower or higher SES, 30-day in-hospital mortality outcomes related to hscTnT. Admissions from high SES were older and with higher Comorbidity Score. Admissions from low SES areas had a median age of 66.4 years (IQR 48.2, 79.6) *vs.* 80.5 years (IQR: 65.6, 86.5) from higher SES areas. The

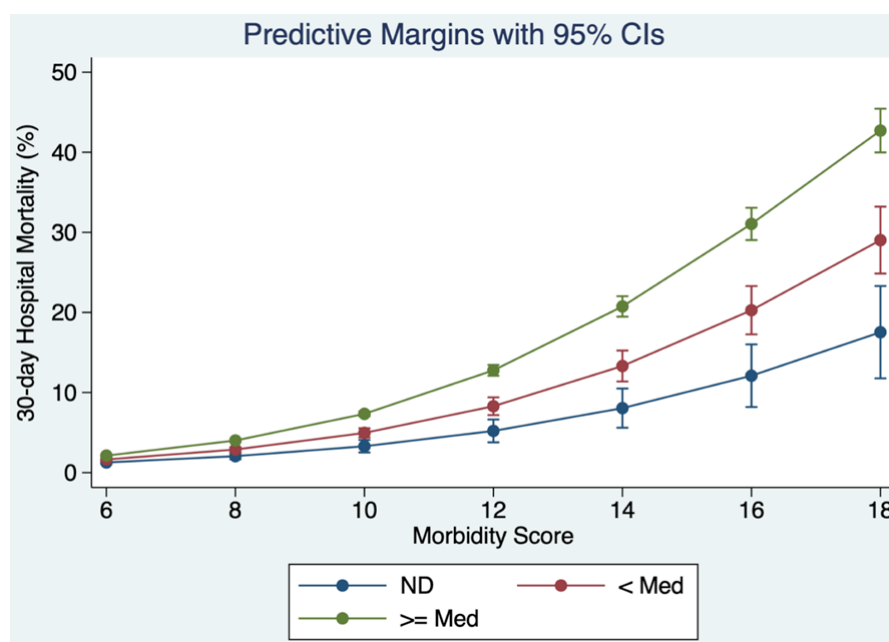


Figure 3. 30-day in-hospital mortality (per admission) related to Comorbidity Score and by hscTnT from the multivariable logistic regression model. The predicted probabilities were derived from and plotted based on the model prediction. Data was adjusted for Acute Illness Severity Score, Sepsis status and hscTnT category [not performed (ND) and hscTnT above or below median]

data demonstrated, that irrespective of SES, the older patients from higher SES had worse outcomes [Figure 4] and that the predictive effect of hscTnT was not modulated by SES.

DISCUSSION

Our study has demonstrated the prognostic value of hscTnT in emergency medical admissions. Patients with hscTnT ≥ 25 ng/L were more likely to have sepsis, high illness severity, and greater levels of comorbidity. Nonetheless, whether analysis was performed on the basis of an admission or unique patient basis, hscTnT was an independent predictor of 30-day in-hospital mortality in the multivariable model with a direct relationship to the level of hscTnT elevation. The decision to perform a hscTnT was associated with increased mortality although this observation mainly applied to high risk individuals; undertaking the test defined a risk group selected on clinical criteria or concern. Patients admitted from areas of low SES had better outcomes paradoxically, irrespective of Comorbidity Score, most likely because they were much younger in general compared to admissions from high SES areas.

Our results showed a clear relationship between the degree of hscTnT elevation and outcomes. Our data may then be seen to be quite different from other studies that have debated the significance of, and the level at which troponin elevation becomes a concern. These have focused on cut-points based on analysis of the literature. Khan *et al.*^[42] performed a meta-analysis of 28 studies; the troponin T assays chose similar cut points (i.e., 0.1 ng/mL) to detect abnormal levels. This cut point was close to or greater than the threshold at which there is a 10% total coefficient of variance; it would be ~ 5 -fold higher than the lower limit of detection. Arguments might revolve around a cutoff at the 99th percentile of a healthy reference population with a CV $\leq 10\%$ or a cutoff value of 0.01 $\mu\text{g/L}$ - the lowest value at which the coefficient of variation was $\leq 10\%$. It is therefore very surprising that our data shows not only that any troponin detection above the LOD carries prognostic significance, but selection of a group on the basis of a decision to perform a laboratory test - those with a hscTnT < 25 ng/L have higher risk than those who had no hscTnT test requested.

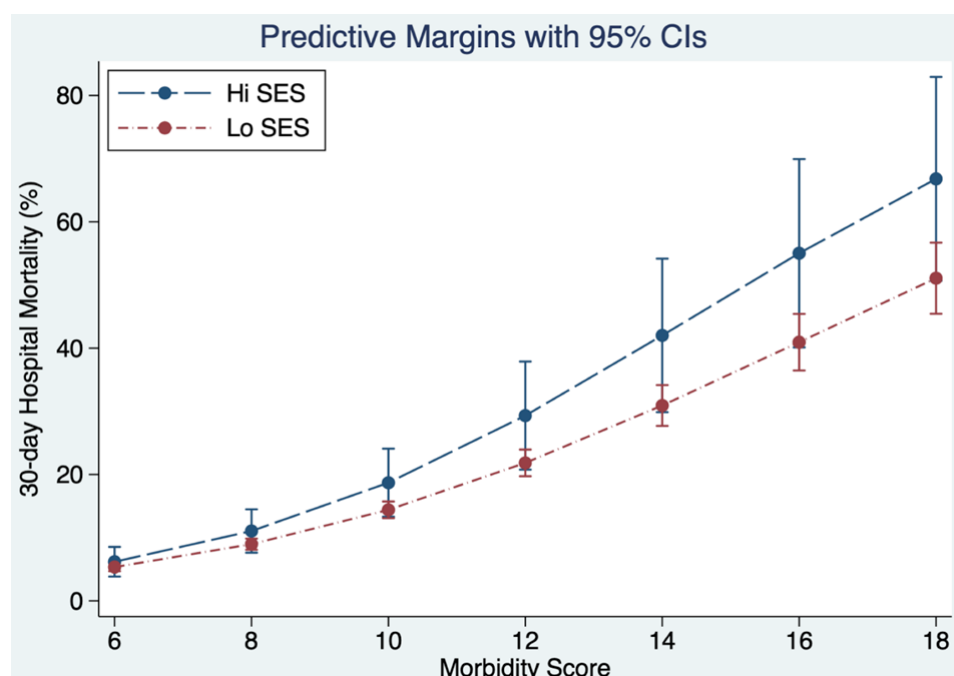


Figure 4. 30-day in-hospital mortality for patients with hscTnT ≥ 25 ng/L related to Comorbidity Score and SES from the multivariable logistic regression model. The predicted probabilities were derived from and plotted based on the model prediction. Data was adjusted for Acute Illness Severity Score, and Sepsis status. Higher SES patients (being older) had worse outcomes. SES: socioeconomic status

However, most previous studies have lacked the ability to measure two of the most important prognostic factors determining hospital survival - illness severity (measured by AISS in our study) and comorbidity (measured by the Comorbidity Score in our study). These two modifiers are of vital importance as prognostic indicators for a very good reason. The AISS, based on admission laboratory data, attempts to quantify homeostatic compensation and thereby relates an individual's status at presentation to the clinical outcome. The fundamental principle relies on the assumption that failure to maintain the internal biochemical milieu, by mounting a counter-regulatory corrective response to a stressor, will have consequence - the extent of departure from the normal status being a measure of the 'at risk' status. Essentially, admission laboratory data is deployed to construct an illness severity score that is predictive of outcomes - AISS is the strongest predictive variable in the multivariable logistic model - OR 2.59 (95%CI: 2.25-2.98). A high comorbidity burden generally speaking is a less significant, but still important, problem in emergency admissions than AISS with an OR of 1.28 (95%CI: 1.25-1.30). The caveat is that the nature of the interaction between the AISS and Comorbidity Score (the threshold effect of the latter relative to mortality outcome lowers as the AISS increases) that one needs both to accurately compute risk. Many studies lack the ability to measure or adjust for such in multivariable logistic models, and so incorrectly attribute the risk of poor outcomes to the prognostic variable of interest.

Published studies on the prognostic value of troponin encompass a range of different clinical conditions^[7-20], many of which are primary cardiac conditions such as congestive heart failure^[20], myocarditis^[18], aortic stenosis^[43] and atrial fibrillation without coronary disease^[17]. Troponin has most obviously been shown to be a prognostic marker in myocardial infarction^[44]. It is also a strong predictor in infective endocarditis with an OR of 3.4^[45]. In 105,338 hospitalized heart failure admissions, Peacock *et al.*^[46] determined troponin levels with 6.2% having positive troponin results; the adjusted OR for death with a positive troponin test was 2.55. In a meta-analysis of community based chronic heart failure patients, troponin T was associated with all-cause mortality with a hazard ratio of 1.48^[47]. In surgical ICU patients, Relos *et al.*^[48] reported that even moderately elevated troponin I levels - below the threshold to diagnose overt myocardial infarction,

were associated with a higher mortality rate and longer hospital and ICU length of stay. A study in acute respiratory distress syndrome reported a high prevalence of elevated cardiac markers^[49] and an associated increased 60-day mortality and organ failure. Thus, occult or subclinical myocardial disease could be implicated as a cause of death in acute respiratory distress syndrome. Other areas, where elevated troponin values have been demonstrated to be predictive of in-hospital mortality have included ischaemic stroke^[50], intra-cerebral haemorrhage^[51], gastro-intestinal haemorrhage^[52], non-cardiac surgery^[53], renal failure^[54], and following renal transplant^[55].

The strengths of our study include the large number of included patients, the comprehensive assessment of all admitted medical patients, and the collection of large volumes of relevant clinical data. The exclusion of patients admitted with ACS is another strength as risk stratification of patients with ACS has been well described and is a potential confounder in any study of this nature. As with any study, there are also potential limitations to our work. We have shown that hscTnT predicts outcome in our multivariable model after adjustment for known collected variables; it is possible that residual unknown or unmeasured confounders remain. This may even be a probable explanation for some of our results, particularly for the association between the performance of hscTnT and mortality, which is likely to be explained by unmeasured factors that are incorporated into the clinicians' gestalt decision to perform a hscTnT test. Our study, while large, was performed in a single centre and the results will require external validation in other settings. We did not have the ability to assess the possibility that patients originally admitted to St. James' may have subsequently been admitted to other hospitals; this may be particularly relevant for those resident outside our catchment area. Additionally, we have examined the relationship in emergency medical admissions only; admissions under other disciplines may not necessarily demonstrate the same relationship. The demonstration of a relationship does not imply that the variable is amenable to intervention, nor does it follow that any attempted intervention may not have deleterious consequences^[56].

In conclusion, we have demonstrated the prognostic value of hscTnT in emergency medical admissions. This suggests potential additive benefit to the inclusion of hscTnT in risk prediction models.

DECLARATIONS

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Authors' contributions

Made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Conway R, Byrne D, Cournane S, O'Riordan D, Coveney S, Silke B

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was approved by the institutional review board of St. James's Hospital. Full ethics committee review was not required as this study used routinely collected, anonymised clinical data and no interventions were performed.

Consent for publication

Not applicable.

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Review

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SNRK: a metabolic regulator with multifaceted role in development and disease

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Abstract

Sucrose nonfermenting 1-related kinase (SNRK) is a serine/threonine kinase and a member of the adenosine monophosphate (AMP)-activated protein kinase (AMPK) family that is involved in the metabolic regulatory mechanisms in various cell types. SNRK is an important mediator in maintaining cellular metabolic homeostasis. In this review, we discuss the role of SNRK in metabolic tissues where it is expressed, including heart and adipose tissue. We discuss its role in regulating inflammation in these tissues and the pathways associated with regulating inflammation. We also discuss SNRK's role in vascular development and the processes associated with it. Finally, we review SNRK's potential as a target in various metabolic dysfunction-associated diseases such as cardiovascular diseases, diabetes, obesity, and cancer. This comprehensive review on SNRK suggests that it has therapeutic value in the suppression of inflammation in cardiac and adipose tissue.

Keywords: Sucrose nonfermenting 1-related kinase, metabolism, inflammation, cardiac function, cardiomyocytes, ischemia, adipocyte, endothelial cells

INTRODUCTION

During embryonic development and cellular differentiation, protein kinases play a vital role in maintaining cellular homeostasis. Protein kinases constitute an exceptionally large family that has been estimated to



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include more than 1000 mammalian proteins. The sequence similarity of protein kinases in their catalytic domains indicates that they have evolved from a common precursor protein^[1]. Protein kinases play an important role in signal transduction by phosphorylating specific amino acids of downstream substrates and catalyzing the conversion of substrate proteins into phosphoproteins. The phosphorylation can be reversed by protein phosphatases. Protein phosphorylation is one of the common forms of cellular regulation during various cellular processes including metabolism, proliferation, differentiation, motility, survival, and death. Protein phosphorylation, first described in eukaryotes, is a post-translational modification of proteins whereby a phosphate group is covalently attached to a serine, threonine, or tyrosine residue^[2,3]. Eukaryotic serine (S), threonine (T), and tyrosine (Y) kinases are grouped together in the eukaryotic protein kinase superfamily based on sequence homology in their kinase domains.

The two main groups of the superfamily, the serine/threonine kinases and the tyrosine kinases can be subdivided further into smaller families which are composed of enzymes that show similar substrate specificities and mode of regulation^[4]. Serine/threonine kinases (STKs) transfer phosphate group from Adenosine triphosphate (ATP) to the OH (hydroxyl) group on the side chain of a serine or threonine amino acid residue in a protein, producing ADP and a phosphoprotein. STKs are involved in the regulation of cellular proliferation, programmed cell death (apoptosis), cell differentiation, and embryonic development^[5]. Similar to STKs, tyrosine-kinase enzymes transfer a phosphate group from ATP to a tyrosine residue in a protein. Tyrosine-protein kinases are classified into two main groups: (1) receptor tyrosine kinases, which are attributed to transmembrane proteins involved in signal transduction and play key roles in growth, differentiation, metabolism, adhesion, motility, death, and oncogenesis^[6]; and (2) cytoplasmic/non-receptor tyrosine kinases, which act as regulatory proteins, playing key roles in cell differentiation, motility, proliferation, and survival^[7]. Several kinases are activated by auto- or trans-phosphorylation on at least one S/T/Y residue in the activation loop by a second kinase^[8].

Sucrose nonfermenting 1-related kinase (SNRK) is a novel member of AMP-activated protein kinase (AMPK) subfamily of STKs. The AMPK family members share sequence homology with other members of the family in their catalytic domain^[4]. SNRK was first identified in 1996 in 3T3-L1 adipocytes where its expression was observed during differentiation into an adipocyte-like cell^[9]. SNRK is a monomeric enzyme containing a nuclear localization signal (NLS) domain, an ATP-binding domain, and an active S/T kinase domain with a conserved T-loop threonine residue (T173)^[10] [Figure 1]. Kinases that regulate SNRK activity have been identified. For example, liver kinase B1 (LKB1) phosphorylates multiple kinases especially the AMPK family, including SNRK. LKB1 phosphorylates substrates (AMPK and AMPK-related kinases) at the T-loop threonine residue^[11]. Unlike many AMPKs, SNRK does not require an additional stimulus for activation such as increased AMP:ATP^[12] ratio within a cell. SNRK also phosphorylates several proteins including Rho-associated kinase (ROCK)^[12-19]. Thus, SNRK regulation and its associated signaling partners and pathways are emerging areas of research. Further, SNRK's role in various cell types in metabolic tissues such as cardiac and adipose is also emerging. We have compiled a list of publications that suggests SNRK's role in numerous cell types, and its influence on the underlying cellular processes [Table 1]. In this review, we focus on SNRK's role in the regulation of metabolism and inflammation in heart and adipose tissue, and the impact of SNRK's dysregulation on metabolic and inflammatory-associated disease conditions.

SNRK: STRUCTURE, EXPRESSION, AMPK FAMILY ASSOCIATION AND ACTIVATORS

SNRK structure

The AMPK family members have been extensively characterized. These kinase enzymes include AMPK α 1, AMPK α 2, MARK1/2/3/4, SIK1/2/3, NUA1/2, BRSK1/2, and MELK^[25]. The AMPK protein family contains similar domain organization, namely, an N-terminal kinase domain and an adjacent ubiquitin-associated (UBA) domain. SNRK is a novel member of the AMPK family, and a 2.9Å resolution crystal structure of its N-terminal fragment containing the kinase and adjacent UBA domain is now available^[10].

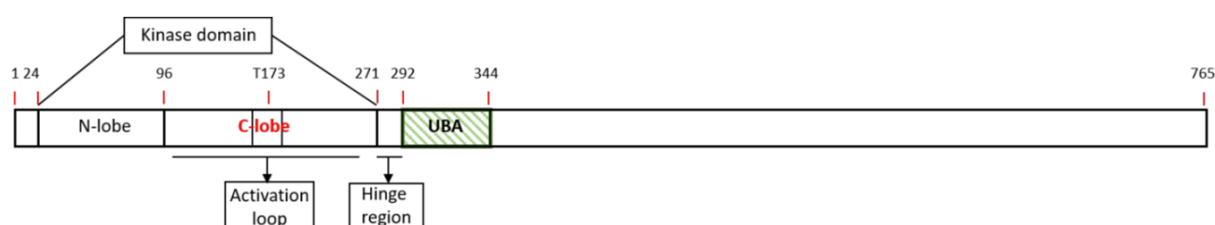


Figure 1. Schematic diagram of SNRK. A linear schematic of the various domains in SNRK is depicted. The numbers on top of the bars denote amino acid. UBA: Ubiquitin-associated domain; SNRK: sucrose nonfermenting 1-related kinase

Table 1. The role of SNRK in various cellular systems is shown

System	Function	Role of SNRK in the system	Ref.
Cardiac system	Cardiac metabolism	Regulates cardiac metabolism through phospho-acetyl-CoA carboxylase (ACC) and phospho-AMPK signaling pathway	[15]
	Cardiac functioning	Regulates Rho-associated kinase (ROCK) signaling pathway and mitochondrial efficiency through uncoupling protein 3 (UCP3) and mitochondrial uncoupling	[13,16]
	Cardiac inflammation	Represses inflammation by regulates NF- κ B phosphorylation	[14]
Adipose system	Adipocyte glucose metabolism	Regulates insulin signaling mediated glucose uptake through PPP2R5D and Akt phosphorylation	[17]
	Adipocyte inflammation	Represses inflammation in white adipose tissue through JNK and IKK β pathways	[19]
	Adipose thermogenesis	Represses WAT inflammation and regulate BAT thermogenesis through UCP1 and PGC1 α	[18]
Vascular system	Vasculogenesis	Maintain angioblast populations and control angioblast numbers in embryonic vascular development through DUSP5	[20]
	Angiogenesis	Promote endothelial angiogenesis by activating ITGB1 (β 1 integrin)-mediated endothelial cell migration	[21]
Renal system	Kidney inflammation	Represses inflammation by directly interacting with NF- κ B phosphorylation	[22]
Colorectal system	Colon cancer	Inhibits colon cancer cell proliferation through upregulation of calcyclin-binding protein (CacyBP) and β -catenin degradation	[12]
Ovarian system	Ovarian cancer	Omental adipocytes transport fatty acids for rapid growth, progression, and metastasis of ovarian cancer cells	[23]
Neuronal system	Neuron apoptosis	Regulates low K ⁺ -induced apoptosis in cerebral neurons	[24]

SNRK: sucrose nonfermenting 1-related kinase; AMPK: AMP-activated protein kinase; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; PPP2R5D: serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform; Akt: protein kinase-B; JNK: Jun N-terminal kinase; IKK β : I κ B kinase β subunit; WAT: white adipose tissue; BAT: brown adipose tissue; PGC1 α : peroxisome proliferator-activated receptor γ isoform α ; DUSP5: dual-specificity phosphatase 5; ITGB1: Integrin beta-1

The SNRK sequence is annotated to include a putative kinase domain (residues 24-270) and a hinge region (residues 271-291) which connects to the UBA domain (residues 292-344) [Figure 1]. The kinase domain consists of two lobes namely a N-lobe and a C-lobe. The N-lobe of the kinase domain consists of β -sheets [β 2 to β 5] and a prominent α C helix. The C-lobe of the kinase domain is mainly α -helical and contains the activation loop^[10] [Figure 1]. The UBA domain of the SNRK is composed of three α helices (α 1 to α 3) and binds to the kinase domain through the hinge region. This binding facilitates interaction of both the N- and C-terminal lobes, which is unique compared to other UBA: kinase domain interactions in the AMPK family. The structure of the UBA domain in SNRK inhibits the kinase activity and thus regulates SNRK's activity^[10]. Further, the UBA domain is unique among AMPK family members, and this characteristic triggers and defines specific downstream signals^[26-28].

SNRK activation by upstream kinases

SNRK possesses a conserved threonine (T) residue within its activation loop sequence. However, the identity of the activation loop sequence is not highly conserved among other AMPK-related kinases. LKB1 activates SNRK by phosphorylating its T-residue 173 (T173). The T residue in the activation loop is

referred to as “T-loop,” and is part of the three residues Leu (L)-Arg (R)-T that is conserved in the AMPK protein family. An important step in LKB1 activation is its export from nucleus to the cytoplasm, and this nuclear transport of LKB1 requires L-rich peptides. Kinases without a -2 L residue before the T-loop residue cannot get phosphorylated or activated by LKB1 substrate. LKB1 possesses a strong preference to phosphorylate T compared to the L residue at the -2 position^[29] in the T-loop. Interestingly, SNRK and the 13 other AMPK subfamily kinases that are phosphorylated and activated by LKB1 (AMPK α 1, AMPK α 2, MARK1/2/3/4, SIK1/2/3, NUAK1/2, and BRSK1/2) has the L residue at the -2 position in the T-loop. LKB1 gets phosphorylated at S325, T366, and S431 residues by upstream kinases and is auto-phosphorylated at S31, T185, T189, T336, and S404^[30] residues. Interestingly, mutation in any of these phosphorylation sites does not significantly affect its intracellular localization^[31,32].

LKB1 was originally identified as a mutated gene in the inherited Peutz-Jeghers Syndrome (PJS), in which subjects are susceptible to developing benign and malignant tumors^[33] in the gastrointestinal organs stomach and intestines. LKB1 protein is complexed with STRAD-related adapter (STRAD), an inactive pseudokinase^[34], and mouse protein 25 (MO25)^[35], a repeat domain scaffold protein that is responsible for activation of AMPK family kinases. Phosphorylation of S307 residue in LKB1 facilitates the binding of LKB1 to the STRAD and MO25 complex, which enables the nucleocytoplasmic transport of LKB1-complex^[36,37] LKB1:STRAD:MO25 complex and Mg-ATP results in a rapid (20 min) 5-fold activation of SNRK in the cytoplasm^[11]. Therefore, the heterotrimeric complex of LKB1, STRAD α or STRAD β , and MO25 α or MO25 β is required to obtain maximal activation of SNRK.

Role of SNRK in cardiomyocyte and adipocyte metabolism

To facilitate cell growth and maintenance, chemical reactions associated with cellular metabolism such as glucose, fatty acid, and amino acid metabolism occurs within a cell. During periods of stress such as low nutrient environment, the maintenance of cellular metabolism - in turn energy reserves in a cell - is critical. AMPK is a key sensor of energy needs in a cell. SNRK like AMPK is beginning to show similar critical roles in metabolism, and is observed in multiple tissues including adipose and cardiac tissues^[13-19]. Central to maintaining cellular energy homeostasis is the control of ATP generation and utilization. We discuss next the emerging evidence regarding SNRK's role in maintaining cellular energy homeostasis [Figure 2].

SNRK in cardiac metabolism

In the heart, the myocardium needs to function throughout the life of the organism. In the myocardium, cardiomyocytes (CMs) are the powerhouses that generate energy. In the prenatal or *in utero* heart, glucose and thus glycolysis is necessary for CMs growth. In late gestational and early postnatal stages, glucose uptake is significantly decreased, creating an intracellular glucose deprivation during development^[38]. In the late phase of cardiac fetal development, circulating lactate contributes to the majority of cardiac oxygen consumption^[39,40] while glucose and fatty acid oxidation (FAO) contribute relatively less^[41,42]. In terms of *Snrk*, during embryonic development, it is expressed at both mRNA and protein levels in tissues that have high demand for metabolic activity^[19]. The heart is comprised of vascular endothelial cells (ECs) and smooth muscle cells, in addition to CMs, all of which express SNRK^[15]. Loss of *Snrk* in all tissues [global knockout (KO)] results in neonatal lethality with enlarged hearts observed at E17.5 and P0^[15]. Microarray analysis of E17.5 hearts revealed systemic metabolic dysregulation^[15]. Glycogen, a polysaccharide (serves as glucose storage) was decreased in E17.5 *Snrk* global KO hearts. Similarly, lipid storage deposits demonstrated by oil red O (ORO) staining was also less in E17.5 *Snrk* global KO heart tissue. Circulating lipid plasma levels were also lower in *Snrk* global KO mice. Thus, global loss of *Snrk* results in defects associated with cardiac tissue energy sources.

The phospho-AMPK-phospho-acetyl-CoA carboxylase (ACC)^[15] is one of the key signaling pathways associated with cardiac metabolism in neonates and adults. AMPK decreases FAO by phosphorylation

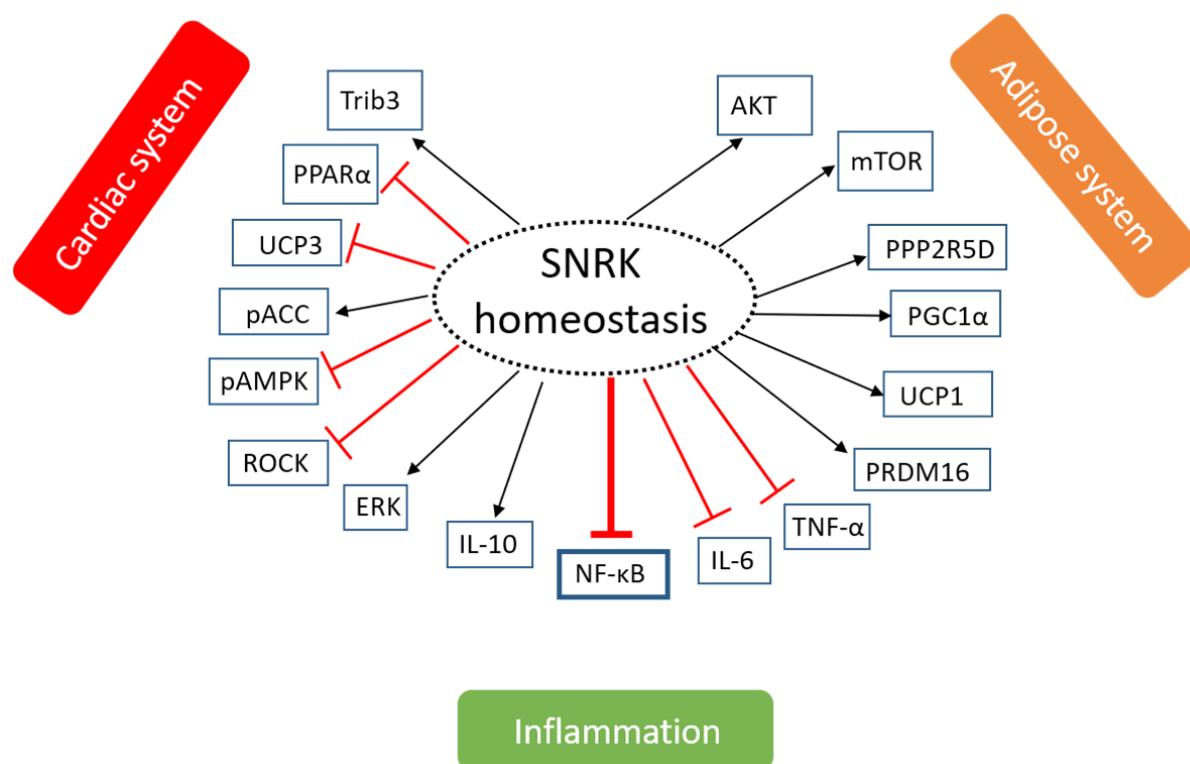


Figure 2. The role of SNRK in maintaining tissue homeostasis. Phosphorylation or signaling of SNRK to maintain homeostasis in three systems - cardiac system, adipose system, and inflammatory system is shown. The arrow indicates whether the phosphorylation is activating (black) or inhibiting (red) for the function of the target protein. The proteins under each system have been implicated to communicate with SNRK in that system. SNRK: sucrose nonfermenting 1-related kinase; Trib3: tribbles homologue 3; PPARα: peroxisome proliferator-activated receptor α; UCP3: uncoupling protein 3; ACC: Acetyl-coA carboxylase; AMPK: adenosine monophosphate-activated protein kinase; ROCK: Rho-associated kinase; ERK: extracellular-signal-regulated kinase; IL-10: interleukin 10; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; IL-6: interleukin 6; TNF-α: tumor necrosis factor α; PRDM16: PR domain containing 16; UCP1: uncoupling protein 1; PGC1α: peroxisome proliferator-activated receptor-γ co-activator 1α; PPP25RD: protein phosphatase 2 regulatory subunit B'Delta; mTOR: mammalian target of rapamycin; AKT: protein kinase B

of ACC1 at S79 and ACC2 at S212 residue. ACC enzymes generates malonyl CoA from acetyl CoA (a byproduct of FAO pathway). Phosphorylation of ACC is inhibitory and thus prevents the generation of malonyl CoA. This relieves the inhibition on a transporter (carnitine palmitoyltransferase) that allows acyl CoA to enter mitochondria for b-oxidation and tricarboxylic acid (TCA) cycle to generate acetyl CoA and ATP respectively. In Po *Snrk* global KO hearts, pAMPK and pACC levels were down compared to wild type hearts suggesting malonyl CoA accumulation and thereby inhibition of carnitine palmitoyltransferase and loss of b-oxidation or FAO^[15]. Thus, SNRK promotes FAO via the pAMPK-pACC pathway in the neonate Po hearts.

As the transition from the neonate to postnatal stage ensues, the heart of a newborn will adapt to the changing environment which is characterized by an increase in contractile demand due to the rapid growth and increase in activity of the newborn^[43]. This results in an increase in energy demand that can be provided by FAO and mitochondrial ATP production. The high fatty acid content of the maternal milk in many species is an effective way to supply the high energy demand of the newborn heart. As the newborn heart matures, the utilization of FAO to meet the overall energy production needs increases, and thus becomes the dominant substrate in the adult heart. Under normal metabolic conditions, over 95% of ATP generated in the heart is derived from oxidative phosphorylation. Only 5% of the remaining comes from glycolysis and to a lesser extent from the TCA cycle^[44]. Accordingly, the high-energy phosphate pool in the heart, ATP, is relatively small and can be exhausted within a few seconds. Therefore, cardiac work depends

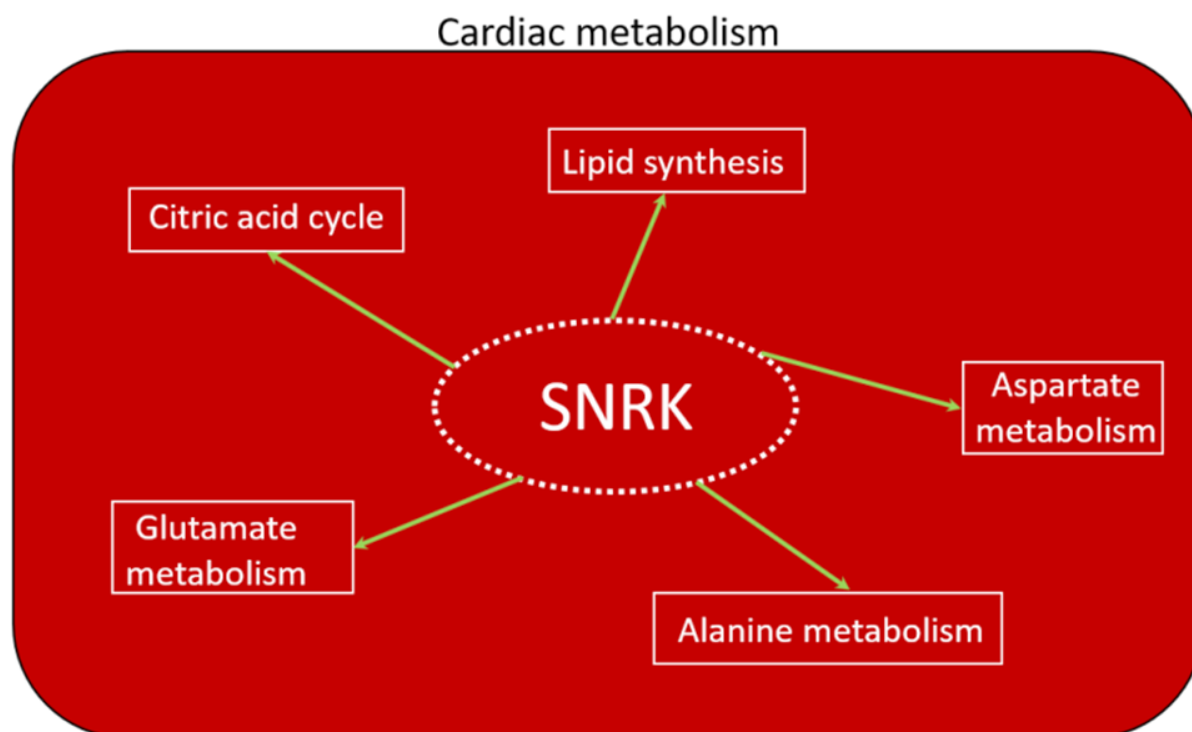


Figure 3. SNRK in cardiac metabolism. The role of SNRK in various metabolic pathways involved in cardiac functioning is depicted. SNRK: sucrose nonfermenting 1-related kinase

strongly on ATP generation, and impairments in this process can rapidly induce contractile dysfunction. Of the ATP generated in the adult heart, 70% to 90% is produced by the oxidation of fatty acids (or FAO). The remaining 10% to 30% comes from the oxidation of glucose and lactate, as well as small amounts of ketone bodies and certain amino acids^[45,46]. Because *Snrk* global KO mice die at or before birth, studying SNRK function in adult requires conditional deletion of SNRK in cardiac tissue using the CRE-LoxP system. Cardiomyocyte-specific (myh6-CRE) *Snrk* KO mice (*Snrk* cmcKO) show cardiac functional deficits at 6 months and die at 9 months. No neonatal lethality was observed in these mice^[15]. Neonate hearts from the *Snrk* cmcKO mice showed higher ORO retention and no change in pACC-pAMPK signaling pathway. However, adult *Snrk* cmcKO hearts at 6 months showed no change in ORO but showed higher pACC (unpublished data) levels. Thus, the switch in energy source for the heart from glucose in neonates to fatty acids in adults may partly reflect SNRK's role at these time points. In terms of cell type, where SNRK function is critical in the heart, there is little doubt that SNRK-CM function is dominant. This is supported by the following evidence: (1) *Snrk* cmcKO show profound cardiac functional deficits at 6 months, die at 9 months, and when stressed by Angiotensin II (Ang II) at 4 months, they show cardiac function deficits within 14 days and die; (2) *Snrk* endothelial (TIE2) cell conditional KO (*Snrk* ecKO) do not show cardiac functional deficits at 6 months, are alive, and when stressed with Ang II at 4 months do not show cardiac functional deficits; and (3) SNRK knockdown cardiomyocytes *in vitro* show metabolic deficits, and NMR (nuclear magnetic resonance)-based metabolomic analysis revealed SNRK is essential for alanine, aspartate, and glutamate metabolism [Figure 3] as well as TCA cycle metabolism and it also regulates metabolites involved in lipid synthesis such as glycerol^[47]. It is also noteworthy that pAMPK-pACC levels were significantly altered in *Snrk* ecKO neonate hearts and also in adult hearts (unpublished data). But, despite these alterations, the SNRK in CMs seem to compensate for cardiac function, and thus prevents functional deficits.

As for the targets of SNRK that are involved in cardiac function, we had previously reported that ROCK was a putative SNRK substrate in CMs [Figure 2], and showed that Fasudil (ROCK inhibitor) can rescue

cardiac functional deficits in *Snrk* cmcKO hearts^[13]. ROCK signaling pathway^[13] activation is also implicated in major cardiovascular disorders such as atherosclerosis, restenosis, hypertension, pulmonary hypertension, and cardiac hypertrophy^[48]. Tribbles homologue 3 (Trib3) is another substrate of SNRK in the heart [Figure 2], and SNRK overexpression in the heart decreases oxygen consumption and improves cardiac function^[16]. Trib3 is also a known inhibitor of AKT signaling^[49] and metabolic flux is maintained by PPAR α -dependent UCP3 (uncoupling protein 3) downregulation^[16]. Thus, SNRK improves cardiac mitochondrial efficiency and decreases mitochondrial uncoupling^[16]. Collectively, SNRK acts as a cardiomyocyte-centric metabolic sensor in cardiac tissues to maintain cardiac function and homeostasis, and is a novel candidate to target in order to improve cardiac health.

SNRK in adipocyte metabolism

Adipose tissue is a loose connective tissue composed of adipocytes, cells which contain either a single large lipid droplet (white adipose tissue) or multiple lipid droplets (brown adipose tissue). Adipocytes release fatty acids into the bloodstream via lipolysis of lipids. Adipose is a highly dynamic tissue and contains adipocytes of various size referred to as small and large adipocytes. The properties of adipocytes cells have been extensively explored for the relationship between cell size and various disease conditions such as inflammation^[50,51], insulin resistance^[52,53], and diabetes^[54,55]. The correlation between its size and cellular function as well as in metabolic disease concluded that the size of the adipocyte is an important factor in predicting pathophysiological conditions^[56].

The most known and recognized function of adipose is its role in the storage and release of lipid species, particularly free fatty acids^[57]. SNRK is ubiquitously and abundantly expressed in both white adipose tissue (WAT) and brown adipose tissue (BAT)^[19]. Phosphoproteomic analysis revealed SNRK knockdown in adipocytes significantly decreased phosphorylation of 49 proteins by 25% or more and increased phosphorylation of 43 proteins by onefold or higher. Among these proteins, several were involved in the inflammatory pathways. Pathways such as mTOR signaling were implicated in addition to those that reduce adipocyte function^[19]. In adipocytes, acute inhibition of mTOR signaling by rapamycin increases insulin-stimulated glucose uptake, but chronic inhibition of mTOR signaling (rapamycin) impairs insulin-stimulated glucose uptake^[58]. Thus, SNRK's role in regulating insulin-mediated glucose uptake is context-dependent. In support of this hypothesis, a recent study, from Li *et al.*^[17], reported that SNRK controls insulin signaling through Protein Phosphatase 2 Regulatory Subunit B'Delta (PPP2R5D) phosphorylation [Figure 2], which subsequently influences protein phosphatase 2A (PP2A) activity and phosphorylates AKT in both WAT and BAT. PPP2R5D is one of the four major Ser/Thr phosphatases implicated in the negative control of cell growth and division. This implies that SNRK activates insulin-stimulated AKT phosphorylation and glucose uptake in adipocytes. Further, SNRK, specifically in adipocytes, maintains body weight but it does not change the size of the WAT depot^[18]. SNRK keeps circulatory triglycerides and free fatty acids in check in order to regulate the body weight. These data imply that SNRK is a potential target for interference in adipocytes to check body weight.

SNRK and inflammation

Tissue inflammation is a key protective mechanism to promote repair caused by ischemia and to prevent further damage. SNRK appears to control tissue inflammation especially by suppressing the inflammatory pathways mediated through nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling^[14,22] [Figure 2]. To restore normal tissue architecture, post-injury inflammation occurs in three distinct phases. In the early proinflammatory first phase, components of the innate immune response initiate the repair by mobilizing the recruitment of key inflammatory cells. In the second phase, the proinflammatory response begins to diminish and inflammatory cells such as macrophages switch phenotype to a reparative mode. In the final phase, tissue homeostasis is reestablished when the inflammatory cells either withdraw from the site of injury or are abolished through apoptosis. However, the

degree and duration of the response varies, and this dictates whether the final outcome of the inflammation will be beneficial or harmful. A prolonged inflammatory response has negative consequences such as activation of a fibrotic response where excessive and aberrant accumulation of collagenous connective tissues occurs in the organs that can weaken tissue function, and in some cases, lead to organ failure (e.g., chronic hepatitis B)^[22,59,60].

Often, inflammation is associated with fibrosis in cardiac, adipose and renal tissues^[8,14,22,61]. Evidence in recent years point to a strong link between chronic low-grade inflammation in the heart and metabolic dysregulation^[62-64]. In the case of heart failure and cardiac hypertrophy, the progression of inflammation usually involves a local rise of cytokines in cardiac cells such as CMs, ECs, and fibroblasts and the activation of the proinflammatory transcription factor nuclear factor NF- κ B^[14,65,66]. In the cardiac system, inflammation is often associated with the deposition of collagen, leading to fibrosis in the heart. Removal of *Snrk* in CMs increases the phosphorylation of NF- κ B p65 and increases proinflammatory cytokine signaling which is partially mediated through Akt. This suggests that SNRK represses inflammation signaling in CMs, which when unchecked, progresses to fibrosis and death. However, when *Snrk* is deleted in cardiac ECs, these hearts show increases in NF- κ B p65 and proinflammatory cytokine signaling, which does not progress to fibrosis. These data collectively suggest that SNRK in CMs compensates for SNRK loss of function in ECs^[14], and also implies crosstalk signaling between CMs and ECs in cardiomyocyte remodeling.

Healthy adipose tissue is vital for metabolic homeostasis, whereas dysfunctional adipose is a contributing factor for metabolic disorders such as obesity, type 2 diabetes mellitus, and cardiovascular diseases^[67-69]. SNRK expression is high in the metabolic adipose tissue. In the adipocytes, SNRK protein is localized to lysosomes, the site for degradation of large intracellular organelles or assembly of protein aggregates^[19]. Adipocyte-specific SNRK represses inflammation in WAT through the JNK and IKK β signaling pathways. Under obesity conditions, the expression level of SNRK is low because of obesity-induced adipose inflammation and/or lipid toxicity. Removal of SNRK specifically in adipocytes leads to metabolic disturbances with decreased energy expenditure, higher body weight, and increased insulin resistance^[18,19], suggesting that SNRK regulates and controls these metabolic pathways.

In the renal system, SNRK in glomerular ECs binds directly to p65 subunit of NF- κ B (activated by Ang II) to suppress inflammatory signaling. Activated NF- κ B plays a critical role in renal damage which occurs as a result of unchecked inflammation and fibrosis^[22]. These evidences suggest that SNRK acts as a repressor of inflammation in cardiac, adipose, and renal tissues, all of which are critical for the maintenance of organismal homeostasis and function.

Role of SNRK in vascular development

During embryonic development, vascular networks permeate the entire body and this conduit is used for the circulation of metabolites and removal of waste products. The vascular system emerges as one of the earliest networks in the embryo to support the rapid growth of tissues. The adult vasculature is generally quiescent but retains the capacity to shift from this dormant state to an expansion and remodeling state. This occurs during normal physiological conditions such as wound healing or pathological states such as tumor neovascularization. The differentiation of angioblasts (precursor cells) into ECs and the *de novo* formation of a primitive vascular network is called vasculogenesis. After primary vascular plexus is formed, a second process during development occurs wherein more ECs are generated via a process called angiogenesis which is the growth of new blood vessels from existing vasculature^[70]. Thus, vasculogenesis and angiogenesis are the two predominant sequential coordinated processes for blood vessel formation during development^[70,71].

SNRK in vasculogenesis

The initial loss of function studies *in vivo* for *Snrk* was performed in the vertebrate zebrafish model system. Zebrafish offers various advantages as a model system including established genetics, loss and gain of function technology, transparency of embryos, fluorescent transgenic reporter lines, *ex vivo* development of embryos, and several others^[72-74]. During embryonic development, angioblasts migrate from lateral plate mesoderm to the midline, differentiate in arterial or venous endothelial cells and coalesce to form cord-like structures which eventually mature to form the major axial blood vessels, namely dorsal aorta (DA) and posterior cardinal vein (PCV)^[75]. The kinase function of SNRK was shown to be essential for angioblast migration and is required for localization and maintenance of these cells in the lateral plate mesoderm^[76]. SNRK also functions later (19-22 h post-fertilization) during angioblast differentiation to arteries and veins in zebrafish where it is involved with Notch signaling-mediated arterial or venous (A/V) specification. Studies in zebrafish reveals that within 2 h of the formation of angioblasts, angioblasts begin their migration to the dorsal midline, and by 18 h post-fertilization, angioblasts coalesce at the midline to form the two major axial vessels (DA & PCV)^[77,78]. Thus, sufficient number of angioblasts are needed in the embryo to facilitate this process. As described earlier, most kinases are counter balanced by phosphatases. In the lateral plate mesoderm, dual-specific phosphatase 5 (DUSP5), a member of MAPK phosphatases was identified as a vascular-specific gene in 2 independent microarray studies^[74,79]. Subsequent analysis shows that Dusp-5 phosphatase and SNRK kinase function together in maintaining angioblast populations during embryonic vascular development^[20]. Thus, the signaling pathway and the targets that are regulated by SNRK and DUSP5 are likely to reveal more understanding of the basic vasculogenesis process during vertebrate organogenesis.

SNRK in angiogenesis

The main mediator of angiogenesis is the arrangement of ECs in tip and stalk cells^[80]. Tip cells containing filopodia invade surrounding tissue leading to the path of neo-vessel formation. Vascular endothelial growth factor and Notch signaling pathways are vital for tip cell differentiation^[80]. Most of our current knowledge about the morphological processes and molecular regulation of angiogenesis are from the developing zebrafish embryos or the vascularization of postnatal mouse retina^[80,81]. In zebrafish, the accessibility of embryos for live imaging provided unprecedented observations which have unraveled new concepts in angiogenesis. Analysis of the zebrafish head and trunk vasculature reveals that SNRK controls patterning of vessels in both locations^[20]. In retina, the segregation of vascular and avascular compartments and the visualization of the progressive expansion of blood vessels from the center to periphery makes it an attractive model to study tip versus stalk cell formation during angiogenesis. In this model, endothelial SNRK was found to be essential for vascular patterning in that it controls vessel diameter and vascularization area in the retina^[21]. This regulation is partially mediated through transcription factor hypoxia-inducible factor-1 α (HIF1 α), which is induced under a physiological drop in oxygen concentration below 60 mmHg, a condition referred to as hypoxia. The hypoxia state in tissue often induces angiogenesis. Similarly, during both acute and chronic myocardial ischemia, angiogenesis is stimulated. Ischemia-driven angiogenesis is primarily an adaptive physiological response to either an increase in tissue mass or elevated oxygen consumption^[82]. Under ischemic condition, HIF1 α is upregulated and was shown to interact directly to *SNRK* promoter^[21]. This binding results in transcriptional upregulation of *SNRK* mRNA, which then promotes angiogenesis by activating ITGB1 (β 1 integrin)-mediated EC migration^[21]. Thus, SNRK plays a vital function in developing vasculogenesis and ischemic angiogenesis. However, its exact role and the underlying mechanisms associated with facilitating normal angiogenesis remains unknown.

Role of SNRK in disease

Inflammation and metabolic dysregulation are major contributing factors to disease. Because SNRK participates in both processes, it is considered an important target for intervention. Based on SNRK's preponderance as a repressor of cellular functions, we consider SNRK as a checkpoint in cells. Thus,

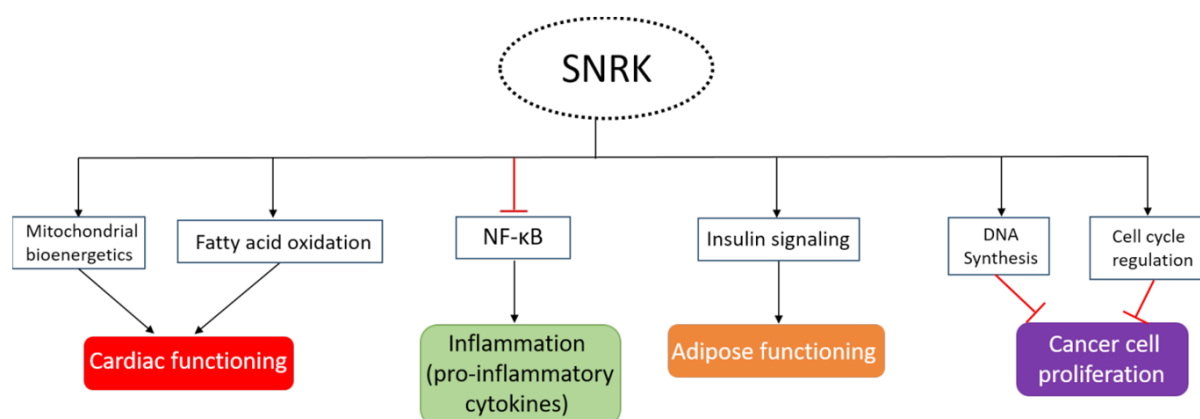


Figure 4. Important functions of SNRK. The important function of SNRK in various signaling paradigms, and consequence associated with that in respective tissues is depicted. The arrows indicate activation (black) and inhibition (red). SNRK: sucrose nonfermenting 1-related kinase

therapeutic approaches that support SNRK agonist development is preferred. Further, SNRK's role at the interface of inflammation and metabolism will benefit conditions such as heart failure or diabetes. We hypothesize that molecules such as SNRK that work at interfaces of inflammation-metabolism will help reduce the progression of the disease. In addition, SNRK's ability to suppress inflammation in multiple systems, such as cardiac, adipose, and renal, opens avenues for therapeutic development in these organ systems.

SNRK in heart failure

Progressive heart failure (HF) is a chronic condition, and current therapies targeting HF are insufficient. HF is a leading cause of morbidity and is associated with increasing mortality rate worldwide. HF is often accompanied with significant perturbations in energy metabolism that can affect both cardiac energy supply and efficiency^[83]. HF is also associated with several underlying comorbidities including dilated cardiomyopathy, myocardial infarction, hypertension, and myocarditis. Metabolic changes or dysfunctions in cardiac tissue are one of the main reasons for HF progression. Prolonged exposure of metabolic stress in the heart decreases its functional ability, especially by reducing mitochondrial function, which is the main energy generating source in the heart. Mitochondrial dysfunction appears to be a vital target for direct intervention to improve cardiac function because it primarily uses fatty acids for ATP production^[84]. SNRK is involved in the regulation of the mitochondrial substrate usage and oxygen consumption to maintain cardiac energy and functioning^[16]. Progressive HF reduces free fatty acid breakdown resulting in less ATP production, more inflammation, and increased fibrosis. SNRK in CMs regulates cardiac energy homeostasis by maintaining FAO^[15] [Figure 4]. Loss of functionally active SNRK in CMs makes the heart vulnerable and the mice succumb in approximately 9 months. Any additional stress such as angiotensin II (Ang II) accelerates HF and the mice dies in two weeks post Ang II infusion. Cardiac functional parameters are severely compromised with upregulation of inflammation and fibrosis markers^[14] in the heart tissue. HF is typically associated with cardiac remodeling where inflammation and fibrosis are thought to play crucial roles^[60]. These studies suggest that maintaining SNRK function in CMs is key to preventing HF. In the heart, in addition to CMs, ECs are also present in higher numbers than previously thought^[85]. Interestingly, when SNRK was deleted in ECs, the hearts from these mice did not show cardiac function deficits in wild type state or in Ang II-induced state. As mentioned earlier, the NF-κB pathway was activated in these hearts [Figure 4], but no fibrosis was observed^[14]. These studies suggest an overriding role for SNRK in CMs and its compensation of functional defects elicited by other cell types in the heart. Further, SNRK in CM keeps inflammation and fibrosis under check which allows the heart to continue its function. These studies suggest important concepts that require more investigation as it relates to SNRK's role in HF, which

include: (1) SNRK-mediated cell-cell communication in the heart; (2) pharmacological activation of SNRK selectively in CMs to suppress inflammation and metabolic dysregulation; and (3) SNRK activation in CMs to promote cardiac output via mitochondrial or other mechanisms. Some of SNRK's cardiac function are reminiscent of mitochondrial sirtuin proteins specifically SIRT3, wherein *Sirt3* knockout mice are highly sensitive to stress, which leads to cardiac hypertrophy, fibrosis, and increased mortality^[86]. Compounds such as Honokiol that activate mitochondrial Sirt3, block and reverse cardiac hypertrophy in mice^[87] and show protective cardiac function^[88] could be candidates for testing in *Snrk* cmcKO mice. Collectively, the therapeutic value of SNRK-CMs-mediated signaling to prevent HF is an emerging area of translational research in cardiovascular medicine.

SNRK in diabetes

Diabetes mellitus is often referred to as a metabolic condition that results in high blood glucose levels. Type 1 diabetes (T1D) is a severe form of the disease and is often referred to as juvenile diabetes or “insulin-dependent diabetes” which is the result of loss of insulin-hormone producing islet cells in the pancreas which normally promotes glucose metabolism. Type 2 diabetes (T2D), the most common form of diabetes, is also referred to as adult onset diabetes or “non-insulin-dependent diabetes.” In T2D, the insulin receptor is defective and insulin produced by the pancreatic cells cannot function to facilitate efficient glucose metabolism. This is often referred to as “insulin-resistance” state. Insulin secreted by pancreas influences other organs including muscle (glucose uptake and storage), liver (decrease glucose production), and adipocytes (increased lipogenesis). SNRK's connection to diabetes was first identified by studying its role on adipocytes^[19]. SNRK is abundantly expressed in adipose tissue (WAT and BAT), and its expression is induced by insulin. *SNRK* knockdown in adipocytes promotes lipolysis, impairs glucose uptake^[17], and activates NF- κ B inflammatory signaling pathway. Thus, SNRK, like in CMs, seems to function in adipocyte as a repressor of adipocyte inflammation. The specific mechanism utilized by SNRK to prevent insulin resistance in adipose tissue is through protein phosphatase 2 regulatory subunit B' delta (PPP2R5D) phosphorylation, which impacts PP2A activity and phosphorylation of AKT^[17] [Figure 4]. The SNRK-AKT connection is intriguing given that this signaling nexus was also observed in heart tissues from *Snrk* cmcKO mice^[14]. Thus, further investigations is needed into the direct versus indirect regulation of SNRK-AKT pathway in CMs and adipocytes.

Obesity is a key risk factor for insulin resistance T2D^[89]. Interestingly, in humans and mouse models of obesity, adipose SNRK expression levels are diminished. Further, adipocyte-specific deletion of *Snrk* causes inflammation in WAT along with ectopic lipid deposition in liver and muscle^[18]. Homozygous loss of *Snrk* in adipocytes decreases the expression of uncoupling protein 1 (UCP1), PR domain containing 16 (PRDM16), and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) in BAT. One of the primary functions of adipocytes is to insulate the body, a process also referred to as “thermogenesis”. All three molecules UCP1, PRDM16, and PGC-1 α play an important role in BAT thermogenesis^[18]. To increase heat production, lipids in adipocytes are catabolized, a process that gets dysregulated in obese conditions, which results in increased inflammation and insulin resistance. Adipocyte-specific deletion of *Snrk* cause impairment in adaptive thermogenesis in BAT leading to decreased energy expenditure, elevated body weight, and insulin resistance. Importantly, a significant association in *SNRK* genetic variants and obesity risk was identified in humans^[18]. These studies collectively make a case for SNRK as a novel target for treating obesity and insulin resistance-related metabolic disorders including diabetes.

SNRK in cancer

Reprogramming of cellular energy metabolism is one of the principal hallmarks of cancer^[90]. We think that tumor cells can exploit SNRK's role in controlling metabolic pathways in various tissues. For example, LKB1, an upstream regulator of SNRK function, has been identified as a critical cancer suppressor protein

and is mutated in several types of cancers^[91-96]. LKB1 may inhibit cancer cell growth through regulation of HIF-1 under hypoxic condition. Hypoxia is an important characteristic in most cancers, and induces the expression of HIF-1 transcription factor. HIF-1 can subsequently activate genes that permit cancer cells to survive and grow in the hypoxic tumor environment^[97]. HIF1 α binds to the *SNRK* promotor during ischemia, and induces its expression. SNRK protein is found in both cytoplasm and in the nucleus and regulates genes involved in DNA synthesis and cell cycle regulation [Figure 4]. Overexpression of *Snrk* decreases cell proliferation, whereas downregulation of *Snrk* increased cell proliferation in colon cancer cell lines. Mechanistically, SNRK inhibits the proliferation of colon cancer cells through upregulation of calcyclin-binding protein (CacyBP) and β -catenin degradation^[12]. CacyBP is a tumor suppressor which has been implicated in reducing cancer cell proliferation through regulating cell cycle G1 check point in breast^[98], gastric^[99], and kidney cancers^[100].

Another tumor type that depends extensively on host metabolism are ovarian cancer cells^[23]. Adipocytes in the omentum (fat layer underlying the belly) microenvironment^[101] provide fatty acids as source of energy to ovarian cancer cells to support their rapid growth, progression, and metastasis^[24]. In ovarian cancer, the expression of SNRK is lower in metastatic tumors and is differentially expressed depending on the stage of the disease. This suggests that SNRK has specific roles in the disease progression of ovarian cancer^[98] and may have diagnostic value for stratifying ovarian tumors of varying types. However, much work is needed to realize SNRK's full potential and its importance in cancer biology.

In summary, therapeutic strategies directed towards the enhancement of SNRK function could significantly improve the clinical conditions associated with inflammation and metabolic dysfunction.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

A considerable amount of evidence supports the notion that SNRK activation may act as a suppressor of inflammation and metabolic processes. Inflammation suppression comes with direct inhibition of NF- κ B-mediated inflammatory signaling, one of the signature pathways that SNRK controls. SNRK's role in regulating glucose and fatty acid metabolism is considered significant for the function of cardiac and adipose tissues. Given that mitochondria is a major source of ATP production in the cell, it is not surprising that SNRK is involved in regulating mitochondrial bioenergetic potential in CMs. Emerging evidence in SNRK biology also suggests that it plays a defining role in cell-cell communications in various tissues that will extend beyond adipose, renal, and cardiac tissues. In adipose tissues, SNRK plays a predominant anti-inflammatory role. In renal tissues, endothelial SNRK protect the kidney epithelium from becoming fibrotic. In the cardiac tissues, CMs SNRK is necessary for protecting ECs from undergoing fibrosis. In addition, SNRK role in phosphorylating proteins (substrates) whether directly or indirectly to either promote or inhibit its target activity/signaling pathway will need extensive evaluation and is the future of SNRK signaling. Finally, SNRK's role in causative human disease phenotypes will cement the importance of this molecule from a translational biology perspective. The next 10 years of SNRK biology will be interesting and exciting to witness, and our group along with many others will benefit from this knowledge which we hope one day will impact patients' lives in the form of SNRK-centric novel treatments.

DECLARATIONS

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Authors' contributions

Wrote drafts of the manuscript, conceptualized ideas, and provided inputs for all aspects of the manuscript preparation: Thirugnanam K

Edited and wrote parts of the manuscript, conceptualized ideas, provided input for scientific content and presentation, and provided financial support for the project: Ramchandran R

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Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

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Not applicable.

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Review

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Oxidative stress and inflammation in the development of cardiovascular disease and contrast induced nephropathy

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Abstract

Utilization of contrast media to visualize vasculature structures in the setting of cardiovascular disorders (CVD) can lead to acute kidney injury, referred to as contrast-induced nephropathy (CIN). CIN can potentiate mortality and hospitalization in aged individuals, patients with CVD, nephropathy, enhancing kidney damage, and cardiac events. Preventing CIN by identifying risk factors is important. The underlying mechanisms of CIN pathology are unclear, but the key factors include direct cytotoxicity, oxidative stress, vascular and endothelial dysfunction and inflammatory processes. Reactive Oxygen Species and inflammatory mediators have been proposed as key factors influencing the development of CIN and CVD, and the elucidation of the interplay between the mechanisms evoked by them may provide a better understanding of the signaling processes happening in these conditions, thereby potentially enabling early identification, prevention and characterization of novel drug targets.

Keywords: Contrast induced nephropathy, cardiovascular disorders, oxidative stress, inflammation, reactive oxygen species



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INTRODUCTION

Inflammation is an immune system response to pathogenic insults and is physiologically important to protect the organism from injury. Inflammatory responses are triggered by harmful stimuli and lead to a removal of invading pathogens and initiation of the healing process^[1]. Reactive oxygen species (ROS) modulate the inflammatory processes^[2-5]. ROS include chemically heterogeneous free radicals (e.g., superoxide) and non-radicals (e.g., hydrogen peroxide) vital for cell development, survival and signaling^[6]. Redox signaling occurs through posttranslational oxidation of proteins (e.g., cysteine residues)^[7,8]. Moreover, there is also a known cross-talk between ROS and neutrophil inflammation clearance and pro-inflammatory markers^[5,9]. Usually, these mechanisms are tightly regulated and when sustained and aberrant, inflammatory responses and ROS can lead to tissue damage and disease.

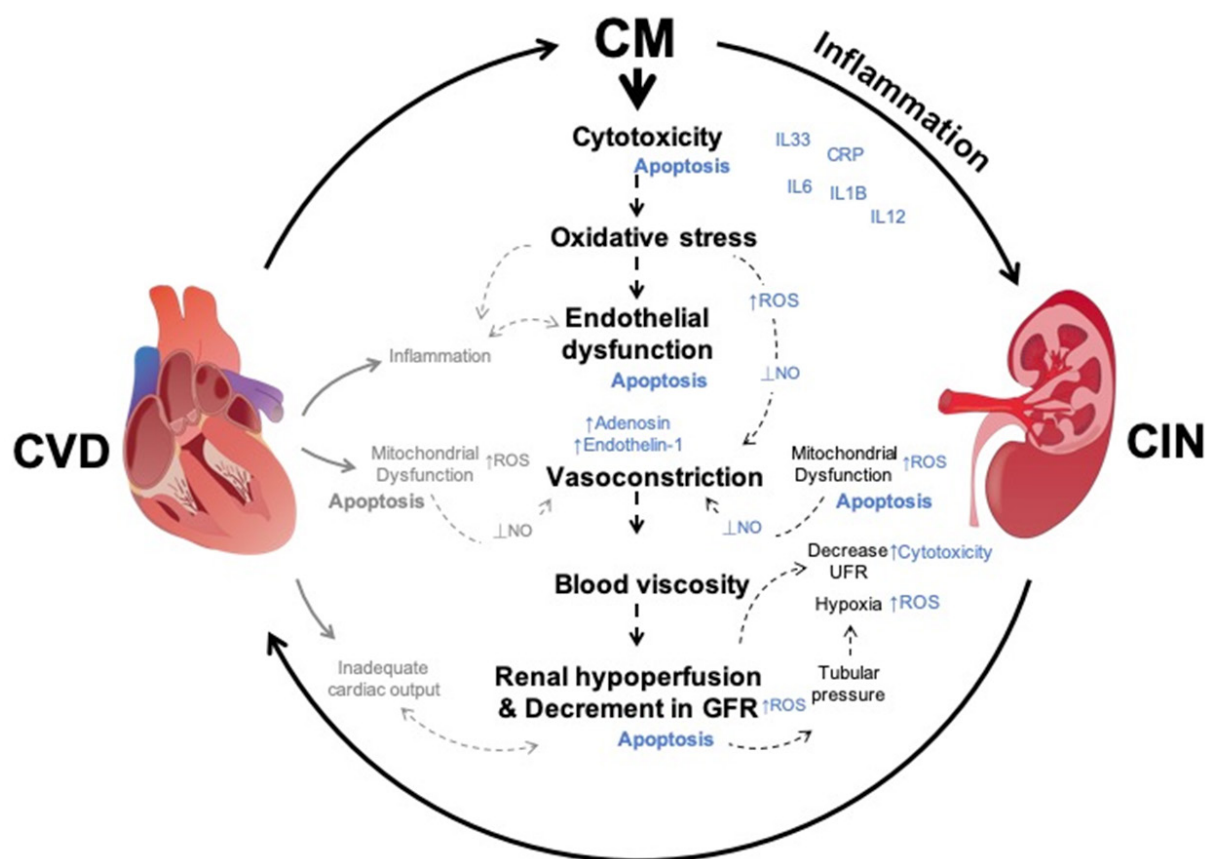
Environmental stress can cause oxidative stress, often defined by cell/tissue injury and attendant oxidative macromolecule damage^[10]. Moreover, ROS have been highlighted as a cause of several inflammatory diseases like cardiovascular diseases (CVD), type II diabetes and cancer.

Due to its role promoting inflammation and lipid peroxidation, ROS have been tightly linked to CVD^[11]. Thus, both inflammatory elements and ROS are CVD risk factors, described as underlying participants in the progression of atherogenesis. In addition, chronic inflammatory diseases, characterized by an involvement of oxidative stress in their pathogenesis, promote high risk and influence CVD susceptibility^[12,13]. Inflammatory molecules and ROS have been proposed as possible predictors and drug targets in CVDs, reviewed by Cervantes Gracia, Llanas-Cornejo, & Husi, 2017^[14,15]. Interestingly, target organ damage, described as the strong association with high blood pressure and functional changes in the heart, brain, eyes and kidney, is known to have significant implications in CVD onset^[16,17]. Furthermore, CVD is a characteristic hallmark of severe kidney failure. Patients with chronic kidney disease (CKD) have been well characterized to carry a significantly higher risk of developing and dying from severe CVDs^[18-20]. Therefore, management of chronic kidney disease progression has been proposed as strategy to reduce the incidence of cardiovascular events^[21]. Conversely, the presence of CVDs have also been associated with a higher risk of renal impairment and CKD progression^[22]. However, the influence that one disease has over the other, as well as the underlying molecular mechanisms remain to be elucidated.

To add to this pathology, kidney failure exacerbated by coronary intervention procedures relying on contrast media (CM), known as contrast induced nephropathy (CIN), constantly increases the incidence of comorbidities in this group of patients undergoing interventions and its prevention is challenging^[23-26]. Since pre-existing CKD is the most common cause of CIN^[27,28], the interplay among the underlying mechanisms of CVD and kidney failure are important. Additionally, inflammation and ROS have been identified as risk factors of CIN and as potential targets for prophylaxis or treatment^[29-34]. Hence, the elucidation of CIN/CVD interplay in this setting would improve understanding of the signaling processes and progression of the diseases, leading the way to different approaches to either early detection or to identification of novel drug targets.

CIN PATHOPHYSIOLOGY IN THE CONTEXT OF CVD

According to the WHO, non-communicable diseases (NCD) account for 71% of all deaths world-wide and CVDs are responsible for most NCD deaths. CVDs were responsible for about 17.8 million deaths in 2017^[35,36], and are the primary cause of death globally. Notably, angioplasty is the most common percutaneous coronary intervention (PCI) method for CVD treatment^[37,38], and diagnostic angiography and PCI routinely utilize iodinated CM for vascular visualization^[39,40]. Although angiograms and PCI can effectively diagnose and treat CVD patients, this can potentially lead to acute kidney diseases such as CIN induced by CM^[41-46]. CM can be retained by the kidney where they have the potential to cause toxicity,



resulting in acute renal injury^[47]. Alternative CM have been developed to perform these procedures, but patients with risk factors such as kidney malfunction, diabetes, advanced age, CVD, anemia and hypotension are at high-risk and remain vulnerable to CIN^[48,49].

Although the precise pathophysiology of CIN is incompletely understood, crucial mechanisms have been associated with CIN, such as vasoconstriction in the renal vasculature, oxidative stress, renal medullary hypoxia, direct renal tubular cytotoxicity, and viscosity^[45,53,61] [Figure 1]. It has been proposed

that the interplay of cytotoxicity and viscosity caused by CM, may be key in CIN pathophysiology. CM causes damage and apoptosis in surrounding endothelial cells (EC) and tubules of the nephron through iodine^[62,63]. Moreover, vasoconstriction is known to increase blood viscosity after CM administration. CM increased viscosity and tubular pressure, exacerbating renal hypoperfusion and promote a decrease in urine flow rate, leading to its retention and allowing its continuous cytotoxicity [Figure 1]^[64,65]. Furthermore, blood viscosity is a key player in CVD pathophysiology and is associated with increased risks of CVD^[66,67]. It has also been reported in the context of renal dysfunction and is associated with an increased risk of CVD and CKD development^[68].

Vasoconstrictor mediators (endothelin, adenosine, angiotensin II, vasopressin) are known to play a key role CIN and CVD pathogenesis^[65,69-73]. CM is known to cause immediate vasoconstriction and vasodilation impairment, reduce renal blood flow, decrease glomerular filtration rate (GFR) and cause renal hypoperfusion, which leads to an inadequate delivery of oxygen, promoting ischemic injury [Figure 1]. These processes are associated with oxidative stress promoted by CM^[74,75]. Decrease in GFR has also been associated with increased risk in CVD mortality, a feature that reflects kidney damage^[76]. Regarding vasodilatation impairment, it has been suggested to be induced by CM through decreased nitric oxide (NO) bioavailability. This event has been proposed to be a result of loss of vasoactive NO and cellular damage on account of generation of peroxynitrite (ONOO⁻). Under physiological conditions, ROS production is attributed to nephron tubular transport regions with dense mitochondria populations, an important source of ROS^[77,78]. Additionally, mitochondrial dysfunction is a key player in acute kidney injury^[79,80] and is a characteristic feature of ageing, and chronic diseases, including diabetes and CVD, which are considered to be major risk factors for CIN^[81,82]. Mitochondria are also abundant within cardiac cells due to the high energy demands, and notably mitochondrial ROS production is associated with CVD development^[15,83]. Deleterious events such as arterial hypertension, endothelial dysfunction, atherosclerotic plaque formation and heart failure are associated with mitochondrial dysfunction^[84-86]. Mitophagy removes damaged mitochondria and its impairment is a feature in CVD development as well. Moreover, ROS can induce damage in mitochondrial DNA, and damaged mitochondria are important sources of ROS; therefore, ROS overproduction due to mitophagy impairment disturbs homeostasis and leads to inflammation and apoptosis^[87,88]. As in CIN, excessive ROS production from mitochondria is also associated with NO vasodilator impairment and it is tightly linked with endothelial dysfunction in cardiac event^[89]. To add to CIN pathophysiology, oxygen imbalance under hypoxic conditions also leads to ROS production by the conversion of adenosine triphosphate (ATP) into hypoxanthine and its further reduction by xanthine oxidase. Mitochondrial dysfunction is also responsible for reduction in ATP synthesis, and will add to the cellular apoptotic state [Figure 1]. Interestingly, it has been recently reported that CKD in a rat model can influence cardiac pathologies by changing the function of cardiac tissue and inducing mitochondrial swelling and damage^[90].

Inflammation is also a CIN hallmark, since the mechanisms previously described can trigger inflammatory processes. Notably, the presence of inflammatory elements has also been set as a feature for the population at high risk of CIN. Several studies have reported that the presence of active inflammatory processes biomarkers in patients with CVD may attribute its high-risk to developing CIN after CM exposure^[30,31,91-94].

Cardiac insufficiency is also accountable for renal function impairment, emphasizing the complex interactions between heart and kidney where dysfunction in one organ can result in injury of the other^[95]. Since CVD is a high-risk factor in CIN, and CIN can exacerbate CVD mortality, it is important to identify potential biomarkers for early detection and development of appropriate treatments. CIN processes that induce the release of vasoconstrictors, ROS and inflammatory cytokines have also been defined as hallmarks in CVDs due to the promotion of myocardial damage^[50,96,97]. Additionally, a drastic decline in renal function may accelerate cardiovascular impairment by triggering inflammatory pathways^[95,98].

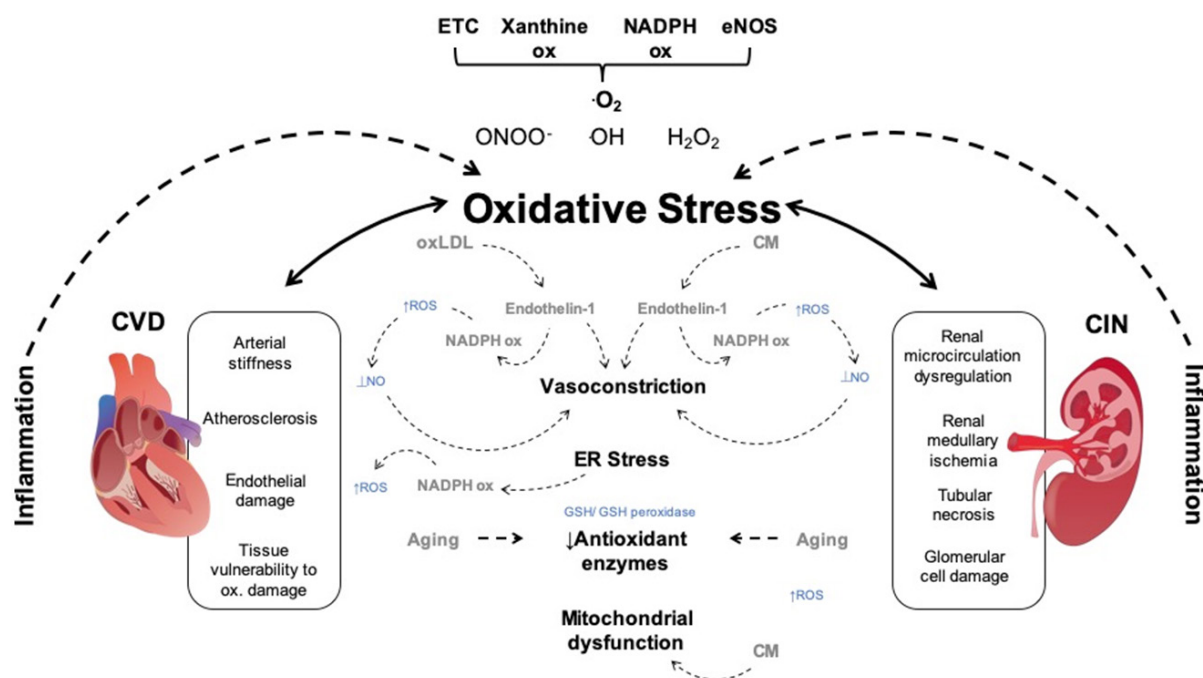


Figure 2. Oxidative stress mechanisms in contrast induced nephropathy and cardiovascular disorders. ONOO^- , OH , O_2^- and H_2O_2 are physiologically relevant ROS in the vascular endothelium. Processes involved in oxidative stress are represented in bold black. Left side represent mechanisms described in CVD, right side represent mechanisms described in CIN. Boxes show effects of oxidative stress in CIN and CVD. Oxidative stress mechanisms lead to inflammation which in turn generates a feedback loop in ROS production. ROS, NO, and antioxidant enzymes are represented in blue. \downarrow : repression/reduction; \uparrow : overproduction; \downarrow : decrease; ROS: reactive oxygen species; NO: nitric oxide; ER: endoplasmic reticulum; ox: oxidases; ETC: electron transfer chain; eNOS: endothelial nitric oxide synthase; ONOO^- : peroxynitrite; OH: hydroxyl radical; O_2^- : superoxide anion; H_2O_2 : hydrogen peroxide; CIN: contrast induced nephropathy; CVD: cardiovascular disorders

Although an association of these events has been suggested for many years, its interplay remains to be described. Elucidating the possible interplay between oxidative stress and inflammation is important.

OXIDATIVE STRESS IN CVD AND CIN

ROS play a significant role as second messengers within cells and regulate normal cellular functions, including gene transcription, signal transduction and homeostasis^[99]. Many sources of ROS exist within cells and amongst ROS, the free radical superoxide (O_2^-), is often a proximal ROS. O_2^- can lead to peroxynitrite (ONOO^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) production. Univalent reduction of molecular oxygen (a diradical) by the mitochondrial electron transport chain (ETC), as well as by xanthine oxidase, uncoupled endothelial nitric oxide synthase and Nicotinamide adenine dinucleotide phosphate oxidases (NOXs) leads to O_2^- production^[100].

Mitochondria are responsible for the bulk ATP synthesis via chemiosmotic oxidative phosphorylation (OXPHOS). OXPHOS involves mobile electron carriers shuttles (NADH, cytochrome C and Coenzyme Q), protein complexes (complexes I-IV and the ATP-synthase complex) and a sequence of redox reactions where electrons are transported across the complexes of the respiratory chain up to complex IV, where molecular oxygen is reduced to water. The proton pumps establish an electrochemical proton motive force necessary for OXPHOS. Mitochondrial ROS can directly disturb the functionality of the ETC complexes by oxidizing iron-sulfur clusters and protein thiols [Figure 2]^[101-103]. Although mitochondria are a major source for ROS production, no clinical studies have been reported for mitochondrial-targeted antioxidants. This is largely due to the complications surrounding the targeted antioxidant delivery of injured mitochondria. Another cause for concern is that the role of mitochondrial ROS differs from cytosolic ROS as they

are responsible for intracellular functions, which are maintained at a delicate equilibrium that could be negatively influenced by the careless use of antioxidants^[104].

It is understood that ageing is associated with cardiovascular oxidative stress^[105]; tissue vulnerability to oxidative damage and is likely to be a key contributor in the development of cardiovascular disease^[106]. Direct CM-induced toxicity on renal tubular epithelial cells appears to be a major contributing factor in CIN. CM induces renal vasoconstriction, through increased adenosine and endothelin-1 secretion, and diversion of blood flow from the medulla to the cortex [Figure 2]. Consequently, renal blood flow to the medulla and GFR is reduced, followed by ischemia in the renal medulla^[107,108].

Atherosclerosis is the main cardiovascular disorder in which the association with oxidative stress became evident. Oxidized low density lipoprotein (oxLDL) plays a critical role in the pathogenesis of atherosclerosis. Studies have shown a clear link between arterial stiffness and oxLDL concentration, independent of the typical CVD risk factors^[109]. It remains uncertain whether oxLDL as an oxidative stress biomarker has any predictive property in cardiovascular patients^[110].

Vascular NOXs are important ROS generating enzymes and in human vascular cells, NOX1, NOX2, NOX4 and NOX5 are expressed. NOX are transmembrane enzyme complexes with a few regulatory subunits and a core catalytic subunit, except for NOX5^[111]. NOX activation results in the generation of O₂ from molecular oxygen by the transfer of electrons from NADPH^[112]. NADPH oxidase in humans was thought to be phagocyte specific as the two membrane bound units, gp91^{phox} and p22^{phox} form a heterodimer and mediate bacterial killing by generating O₂ (gp91^{phox} produces a burst of O₂ and p22^{phox} acts to stabilize gp91^{phox}, enhancing O₂ production)^[113]. P22^{phox} expression in non-phagocytic cells directed the discovery of NOX1 in non-phagocytic cells which then led to the identification of the other NOX proteins^[111].

NOX4 plays a key regulatory role, generating athero-protective ROS that inhibits inflammation and vascular remodeling. Decreased levels of effector T cells and chemokines, increased regulatory T-cells and reduced lesion formation was seen in apolipoprotein E-deficient mice expressing ectopic endothelial NOX4^[114]. However, reduced levels of endothelial H₂O₂ and phosphorylated mothers against decapentaplegic homolog 3 (p-SMAD3), along with the elevated expression of profibrotic connective tissue growth factor has been seen when NOX4 was downregulated in human aortic endothelial cells^[115]. NOX4 knockdown *in vivo* has also been shown to elevate fibrillar collagens I and III production in plaques, which is linked to increased p-SMAD3 levels and transforming growth factor-β expression in diabetic lesions^[116]. During the development of arteriosclerosis, NOX4 and H₂O₂ regulate the response of EC to endoplasmic reticulum (ER) stress [Figure 2]^[117]. ER stress leads to elevated ER H₂O₂ in a NOX4-dependent manner which then results in Ras-specific guanine nucleotide releasing factor (RasGRF) activation, the oxidation of thiols in the Ca²⁺-ATPase of sarcoplasmic reticulum microsomes and increased cytosolic calcium levels. In addition, NOX produced ROS affects X-box-binding protein 1 (KBP1) splicing, a key protein that promotes EC apoptosis and atherosclerosis formation^[118].

As well as the increased production of oxLDL, an additional contributor to cardiovascular morbidity appears to be oxidative endothelial damage. In healthy adults of varying ages, brachial artery flow-mediated dilation appeared to inversely correlate with the concentration of nitrotyrosine (produced, for example, via nitrogen dioxide radical and tyrosine radical recombination) in vascular EC^[119]. ET-1, as well as being a powerful vasoconstrictor, has also demonstrated proinflammatory and prooxidant properties and consequently, it has been associated with oxidative endothelial damage^[120]. In EC, oxLDL has been shown to stimulate endothelin-1 production, and elevated levels of endothelin-1 is known to generate ROS by NADPH oxidase [Figure 2]^[121]. Furthermore, the cardiovascular system inflammatory response is induced by oxidative stress and proinflammatory cytokines additionally induce oxidative damage in a positive, reverse feedback mechanism [Figure 2]^[122].

Antioxidant defense mechanisms decrease with age^[123], therefore age is a major risk factor of CIN. The unique anatomy of the renal medulla requires the thick ascending limbs of the loop of Henle to carry out energetically challenging ion transport in a state of relative hypoxia compared to the renal cortex. It has been proposed that a discrepancy between the metabolic requirements of these thick ascending limbs and the medullary blood supply could generate O_2 ^[124]. The thick ascending limb is associated with ROS generation mostly due to the extremely high mitochondrial density and therefore, mitochondrial ROS generation^[125]. Reduced renal blood flow can induce oxidative stress and osmotic necrosis consequently generating ROS, via a positive feedback mechanism, leading to acute tubular necrosis^[114,123]. Renal microcirculation is compromised by ROS production, which affects renal vascular function by facilitating the production of vasoconstrictors such as endothelin-1 and ameliorating the effects of vasodilators, such as NO ^[126]. Direct toxicity of CM in renal tubular cells can also result in mitochondrial dysfunction and, combined with elevated levels of ROS, leads to extensive damage of glomerular cells by compromising the cellular membrane, ultimately resulting in apoptosis^[127].

A crucial factor in the production of ROS in the kidney is renal hypoxia. There are, however, conflicting reports relating to the extent to which oxidative stress is a cause or epiphenomena. ROS are regularly involved in cellular inflammatory responses and it is proposed that ROS are formed during renal parenchymal hypoxia, following CM exposure, resulting in vascular endothelial injury. This aggravates renal parenchymal hypoxia resulting in endothelial dysfunction^[125]. O_2 can lead to the accumulation of $ONOO^-$, the production of which reduces NO bioavailability. In addition, ROS activate p38 MAPK stress kinases and c-Jun N-terminal kinases, that are involved in the activation of caspase-3 and caspase-9, which are associated with the induction of apoptosis^[128]. Mitochondrial dysfunction can induce apoptosis by releasing cytochrome c and activating caspase-9, which in turn activates caspase-3. Caspase-3 plays a major role in apoptotic signaling by mediating death receptor-dependent and mitochondria-dependent apoptosis pathways^[129].

In response to excessive oxidative stress, cells activate/induce their own antioxidant defense mechanisms. Glutathione (GSH), is an important endogenous thiol that is essential to a variety of detoxification processes. Mammalian cells contain high concentrations of GSH (3-5 mmol/L) which is used in numerous diverse roles as well as hepatic detoxification. GSH can donate reducing equivalents for the activity of specific antioxidant peroxidase enzyme, such as GSH peroxidase (GPx), and can react directly with certain ROS (e.g., carbonate radical). Intracellular levels of GSH are tightly controlled by the enzymes glutamate-cysteine ligase and GSH synthase (involved in synthesis), GSH reductase (involved in recycling of oxidized glutathione back to GSH) and GSH transferases (involved in utilization)^[130]. Redox enzymes include thioredoxin, catalase, GPx, peroxiredoxins and superoxide dismutase (SOD)^[100].

ROLE OF INFLAMMATION IN CIN/CVD

One of the factors that is central to the prevalence of CIN is chronic inflammation. The role of inflammation in CIN has been extensively studied and clinical trials in humans and animal models have been performed to help elucidate this role^[131-134]. One of the main features of intravascular iodinated CM is that it causes vasodilation followed by a prolongation in vasoconstriction^[135,136]. The vasodilation/vasoconstriction occurs in all patients that require a CM procedure, but this effect has not been found to work alone in the increase of CIN risk among patients. Two additional pathways suggested to promote this increase are cellular toxicity and elevated urinary viscosity that can cause obstructions through stone formation^[137].

Although the global prevalence of CIN does not constitute a public health threat, at risk populations, such as those suffering from higher presence of infectious diseases, have a higher incidence of inflammation than populations that are not affected by these diseases^[138]. A close relationship between inflammatory

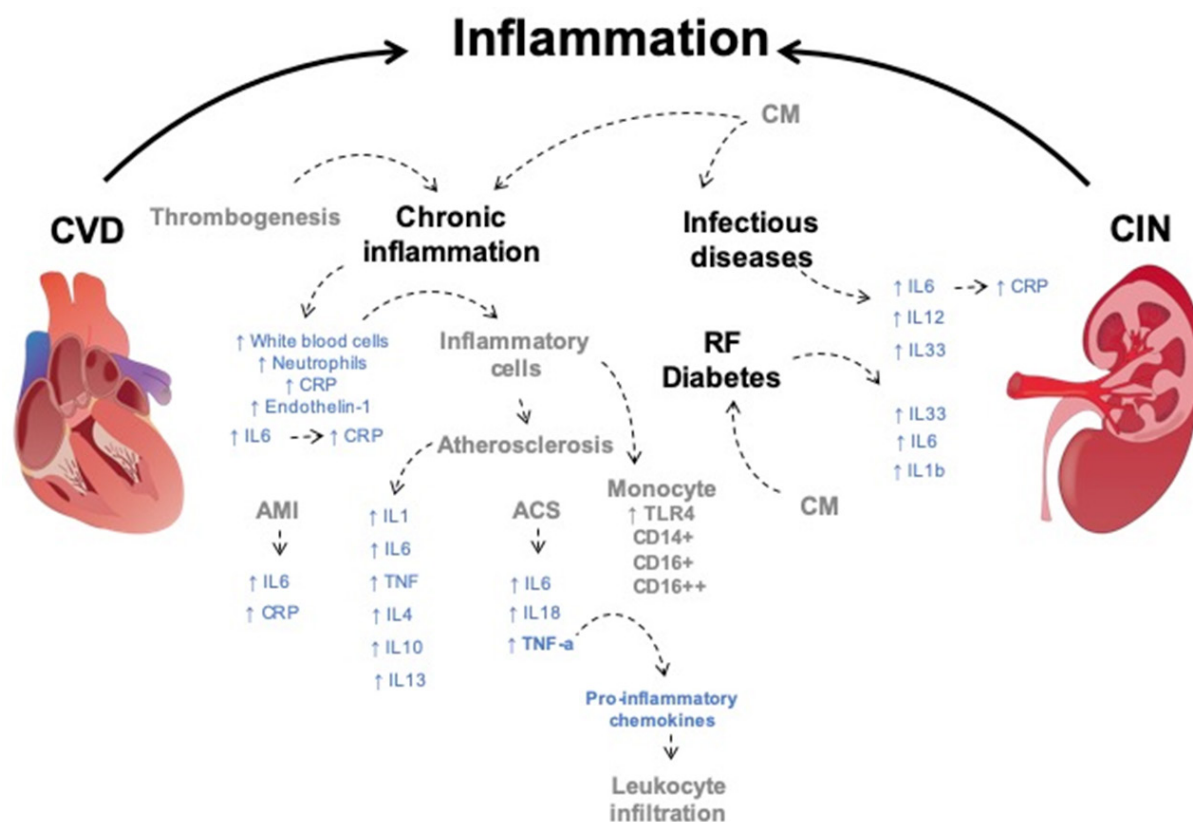


Figure 3. Inflammatory molecules in CIN and CVD. Inflammatory states have been associated with CIN and CVD risk factors. Inflammatory cells and molecules are considered as potential risk factors in CVD and CIN. Inflammatory risk factors highlighted in blue. CM, Disease states and cellular types related to inflammatory risk factors represented in grey. ↑: overproduction. CM: contrast media; RF: risk factors; AMI: acute myocardial infarction; ACS: acute coronary syndrome; IL: interleukin; CRP: C reactive protein; TNF- α : tumor necrotic factor- α ; TLR4: toll like receptor 4; CIN: contrast induced nephropathy; CVD: cardiovascular disorders

molecules and thrombogenesis has been well reported^[139]. The acute inflammatory state is a landmark of infectious diseases and one of the main type of molecules that derive from it are interleukins (ILs). IL-6 and IL-12 have been targeted as disruptors of homeostasis within inflammatory processes. IL-6 promotes the expression of the C reactive protein (CRP), which is being used as a current acute inflammation marker [Figure 3]^[140].

One of the studies that assessed the increased risk for CIN due to inflammation was performed by Kwasa *et al.*^[132]. They performed a prospective cohort study of patients undergoing a contrast-enhanced CT (CECT) scan. 423 patients were recruited and grouped into those without inflammation having serum CRP levels ≤ 5 mg/dL and those with evidence of inflammation having CRP levels > 5 mg/dL. Serum creatinine (SCr) was measured before the CECT and 48 h following the CECT with CIN diagnosed by an increase of $> 25\%$ in SCr from the baseline [Figure 3]. The observed incidence of CIN was 9.92%. Of the patients with inflammation, 29 (13.5%) developed CIN, while 13 (6.25%) of those without inflammation developed CIN. No significant relation was found between the increase of CIN prevalence and biophysical variables (age, sex, height, weight, *etc.*)^[132]. Another study reported by Oweis *et al.*^[30] showed serum levels of IL-33 as significant predictor for development of CIN. Of the total 202 patients, 30 (14.8%) developed CIN. The incidence rate was 21.1% among females and 12.4% among males [Figure 3].

Additional biomarkers of inflammation have been studied to assess their potential as predictors of CIN in different conditions. Cell types that are associated chronic inflammation have been proposed as predictors

of increased risk of developing CIN: the study published by Yuan *et al.*^[92] in 2017 found in 1,061 patients that white blood cell count, neutrophil count, neutrophil lymphocyte ratio, CRP level, and big ET-1 level were all associated with an increased risk of CIN development. It is important to mention that all of the patients in this study went through emergency PCI.

Regarding the assessment of multiple markers to predict the development of CIN, different studies have reported combinations between proteins that can be measured in human serum. The study performed by Satilmis *et al.*^[141], presented an assessment of the ratio between 2 inflammatory markers, CRP and albumin. 205 patients with non-ST-elevation myocardial infarction that underwent PCI were subsequently assessed for development of CIN. The prevalence of CIN in this study was 10.2%. Multivariate logistic regression analysis showed significant association between CRP: albumin ratio and the development of CIN; advanced age, diabetes, dyslipidemia and left ventricular ejection fraction were also associated with the condition.

Animal models have also been used in the search for the potential role of inflammation in the development of CIN. Demirtas *et al.*^[29] evaluated the role of IL-33 in the pathogenesis of CIN in diabetic rats. 30 male Sprague-Dawley rats were divided into 3 groups (healthy, diabetic and diabetic with CIN). Significantly increased presence of IL-33 was found in the kidney tissue of the diabetic group after induction of CIN when compared with the healthy and diabetic groups. Serum levels of IL-33, IL-6, and IL-1 β were also significantly increased in the diabetic + CIN group when compared to the healthy and diabetic groups [Figure 3].

Prophylactic use of carotenoids has been studied in animal models to assess the relation between oxidative stress induced inflammation and CIN development. The studies presented by Buyuklu *et al.*^[142,143] aimed to investigate the effects of lycopene and curcumin as protection against the development of CIN in rats. 28 male Wistar albino rats were divided into 4 groups, they included a normal control group, CIN group, CIN + lycopene and CIN + curcumin groups. Significant increase in urea, creatinine and malondialdehyde were observed in the CIN group when compared with the control group. Additionally, histological tests showed significant increase of infiltrated inflammatory cells and necrotic degenerative changes in the CIN group when compared against the control^[142,143].

The role of the inflammatory state in CVD was addressed in an extensive literature^[14]. The search for markers has two principal aims: to look into the understanding of the mechanisms of disease and to identify molecules that can be detected more accurately to predict the risk of cardiovascular events. The role of inflammation in CVD development has been assessed throughout different populations and experimental models, critical importance has been given to events such as acute myocardial infarction (AMI) and atherosclerosis due to their high incidence and mortality rates^[144]. Inflammation in CVD includes a vast number of processes which can occur at the site of disease, in the bloodstream and at sites far from the disease^[145]. Immune response takes the spotlight when addressing inflammation and CVD. In AMI a signaling cascade induces the expression and recruitment of proinflammatory molecules, accelerating both damage and further repair of injured cardiac tissue. Elevated levels of high-sensitivity CRP and IL-6 in plasma have been found correlated with unfavorable outcomes in patients [Figure 3]^[146].

Rajendran *et al.*^[147] assessed both IL-6 and hs-CRP in a Chennai based population. 93 patients with AMI and 102 healthy subjects as a control group were analyzed. Both IL-6 and hs-CRP were found to be significantly increased when compared with the control group. Pro-inflammatory cytokines IL-6, IL-10, IL-18 and TNF- α were evaluated in a study published in 2019 including 120 patients with acute coronary syndrome (ACS) and 60 healthy controls. Serum levels of IL-6, IL-18 and TNF- α were significantly higher in the ACS group when compared to the healthy group [Figure 3]. No significant difference in serum levels of IL-10 was found^[148]. Additionally, TNF- α has been found to promote the release proinflammatory

chemokines and adhesion molecule synthesis in damaged myocardium and causing additional leukocyte infiltration in mice^[149].

Toll-like receptors (TLRs) may be key to understanding heart failure. TLR4 deficiency is associated with decreased in size of damage by infarct and reduction of systemic inflammation in mice^[150]. In humans, the activation of TLR4 in monocytes is associated with the development of cardiac failure after AMI [Figure 3]^[151]. By contrast, deficiencies in the function of TLR2 were found to reduce myocardial fibrosis and improve ventricular remodeling after AMI in a murine model^[152].

Atherosclerosis is often described as a chronic inflammatory process. Deregulation in the endothelium is mediated by cell adhesion molecules, such as ICAM1, P-selectin and VCAM1. Additionally, the secretion of cytokines has a role in atherogenesis, namely IL-1, IL-6, TNF, IL-4, IL-10 and IL-13 [Figure 3]. The detection of some of these molecules in plasma has identified associations that could help to predict atherosclerosis severity. Moreover, the identification of cell types through flow cytometry has proven to be a promising predictor for atherogenic levels of severity. The amount CD¹⁴⁺CD¹⁶⁺⁺ monocytes present in circulation has been found to be inversely correlated to plasma HDL levels while CD¹⁶⁺ monocytes levels are proportional to severe atherosclerosis [Figure 3]^[153].

CVD AND CIN BIOMARKERS

The identification of rapid, predictive biomarkers for CIN is essential as current targets are relatively slow to be useful, or the assays are just too expensive to be launched in a clinical setting. Some of the postulated biomarkers for CIN and CVD are shown on Table 1. An early predictive biomarker of AKI is human neutrophil gelatinase-associated lipocalin (NGAL). NGAL is a small protein of the lipocalin superfamily that was initially identified from the supernatant of activated human neutrophils in 1993. Successive studies have recognized renal NGAL as a unique, specific biomarker for the early detection of AKI in critically ill patients and after CM administration. Urinary and serum levels of NGAL increase well before the increase of serum creatinine levels (~2 h). As a result, NGAL is increasingly studied as a marker of AKI^[154-157]. Another proposed sensitive, early, non-invasive biomarker for AKI kidney injury is urinary neutrophil gelatinase-associated lipocalin (uNGAL) also known as lipocalin-2. uNGAL is an iron-transporting protein that rapidly accumulates in the urine and kidney tubules after nephrotoxic and ischemic insults. Zappitelli *et al.*^[158] concluded that uNGAL is an effective predictor of AKI which is triggered in advance of increases in serum creatinine concentration. Despite these findings, the use of uNGAL is still experimental.

Liver type fatty acid binding protein (L-FABP) is an intracellular lipid chaperone and is expressed in renal proximal tubule cells and secreted into the urine in response to hypoxia caused by a decrease in peritubular capillary blood flow. Although L-FABP concentration is significantly increased in CIN patients after 24 hours, the specificity of this biomarker for CIN is low on account of a range of potential confounders^[159].

Tissue plasminogen activator (tPA), a part of the serine protease family, is a plasma protein involved in the breakdown of blood clots and a key fibrinolytic agent that takes part in the recruitment of inflammatory cells. Some other roles of tPA involve the turnover of extracellular matrix components via activation of matrix metalloproteinases and immune-modulatory functions. Plasminogen activator inhibitor-1 is the main physiological inhibitor of endogenous fibrinolysis which functions through the inhibition of tPA and the urokinase type activator (uPA)^[160,161]. A recent study^[162] reported a relationship between increased serum tPA levels with an increased rate of mortality of dialysis-dependent AKI (AKI-D) patients. Elevated tPA expression has been detected in the proximal tubular epithelial cells of ischemic kidneys, in animal models. Removing tPA by antisense treatment had reduced the influx of neutrophils and helped protect renal function during ischemia-reperfusion injury. This suggests tPA inhibition as a novel strategy to improve ischemic AKI^[163]. Many additional studies have also implied the involvement of tPA in the process of kidney fibrosis that leads to progression of CKD^[164-166].

Table 1. Origin and mechanisms of potential biomarkers for prediction of CIN and CVD

Biomarkers	Etiology	Mechanisms	Organism	Ref.
IL-6, IL-12, IL-8	CIN and CVD	Induction of the production of CRP	Human	Alladina <i>et al.</i> ^[171] (2016) Kwasa <i>et al.</i> ^[132] (2014) Rajendran <i>et al.</i> ^[147] (2012)
C reactive protein	CIN and CVD	Response to chronic inflammation	Human	Kwasa <i>et al.</i> ^[132] (2014) Rajendran <i>et al.</i> ^[147] (2012)
TNF- α	CVD	Upregulated in inflammation in acute myocardial infarction, modulates cardiac contractility and peripheral resistance. Promotes leukocyte infiltration in mice	Human Mice	Senguttuvan <i>et al.</i> ^[148] (2019) Maekawa <i>et al.</i> ^[149] (2002)
CD14 ⁺ CD16 ⁺⁺ monocytes	CVD	Presence inversely correlated to plasma HDL levels	Human	Schlitt <i>et al.</i> ^[153] (2004)
CD16 ⁺ monocytes	CVD	Levels proportional to severe atherosclerosis	Human	Schlitt <i>et al.</i> ^[153] (2004)
Neutrophil/Lymphocyte ratio	CIN	Elevated in subclinical inflammation	Human	Yuan <i>et al.</i> ^[92] (2017)
CRP/Albumin ratio	CIN	CRP levels are found increased in chronic inflammation and albumin levels are negatively correlated in the presence of acute inflammation	Human	Satilmis <i>et al.</i> ^[141] (2020)
IL-33 and IL-1 β	CIN and CVD	Proinflammatory cytokines, IL-33 binds to immune cells and promotes secretion of cytokines resulting in inflammation	Human and Sprague-Dawley rat	Oweis <i>et al.</i> ^[30] (2018) Demirtas <i>et al.</i> ^[29] (2016)
NGAL	CIN	Accumulates in urine, blood and renal cortical tubules following ischaemic and nephrotoxic injury. Antioxidant protection against CIN development	Human Wistar albino rat	Malyszko <i>et al.</i> ^[156] (2009) Buyuklu <i>et al.</i> ^[143] (2014)
L-FABP	CIN	Specifically binds to intracellular, free unsaturated fatty acids during hypoxic tissue injury	Human	Nakamura <i>et al.</i> ^[159] (2006)
tPA	CIN and CVD	Tissue type fibrinolytic agent involved in the breakdown of blood clots and the recruitment of inflammatory cells	Human	Baramova <i>et al.</i> ^[160] (1997) and Stringer <i>et al.</i> ^[161] (1997)
uPA	CIN and CVD	Urokinase type fibrinolytic agent involved in the breakdown of blood clots and the recruitment of inflammatory cells	Human	Baramova <i>et al.</i> ^[160] (1997) and Stringer <i>et al.</i> ^[161] (1997)
PAI-1	CIN and CVD	Primary physiological inhibitor of tPA and uPA	Human	Baramova <i>et al.</i> ^[160] (1997) and Stringer <i>et al.</i> ^[161] (1997)
KIM-1	CIN	Localised to the proximal tubules of the human kidney following toxic or ischaemic injury	Human	Nogare <i>et al.</i> ^[172] (2012)
IL-18	CIN and CVD	Proinflammatory cytokine	Human Mice	Ling <i>et al.</i> ^[168] (2008)
CysC	CIN	Produced by all nucleated cells and displays a stable rate of production. Freely filtered by the glomerulus	Human	Soto <i>et al.</i> ^[174] (2010)
Serum Creatinine	CIN	Resulting product of creatine phosphate from protein and muscle metabolism. Exhibits a stable rate of production and is freely filtered by the glomerulus	Human	Slocum <i>et al.</i> ^[173] (2012)

IL: interleukin; TNF: tumor necrotic factor; CRP: C reactive protein; NGAL: neutrophil gelatinase-associated lipocalin; L-FABP: liver type fatty acid binding protein; tPA: tissue plasminogen activator; uPA: urokinase plasminogen activator; PAI: plasminogen activator inhibitor; KIM-1: kidney injury molecule 1; CysC: Cystatin C; CIN: contrast induced nephropathy; CVD: cardiovascular disorders; CRP: C reactive protein

IL-6 is an interleukin that can act as both an anti-inflammatory myokine and a pro-inflammatory cytokine and is encoded by the IL6 gene in humans. Osteoblasts produce and release IL-6. The role of IL-6 as an anti-inflammatory cytokine is facilitated via the interleukins inhibitory effects on IL-1 and TNF- α , and activation of IL-10 and IL-1ra^[167]. Studies have demonstrated a close correlation between AKI and IL-6

expression in many animal models^[168,169]. Resident kidney cells, such as tubular epithelial cells, endothelial cells, mesangial cells and podocytes can all produce and release IL-6. A study has shown that, in a model of ischemia-reperfusion injury, after leukocytes penetrated the injured kidney, maladaptive IL-6 was produced in response to their TLR-4 receptors interacting with high mobility group box 1 protein released by the injured renal cells^[170]. Raised levels of the pro-inflammatory cytokines, IL-8 and IL-6, have been seen early on in AKI patients and were linked to prolonged mechanical ventilation^[171].

The transmembrane protein, kidney injury molecule 1 (KIM-1), recognizes apoptotic cells and leads them to lysosomes. Additionally, it acts as a receptor for oxidized lipoproteins and is therefore adept at recognizing apoptotic cell signals. KIM-1 is undetectable in normal kidney tissue but is highly expressed following toxic or ischaemic injury in differentiated proximal tubule epithelial cells from rodent and human kidneys^[172,173]. Plasma cystatine-C (CysC), is a low molecular weight protein produced at a predictable rate by all nucleated cells. CysC is filtered across the glomerular membrane but is neither reabsorbed nor secreted during its passage through the nephron. Given that CysC is almost entirely catabolized in the proximal tubule, it is impossible to measure its renal clearance. However, the plasma or serum concentration of CysC accurately reflects the GFR and significant increases in CysC are detected in CIN patients after 8 h. However, a similar increment has also been seen in several other conditions, including thyroid dysfunction, age, an increase in muscle mass, systemic inflammation, corticosteroids administration and neoplasia^[174] limiting its utility as a CIN biomarker.

The key diagnostic criterion for CIN is the elevation of serum creatinine concentration by more than 25% over baseline, after eliminating any other possible causes. Other laboratory findings may also be present such as hyperkalaemia and acidosis. Although patients may have normal urine output, they can also suffer from anuria (failure of the kidneys to produce urine) or oliguria (low output of urine > 80 mL/day, < 400 mL/day). Findings on urine analysis are normally non-specific^[175]. Normally a delay of 24-48 h is seen between contrast exposure and changes in serum creatinine concentration, which makes creatinine a late indicator of renal function changes^[176].

Since a close correlation among inflammatory molecules and kidney injury in CIN has been observed, as described above, they have also been proposed as potential CIN biomarkers [Table 1]. IL-8 and IL-6, have been seen early on in AKI patients and were linked to prolonged mechanical ventilation^[171]. Successive studies have recognized renal NGAL as a unique, specific biomarker for the early detection of AKI in critically ill patients and after CM administration^[154-157]. Other proposed biomarkers, despite being effective predictors of AKI, such as uNGAL triggered preceding increases in serum creatinine concentration^[157,158] are still experimental. Other potential biomarkers have been deemed as non-specific, such as L-FABP, although significantly increased in CIN patients after 24 h, where potential confounders lower its specificity^[159].

CONCLUSION

Oxidative stress influences cardiovascular morbidity mainly through increased peripheral vascular resistance [Figure 1]. However, although the generation of ROS could affect renal blood flow by facilitating the production of vasoconstrictors and impacting the effects of vasodilators, the influence of oxidative stress in the development of CIN is uncertain.

Inflammation results in the alteration of homeostasis in both the circulatory and renal systems. These alterations can be intrinsic of cellular damage or can be mediated by external factors such as CM. Immune response to CM cytotoxicity causes a rapid increase in the migration and accumulation of cytokines such as ILs and TNF- α in the progression of both CVD and CIN. Additionally, the presence of cellular types found in response to inflammation is a feature in early development of CVD and CIN. The main interplay

between CIN and CVD in the context of inflammation may rely on endothelial dysfunction and immune response. The signaling pathways activated through endothelial dysfunction in cardiac events result in the generation of systemic inflammation which has been found to affect the kidneys and made them more susceptible to local inflammation processes driven by CM cytotoxicity.

Current CIN prevention strategies, such as the use of carotenoids, for instance curcumin and lycopene^[142,143], to limit the oxidative effects of CM are questionable due to the inconclusive evidence to support the oxidative capacity of CM. Existing biomarkers for CIN are either non-specific, such as L-FABP, or late indicators of renal function changes, such as changes in serum creatinine, making them poor predictive markers at best. The relationship between CVD and CIN and the underlying mechanisms responsible for CIN are unclear. Identifying novel biomarkers, be it genetic, redox or serum protein markers, for the early detection of CIN will help gain a better understanding of the underlying mechanisms. Greater mechanistic understanding is required to better predict and treat CIN.

DECLARATIONS

Authors' contributions

Design and conception of the original draft: Cervantes-Gracia K, Raja K, Llanas-Cornejo D

Original draft text editing: Cervantes-Gracia K, Raja K, Llanas-Cornejo D, Cobley JN, Megson IL, Chahwan R, Husi H

Figures design and editing: Cervantes-Gracia K

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Led the study: Husi H

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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Review

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The role of vascular endothelium and exosomes in human protozoan parasitic diseases

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Abstract

The vascular endothelium is a vital component in maintaining the structure and function of blood vessels. The endothelial cells (ECs) mediate vital regulatory functions such as the proliferation of cells, permeability of various tissue membranes, and exchange of gases, thrombolysis, blood flow, and homeostasis. The vascular endothelium also regulates inflammation and immune cell trafficking, and ECs serve as a replicative niche for many bacterial, viral, and protozoan infectious diseases. Endothelial dysfunction can lead to vasodilation and pro-inflammation, which are the hallmarks of many severe diseases. Exosomes are nanoscale membrane-bound vesicles that emerge from cells and serve as important extracellular components, which facilitate communication between cells and maintain homeostasis during normal and pathophysiological states. Exosomes are also involved in gene transfer, inflammation and antigen presentation, and mediation of the immune response during pathogenic states. Protozoa are a diverse group of unicellular organisms that cause many infectious diseases in humans. In this regard, it is becoming increasingly evident that many protozoan parasites (such as *Plasmodium*, *Trypanosoma*, *Leishmania*, and *Toxoplasma*) utilize exosomes for the transfer of their virulence factors and effector molecules into the host cells, which manipulate the host gene expression, immune responses, and other biological activities to establish and modulate infection. In this review, we discuss the role of the vascular endothelium and exosomes in and



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their contribution to pathogenesis in malaria, African sleeping sickness, Chagas disease, and leishmaniasis and toxoplasmosis with an emphasis on their actions on the innate and adaptive immune mechanisms of resistance.

Keywords: Vascular endothelium, exosomes, leishmaniasis, malaria, trypanosomiasis, toxoplasmosis

INTRODUCTION

The vascular endothelium

The vascular endothelium (VE) is a large endocrine organ consisting of a single layer (one cell thick) of endothelial cells (ECs). The VE mediates regulatory functions in cell proliferation, angiogenesis, permeability, blood flow, thrombosis, thrombolysis, coagulation, homeostasis, and inflammation^[1,2]. It also acts as the barrier between vascular and parenchymal compartments of all organs and regulates the exchange of gases, immune cell trafficking, metabolism, and the spread of infections. The entire circulatory system, from the heart to the smallest capillaries, is layered with ECs, which carry out unique functions such as fluid filtration, maintaining the tone of the blood vessel, platelet and leukocyte interactions, neutrophil recruitment, and hormone trafficking^[3,4]. The VE plays a major role in leukocyte recruitment from the vessel lumen and transit into tissue parenchyma^[5,6], and it is also the precursor for both hematopoietic and endothelial lineages^[7]. The role of the VE is governed by the presence of many membrane-bound receptors for molecules such as proteins, lipid transporting particles hormones, and metabolites^[8,9]. Recent studies have identified the immunological role of the VE from the secretion of cytokines and chemokines to the expression of adhesion molecules and antigen presentation^[10-12].

The VE is also involved in bacterial, viral, and protozoan infectious disease processes. However, the interactions between the pathogen and the VE and how they both influence the disease outcome needs to be explored further. A clearer understanding of these relationships may help in identifying potential targets for therapeutic intervention(s) to prevent and/or reduce disease severity.

Role of the VE in innate immunity

The VE serves as the first line of defense against the physical stimuli and chemical agonists that are present in the bloodstream during infection and disease by activating the inflammatory response when receptors on the ECs, such as the toll-like receptors (TLRs) and NOD-like receptors (NLRs), recognize Pathogen associated molecular patterns (PAMPs), damage associated molecular patterns (DAMPs), and pro-inflammatory cytokines such as interleukin (IL)-1 β or TNF- α ^[13,14]. These recognition signals regulate the expression of pro-inflammatory genes (IL-1, IL-6, TNF- α , and IFN- γ), leukocyte recruitment, phagocytosis, and a subsequent adaptive immune response^[14]. During the early phase of an inflammatory response, ECs are stimulated to release nitric oxide (NO), prostacyclin-2 (prostaglandin I₂ or PGI₂), and endothelin-1 to increase vasodilation by relaxing the surrounding smooth muscle cells^[13,15]. Capillary permeability is further facilitated by the removal of occludin between EC junctions and inflammatory mediators such as kinins, cytokines, histamine, arachidonic acid, and complement components produced during this onset of inflammation^[14]. VE activation upregulates expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin by ECs to aid chemokine-guided leukocyte rolling and extravasation through the vessel wall into the target site of the tissue^[14].

Role of the VE in adaptive immunity

The adaptive immune response can be mediated by the VE in multiple ways under certain conditions. For example, thrombin-activated platelets as discussed above, upregulate CD40L similar to activated T cells and are therefore able to interact with CD40 on the EC surface to promote cytokine and chemokine

secretion, adhesion molecule expression, tissue factor release, and leukocyte recruitment^[16,17]. Furthermore, the upregulation of adhesion molecules and chemokines by activated ECs also serves to selectively recruit specific T cell types in circulation^[18]. During this recruitment, antigen presentation may occur between the ECs and T cells mediated by major histocompatibility complex (MHC). It is known that ECs express MHC Class I on their surface, as well as MHC Class II in certain vasculatures of the body^[14]. Due to this presence of MHC, ECs can act as antigen-presenting cells (APCs) to present antigen to effector or memory T cells^[19]. Interaction with T cell co-stimulatory or co-inhibitory molecules expressed on ECs, such as CD80, CD86, LFA-3, ICOS-L, PDL-1, CD40, and CD134L, also mediates the T cell response^[14].

Due to their ability to act as APCs, ECs may generate certain T cell subsets to induce either inflammation or immune tolerance^[14]. For instance, it has been shown that ECs support T cell proliferation and increases in the number of suppressor Treg cells^[20] and can stimulate the production of pro-inflammatory Th17 cells under inflammatory conditions^[21]. ECs are also directly recognized by effector memory CD4⁺ T cells to stimulate IFN- γ production and subsequent CD4⁺ Th1 polarization^[22]. This phenomenon has been further supported by a study involving activation of the ECs mediated by the C3a and C5a anaphylatoxins, which induce a Th1 phenotype, an increase in IFN- γ production, and activation of B lymphoblasts^[23]. Taken together, the evidence suggests that VE plays a critical role in the initial onset of inflammation, innate immunity, and subsequent adaptive immunity in response to diverse stimuli present within the bloodstream.

Exosomes

Exosomes are membrane-bound extracellular vesicles between 40 and 100 nm in diameter that have been recognized as important players in cell-to-cell signaling^[24]. They are secreted into the extracellular space by ECs and various other cells, such as platelets, immunocytes, and smooth muscle cells^[25-27] and are present in almost all biological fluids^[28]. Extracellular exchange of exosomes containing various bioactive molecules takes place continuously between organelles to foster communication during homeostasis and diseased states^[29]. Exosome production begins with the internalization of the cellular membrane through endocytosis to form an endosome, followed by the invagination of the endosomal membrane, which matures into multivesicular bodies (MVBs). The MVBs can then either be degraded by internal lysosomes or are transported to the cell membrane to undergo transcytosis or fusion and release of the contained liberates intraluminal vesicles into the extracellular space, becoming exosomes^[28,30]. Contents carried by exosomes consist of different proteins, lipids, metabolites, RNA, and DNA, which may be exchanged between the exosomes and their target cells.

Originally, exosomes were thought to be involved only in the process of excretion of unnecessary/unwanted proteins, and several studies have revealed that cellular stress triggers an enhanced release of exosomes^[31-34]. However, further studies have suggested the participation of exosomes in cellular communication associated with many physiological and pathological states due to their ability to influence the phenotype of recipient cells^[24,28]. Exosomes can target cells through specific receptor binding to activate cell-to-cell signaling pathways, which induce specific functions^[35]. Information from an exosome may be transferred to the recipient cell either by interacting at the cell surface or by endosomal uptake^[36]. Functions of released exosomes include horizontal gene transfer, inflammation, antigen presentation, tumor progression, and mediation of the immune response during pathogenic states^[35,37].

Exosomes have been isolated from protozoa, bacteria, viruses, and fungi but each has distinct exosome profiles with varying compositions^[38]. Protozoan parasites modulate host cells by producing exosomes containing virulence factors and effector molecules. In this way, parasites can manipulate host gene expression, immune responses, and other factors that favor parasite growth, survival, and pathogenesis^[39]. Exosomes released during infection may also facilitate host immunity^[38,40].

Role of exosomes in innate immunity

Exosomes are thought to play an important role in the host immune response to infection. Reciprocal cross-talk between platelets and neutrophils is enabled by neutrophil-derived exosome transfer of arachidonic acid to platelets, which is enzymatically converted into thromboxane A₂ (TxA₂). Release of TxA₂ from platelets contributes to the upregulation of ICAM-1 on neutrophils, which binds to the EC surface and enables their extravasation to the site of infection to engage microbes^[41].

Exosomes are directly involved in immune signaling in as much as they can cargo pro-inflammatory and anti-inflammatory cytokines to target cells^[42-45] as well as stimulate the secretion of these cytokines from recipient cells^[35,46,47]. For instance, a recent study documented the role of apoptotic exosome-like vesicles in promoting the synthesis of the pro-inflammatory IL-1 β in macrophages, thereby contributing to their activation^[48]. In contrast, exosomes have also been shown to promote the production of the anti-inflammatory transforming growth factor- β (TGF- β) in macrophages leading to the inhibition of the innate immune response^[47,49]. Murine LPS-stimulated macrophages produce exosomes containing endoplasmic reticulum aminopeptidase 1, TNF- α , IFN- γ , and CCL3, which induces phagocytosis and nitric oxide synthesis in adjacent recipient macrophages^[47,50], important cellular mediators in clearing the microbial infection. Additionally, epithelial cell uptake of exosomes secreted by LPS-induced dendritic cells (DCs) has been shown to stimulate their activation and subsequent release of cytokines and chemokines to further the innate response^[51,52].

PAMPs are vital for recognition of pathogens and activation of immune cells and have been found inside exosomes secreted by both infected cells^[53,54] and pathogens^[55-57]. Interaction of PAMP-containing exosomes with the innate immune cells can induce inflammation. This has been observed in bacteria-infected macrophages, which release exosomes-contained pathogen antigens, which in turn promote maturation of DCs and secretion of pro-inflammatory cytokines^[58].

Role of exosomes in adaptive immunity

Exosomes not only influence innate immune responses but can also play a marked role in adaptive immunity by promoting immune activation or suppression. B-lymphocytes infected with Epstein-Barr virus were the first immune cells discovered to release exosomes. These exosomes were shown to contain peptide-bound MHC Class I and II, B7 co-stimulatory, and ICAM-1 adhesion molecules that could induce a specific T cell response through the direct presentation of MHC Class II antigen^[51]. Later, DCs were also found to release exosomes, possessing MHC Class I and II and T cell co-stimulatory molecules, leading to direct antigen presentation and CD4⁺ and CD8⁺ T cell activation. DC-derived exosomes can also mediate antigen presentation through bystander DCs, by either cross-dressing or internalization of the exosomes and subsequent peptide transfer to the recipient cell MHC molecules. It is known that immature DCs have decreased T cell activation ability due to the lack of co-stimulatory molecules but do produce more antigen-presenting exosomes than mature DCs. Therefore, the transfer of exosomes from antigen-containing donor DCs to recipient bystander DCs allows indirect antigen presentation and activation of T cells, especially from the immature DCs, which are unable to successfully present the antigen on their own^[51,59].

Furthermore, the CD4⁺ and CD8⁺ T cells can also constitutively secrete exosomes containing TCR/CD3 complexes, which is enhanced upon TCR activation^[60] and can be either immune-activating or immune-suppressing depending on the microenvironment. Activated T cells can transfer exosomes to induce activation of resting T cells and enhance adaptive immune responses. For instance, CD3⁺ T cells activated with IL-2 and anti-CD3-secreted exosomes promote the proliferation of CD8⁺ T cells as well as their subsequent cytokine secretion^[61].

Exosomes can also promote an immunosuppressed environment under certain circumstances. Exosomes produced by immature DCs have been shown to induce T cell anergy/deletion as well as activate CD4⁺

regulatory T (Treg) cells^[38]. Treg cells promote an immunosuppressive environment by secreting exosomes containing anti-inflammatory molecules that inhibit IFN- γ secretion and CD4+ Th1 cell proliferation, as well as signal other T cells to differentiate into Treg cells. It has also been found that CD4+ T cells and certain B cells whose exosomes contain FasL can induce apoptosis in recipient T cells. Furthermore, ECs transfer anti-inflammatory miRNA through exosomes to mediate T cell responses and prevent chronic inflammation^[62].

HUMAN PROTOZOAN PARASITIC DISEASES

In humans, protozoan parasitic infections represent a substantial threat causing more than one million deaths annually^[63]. According to the World Health Organization (WHO), it is estimated that protozoan parasitic infections occur in billions of people worldwide and are associated with significant mortality and morbidity and negatively impact many countries economically (WHO.org and^[64]). The three most important protozoan diseases in humans are malaria, leishmaniasis, and African trypanosomiasis that cause disability-adjusted life years in millions of patients (WHO.org 2008 and 2010).

Many protozoan infections cause non-self-limiting chronic infections and neglected diseases. There are 20 diseases that affect more than one billion people in almost 149 tropical and subtropical countries and are responsible for approximately 12% of the total global health burden, which are categorized as Neglected Tropical Diseases (NTDs)^[65] (https://www.who.int/neglected_diseases/diseases/en/). Due to the disease burdens, limited available effective treatments, and lack of vaccines, the WHO has classified NTDs such as leishmaniasis, Chagas disease, and Human African trypanosomiasis under the specialized program “Innovative and Intensified Disease Management”^[66]. While most of these infections occur in developing countries, it is evident that developed countries are also affected^[67]. In addition, the emergence of anti-microbial resistant strains, toxicity, and low effectiveness of the currently available treatments pose a substantial problem^[68]. Globalization and socioeconomic conditions also play a major role in the emergence and spreading of specific protozoan parasitic infections^[69]. Furthermore, while many protozoan infections remain asymptomatic, they can lead to death, especially among children. It is important to note that, unlike bacterial and viral infections, many protozoan parasitic infections do not have readily available vaccines^[70]. The lack of reliable drugs, difficulties in vector control, and limited knowledge about these infections are also major barriers to preventing, controlling, and treating these protozoan parasitic infections. Understanding the host immune response to protozoan parasites is very important to develop vaccines and new drugs with low toxicity and high efficacy.

The most severe protozoan infections, such as malaria, leishmaniasis, Chagas diseases, Human African trypanosomiasis (HAT), and toxoplasmosis are transmitted through blood, although Chagas disease and Toxoplasmosis can also be transmitted through the consumption of infected meat and liquid^[71]. Hematophagous arthropod vectors serve as intermediate hosts and transmit the parasites (*Leishmania*, *Trypanosomes*, and *Plasmodium*) between successive vertebrate hosts. Thus, these bloodborne and vector-transmitted infections involve complex interactions between the parasite and insect and mammalian hosts.

Below, we discuss the role of VE and exosomes in malaria, leishmaniasis, toxoplasmosis, Chagas disease, and HAT, as well as their effects on the innate and adaptive immune responses in these infections. The roles of VE and exosomes in these infections are summarized in [Tables 1 and 2](#), respectively.

MALARIA

Malaria is the most prevalent tropical disease, which annually infects 300-500 million individuals worldwide (CDC.org). It is estimated that 2-3 million people are at risk every year, most of whom are children who die from the infection without the proper treatment along with the development of other

Table 1. Roles and mechanisms of vascular endothelium in malaria, leishmaniasis, toxoplasmosis, Chagas disease, and HAT

Parasite name	Disease	Role of VE	Ref.
<i>Plasmodium</i> spp.	Malaria	Expresses receptors for <i>Plasmodium</i> antigens	[72,73]
		Reservoir for epoxide contains lipid signaling molecules and helps in multiplication of parasites	[74]
		Produces low molecular weight growth factors, which enhance the parasite proliferation	[75]
<i>Leishmania</i> spp.	Leishmaniasis	Reservoirs for intra- and extracellular parasites	[76]
		Releases nitric oxide (NO) and limits the spread of the disease	[77]
		Expresses ICAM-1 in skin lesions in cutaneous disease, which helps lymphocyte migration s to sites of inflammation	[78]
		Increases expression of VCAM-1, VEGF-A and VEGF-R in the skin lesions in cutaneous disease	[79-81]
<i>Toxoplasma</i>	Toxoplasmosis	Splenic endothelial cells express Ntrk2, helps in the pathological remodeling of the spleen in visceral disease	[82]
		Serves as replicative niche and provides the entrance to CNS	[83]
		<i>T. gondii</i> infection leads to activation of cerebral endothelial cells, facilitating the spreading of the disease	[84]
<i>Trypanosoma</i> spp.	Chagas disease	Key role in the dissemination of parasites to the other organs	[85,86]
		Produces various inflammatory molecules leading to trans-endothelial migration	[85,87,88]
		Releases vasoactive molecules such as endothelin-1 and pro-inflammatory cytokines IL-1 β , IL-6, TNF- α , and thromboxane A2 leading to the production of iNOS	[89-92]
<i>Trypanosoma</i> spp.	Human African trypanosomiasis	Produces endothelin-1 and IL-1 β , activated ERK1/2 and NF- κ B, resulting in the induction of Cyclin-D1 in uninfected cells	[93-95]
		Serves as replicative niche	[96,97]
		Produces inflammatory cytokines such as TNF- α , IL-6, and IL-8	[98,99]
		Induces the production of ICAM-1, E-selectin, and VCAM-1 to facilitate parasite migration into the central nervous system (CNS)	[96,98,100]
		Facilitates parasite transit across the endothelium of cerebral blood vessels by the production of laminin-8, calcium, and papain-like cysteine proteases	[97,101]

VE: vascular endothelium; HAT: Human African trypanosomiasis; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; VEGF-A: vascular endothelial growth factor-A; VEGF-R: vascular endothelial growth factor receptor; IL: interleukin

Table 2. Roles and mechanisms played by exosomes in malaria, leishmaniasis, toxoplasmosis, Chagas disease, and HAT

Disease	Exosomal Factors	Cell origin	Mode of Action	Ref.
Malaria	Parasitic components (protein, lipid, RNA, DNA)	Infected reticulocytes	Induces antigen presentation and elicit a long-term antibody protective immune response, increase memory CD4+ and CD8+ T cells	[102,103]
	Pathogen genes	Infected RBCs	Facilitates cell-to-cell communication between parasites, promote differentiation to sexual forms	[104]
Leishmaniasis	Virulence factors and effector proteins	Parasite	Induces secretion of IL-8 over TNF- α in host macrophages	[105]
			Alters the cytokine response of monocytes through upregulating IL-10 and inhibiting TNF- α production	[106]
			Inhibits IL-12p70, TNF- α , and IL-10 cytokine functions in monocyte-derived DCs and prevent DC-induced naïve T cell differentiation into mature Th1 cells	[107]
	GP63	Parasite	Exacerbates lesions due to increased production of inflammatory cytokine IL-17 α and over the induction of IL-4 and IL-10	[108]
		Infected macrophages	Regulates PTPs and TFs in target macrophages	[109]
Toxoplasmosis	Antigenic proteins	Infected DCs	Cleaves Dicer1 in hepatocytes to block miRNA-122 production, causing a decreased serum cholesterol level	[110]
	Antigenic proteins	Infected DCs	Induces protective spleen-derived Th1 and humoral immune responses with high levels of IgA antibody	[111,112]
	Exosome	Parasite	Modulates macrophage activation through increased production of IL-12, TNF- α , and IFN- γ and a decrease in IL-10	[113]
	PAMPs	Parasite	Induces protective cellular and humoral immune responses	[53]

	miRNA	Parasite	Activates inflammatory responses in nearby macrophages in a TLR- and MyD88-dependent manner. Interact and modulate host cells through gene regulation	[114]
Chagas disease	Exosome	Infected blood cells	Protects extracellular parasites from complement-mediated lysis by binding the C3 convertase on the parasite surface and inhibiting C3 cleavage	[115-117]
	Virulence factors and soluble proteins	Parasite	Helps parasites to invade host cells through the expression of transforming growth factor-beta (TGF- β)	[118]
	Complement regulatory and inhibitory proteins	Parasite	Enhances cell invasion and parasite survival by invading the innate immune system	[119,120]
HAT	Virulence factors and proteins (SRA)	Parasite	Avoids the complement system and increase the invasion of host cells	[121,122]
			Activates the innate and acquired immune responses and induces rapid clearance of erythrocytes to cause anemia and tissue damage	[123,124]

HAT: Human African trypanosomiasis; IL: interleukin; PAMPs: pathogen associated molecular patterns; TLRs: toll-like receptors; PTPs: protein tyrosine phosphatases; TFs: transcription factors

complications. Malaria is prevalent among 90 countries, which represent 40% of the world's population^[125]. Malaria is caused by an intracellular protozoan parasite of the genus *Plasmodium*, in which five species are known to infect humans: *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi*^[126,127]. *P. falciparum* causes the most severe clinical form of the infection leading to major morbidity and mortality. Female Anopheles mosquitoes transmit this disease to humans when releasing sporozoites while taking a blood meal. These circulate in the blood and invade and mature in hepatocytes. Hepatic forms are released and invade erythrocytes where merozoites further increase in number and are released and invade other red blood cells (CDC.org: <https://www.cdc.gov/malaria/about/disease.html>).

Role of the VE in malaria

The VE plays a major role in host-parasite interactions and the severity of the malarial disease. It has been shown that *P. falciparum* antigens are present on the surface of infected erythrocytes and bind to the receptors expressed on the VE^[72]. After invasion, *Plasmodium* modulates endothelial function either by direct adhesion to the EC receptors or by releasing parasite products that can induce EC activation, leading to the disruption of the EC barrier^[73]. It has also been shown that histones released from merozoites (HeH) stimulate the production of inflammatory mediators by primary human dermal microvascular endothelial cells, supporting the pathogenic role of both host- and pathogen-derived histones in *P. falciparum* caused malaria^[128].

Malaria caused by *P. falciparum* is associated with the cytoadherence to endothelial cells through the parasite ligand *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). *P. falciparum*-infected RBCs sequester in blood capillaries through several endothelial cell cytoadherence receptor molecules such as CXCL1, ICAM1, CD36, and VCAM1^[129] and release exosome-like vesicles to directly communicate between the parasites^[104]. These extracellular vesicles and the other abnormal accumulation of metabolites play a critical role in the damage of the blood-brain barrier (BBB) in determining the severity of cerebral malaria (CM) caused by *P. falciparum*. CM is accompanied by coma, seizures, and focal neurological deficits, which contribute to a mortality rate of 15%-20% despite therapy^[130]. After establishing infection, *Plasmodium* export many proteins, including epoxide hydrolases into the erythrocyte, which results in the alteration of fatty acid composition, leading to perturbed vascular function and sequestration of the parasite in the VE^[74]. Exportation of PfEMP1 mediates the adhesion of infected erythrocytes to VE and placental syncytiotrophoblasts^[131]. In addition, a recent study suggests that brain ECs produce low molecular weight growth factors, which stimulate the growth of *P. falciparum* *in vitro*. These growth factors potentially enhance parasite proliferation in erythrocytes in the brain microvasculature^[75].

Role of exosomes in malaria

Production of exosomes from infected-host cells and *Plasmodium* species during infection correlates with higher malarial disease severity^[39,132]. Supporting this idea, a study of *P. falciparum* revealed that exosome-like particles released from infected RBCs facilitate cell-to-cell communication among parasites through gene delivery. The *P. falciparum* protein PfPTP2 has been identified as a critical player in this mechanism. This cellular communication pathway promotes the multiplication of sexual forms (gametocytes), which is a key process to maintain malaria infection and increase transmission probability^[104]. Furthermore, it has been hypothesized that blocking the synthesis of these exosome-like vesicles as a therapeutic target may lead to decreased parasite transmissibility^[104].

Exosome production may serve as a means of host protection during malaria infection. In a study of *P. yoelii*-infected mice, parasite protein-containing exosomes were released from reticulocytes, which could induce antigen presentation and elicit a long-term antibody protective immune response when administered as a vaccine in naive mice. The production of IgG antibodies and recognition of the parasite-infected RBCs in response to the vaccination with released exosomes reduces the parasite load and leads to increased survival as well as reticulocytosis^[102]. Vaccination of mice *in vivo*, as well as *in vitro* in human spleen cells, with CpG adjuvanted *P. yoelii*-infected reticulocyte-derived exosomes (rexPy) induces a spleen-dependent memory response against the parasite infection. This memory response is associated with the activation of spleen cells through rexPy uptake, leading to changes in the distribution of T cell subsets and, more specifically, an increase in memory CD4+ and CD8+ T cells^[103]. Due to the immunoregulatory action, *Plasmodium* exosome particles are viable candidates in the development of future malaria vaccines^[39].

LEISHMANIASIS

Leishmaniasis are a group of neglected tropical diseases caused by infection with parasites belonging to the genus *Leishmania*, which are transmitted by the bite of infected sand flies. It is estimated that one billion people are at risk of infection and about 1.7 million new cases of leishmaniasis occur each year in 102 countries (https://www.who.int/leishmaniasis/resources/who_wer9122/en/). Due to the lack of efficient treatment options or a vaccine, leishmaniasis has become the second largest cause of death among parasitic infections after malaria. Leishmaniasis consists of a spectrum of clinical syndromes and is dependent on the species of infecting parasite. There are three main forms of the disease: localized or disseminated skin lesions [cutaneous leishmaniasis (CL) or diffuse cutaneous leishmaniasis caused by *L. major* and *L. tropica*], mucocutaneous disease (mucocutaneous leishmaniasis caused by *L. Mexicana*, *L. braziliensis*, and *L. amazonensis*), and systemic disease [visceral leishmaniasis (VL) caused by *L. donovani* and *L. infantum*]. Infection is initiated when infected female phlebotomine sand flies take a blood meal, leading to inoculation with infective promastigotes. Promastigotes are phagocytized by macrophages and neutrophils, which transform into and multiply as intracellular amastigotes, which can metastasize to distant organs. The lifecycle is complete when the infected macrophages are ingested by uninfected sandflies, which transform and replicate as promastigotes in the insect gut (<https://www.cdc.gov/parasites/leishmaniasis/biology.html>).

Role of the VE in leishmaniasis

The VE is critical for the initiation of inflammatory processes and vascular remodeling, including angiogenesis and lymphangiogenesis, which occur in the inflammatory microenvironments of both VL and CL infections^[133,134]. Intra- and extracellular parasites attached to the wall of dermal blood vessels and the capillary lumen lead to the development of secondary infections and the spread of the disease, especially in endemic areas^[135]. During CL, it has been shown that endothelial cells release NO, which counteracts the recruitment of granulocytes and limits the spreading of infection^[135]. In CL infections, the venous endothelium of skin lesions expresses ICAM-1, which helps in the migration of lymphocytes to the site of inflammation^[78]. CL infection with *L. major* has been shown to increase the expression of VCAM-1, which

mediates the adhesion of mononuclear cells to the endothelial cells^[79]. Both human and animal leishmanial infections lead to increased levels of vascular endothelial growth factor-A (VEGF-A) and its receptor (VEGF-R) in the skin^[80,81]. Recently, Weinkopff *et al.*^[133] showed that CL infection by *L. major* induces VEGF-A in macrophages in an ARNT/HIF dependent manner, leading to the limitation of inflammation and lymphangiogenesis. The expansion of the lymphatic network promotes lesion resolution, and inhibition of this process enhances the lesion development. In the VL model, *L. donovani*-infected mice aberrantly express neurotrophic tyrosine kinase receptor type-2 (Ntrk2) on splenic endothelial cells, which plays a role in pathologic remodeling of the spleen^[82].

Role of exosomes in leishmaniasis

The role of exosomes in *Leishmania* infection is well studied, revealing that they serve as a key mode of delivery of *Leishmania* virulence factors and effector proteins to host cells during infection^[136]. Both pathogen and host-derived exosomes have been identified in this process. *Leishmania*-derived exosomes can transport virulence factors into the host macrophages and induce secretion of IL-8 instead of TNF- β ^[105]. Proteomic analysis has revealed that one such virulence factor contained in *L. major* exosomes is the metalloprotease glycoprotein GP63, which regulates protein tyrosine phosphatases (PTPs) and transcription factors (TFs), such as NF- κ B, in target macrophages^[108]. PTPs prevent macrophage activation by inhibiting the secretion of pro-inflammatory IFN- γ , IL-12, and NO^[137,138], which are important in host control of parasite infection. These act to modulate the immune response, diminishing inflammation in favor of parasite growth and survival. Exosomes released from *L. donovani*-infected macrophages contain GP63, which proteolyzes Dicer1 in hepatocytes to block miRNA-122, production leading to disease progression^[109]. Exosomes released from *L. donovani* promastigotes can effectively alter the cytokine response of monocytes through the upregulation of IL-10 and inhibition of TNF- α production. Similarly, it has been observed that monocyte-derived DCs that have been exposed to parasite exosomes have inhibited levels of IL-12p70, TNF- α , and IL-10. Exosome-exposed DCs cannot induce naïve T cell differentiation into mature Th1 cells^[106]. In contrast, Schnitzer *et al.*^[110] showed that that vaccination with *L. major* antigens present in DC-derived exosomes can induce immune-protection against the infection.

Leishmania is also able to modify the production and content of exosomes in response to environmental stress (heat shock and pH) that mimic infection. Silverman *et al.*^[105] showed that vesicle release from parasites can be increased by three-fold in response to heat shock. Interestingly, Atayde *et al.*^[107] showed that *Leishmania* release exosomes within the lumen of the sand-fly midgut, which are ejected in the egested inoculum during by the sand-fly bite. These exosomes lead to exacerbated lesions in *L. major* and *L. infantum* models, possibly due to increased production of inflammatory cytokine IL-17 α , and overproduction of IL-4 and IL-10, which are both known to suppress the Th1 responses and play a role in disease susceptibility.

TOXOPLASMOSIS

Toxoplasmosis is caused by the obligate intracellular protozoan parasite, *Toxoplasma gondii*, which infects healthy and immunocompromised individuals worldwide. It is estimated that more than 11% of the population in the US and 60% of the population throughout the world are infected^[139] (<https://www.cdc.gov/parasites/toxoplasmosis/epi.html>). The transmission of toxoplasmosis mainly occurs through ingesting raw meat containing *T. gondii* cysts (foodborne) or water containing oocysts from feline feces (waterborne). The parasite can also be transmitted congenitally when a woman acquires the infection during pregnancy^[139], or very rarely through transplantation of organs^[140]. Although felines act as definitive hosts for *T. gondii*, it can infect almost all nucleated mammalian and avian cells. The life cycle of *T. gondii* mainly involves an asexual phase within nucleated cells and a sexual phase, which occurs in felines. Ingested oocysts released in feline feces serve as the infectious stage for humans. Toxoplasmosis can cause miscarriage, stillborn infants, or severe central nervous system (CNS) disease; in adults, it can lead

to multi-organ involvement including encephalitis, retino-uveitis, and pulmonary disease, especially in immunosuppressed hosts cerebral toxoplasmosis.

Role of the VE in toxoplasmosis

It is believed that *T. gondii* can modulate the gene expression of brain ECs and promote dissemination through the BBB. *T. gondii* transforms into motile extracellular forms (tachyzoites) that use transcellular or paracellular migration to cross the BBB and infect host cells. In this context, the ECs serves as a replicative niche for the entry of *T. gondii* to the CNS^[83]. The most common clinical manifestation of toxoplasmosis is retinal infection. Recently, Furtado et al.^[141] demonstrated that tachyzoites can cross the retinal endothelium to establish infection and that blocking this entrance leads to reduced diseased burdens. It has also been observed that *T. gondii* infection leads to induction and activation of cerebral blood vessel ECs^[84].

Role of exosomes in toxoplasmosis

T. gondii was the first non-viral protozoan pathogen for which exosomes were identified^[38]. Although *T. gondii* exosomes are continuously released during infection by tachyzoites, the bulk of released molecules are non-exosome associated excretory/secretory antigens constitute during the acute phase of infection^[114]. An earlier study conducted by Aline et al.^[111] revealed exosomes are important in generating protective immunity against *T. gondii* infection; *T. gondii*-pulsed DCs can effectively induce a spleen-derived Th1 immune response that is protective against acute and chronic infection. It has also been shown that exosomes secreted by SRDCs induce a protective humoral immune response against the infection in syngeneic and allogeneic mice associated with high levels of IgA antibodies^[112]. Furthermore, vaccination of mice before pregnancy with exosomes secreted by *T. gondii*-pulsed DCs protects pups from congenital infection due to a robust T cell response^[142].

Li et al.^[113] characterized *T. gondii*-derived exosomes as ~50 nm in size and containing HSP70, CD63, and *T. gondii* surface marker P30. These exosomes were shown to modulate macrophage activation through increased production of IL-12, TNF- α , and IFN- γ and a decrease in IL-10. Mice immunized with these exosomes exhibited cellular and humoral immune responses and were protected against acute infection^[39,113]. Li et al.^[143] also reported that *T. gondii* exosomes activate JNK signaling to elicit this innate immune response. Macrophages infected with *T. gondii* release exosomes containing PAMPs, which activate inflammatory responses in adjacent macrophages in a TLR- and myeloid differentiation factor 88 (MyD88)-dependent manner^[53]. Some *T. gondii* exosomes contain miRNA that may interact and modulate the host cells through gene regulation^[114]. Taken together, *T. gondii*-derived exosomes displayed significant immunogenic properties that make them viable candidates for vaccine production.

CHAGAS DISEASE

The hemoflagellate *Trypanosoma cruzi* is the etiologic agent of Chagas disease, also termed American trypanosomiasis. Chronic infection by this parasite is characterized by chronic myocarditis, cardiomyopathy, and vasculopathy, as well as mega-organ syndromes^[144-147]. It is estimated that ~8 million people are currently infected worldwide and that 20%-30% of those individuals develop sequelae of chronic infection. Chagas disease is the leading cause of heart failure in Latin America^[148-151]. The parasite exists in four morphological forms: epimastigotes, insect metacyclic trypomastigotes, human trypomastigotes, and intracellular amastigotes^[151]. Insect stage trypomastigotes (also termed metacyclic trypomastigotes) are the infective stage of the parasite, which are present in the feces of hematophagous triatomine insects, which contaminate wounded skin or mucous membranes^[148,152]. Metacyclic trypomastigotes invade nucleated host cells to establish the infection. Inside the host cells, they transform and replicate as intracellular amastigotes, eventually differentiating into blood-stage trypomastigotes and exit the host cells to disseminate to multiple organs, including the heart and gastrointestinal tract of the mammalian hosts^[148].

Role of the VE in Chagas disease

T. cruzi infects various types of cells including cardiac myocytes, GI-tract, smooth muscle cells, and the VE^[95,153]. The VE plays a key role in the dissemination of *T. cruzi*, as parasites engage ECs during the initial stages of infection^[85,86]. As the infection progresses, VE release inflammatory molecules by direct physical disruption of ECs, and parasites undergo trans-endothelial migration with the help of the parasite protease, cruzipain^[85,87,88]. Cruzipain cleaves the human kininogen into bradykinin, an inflammatory mediator of endothelial permeability^[85,154,155]. Studies have shown that *T. cruzi* induces ECs release of vasoactive molecules endothelin-1, pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α), and thromboxane A₂, which trigger the production of iNOS and nitrosative stress^[89-92]. Moreover, increased levels of TNF- α exacerbate endothelial COX-2/TXA₂/TP/superoxide signaling^[92]. The infection of *T. cruzi* also activates the NF- κ B that accumulates in the nucleus and activates many genes specific to endothelial pathophysiology^[95].

In addition to the vascular damage, *T. cruzi* invades VE through the secretion of neuraminidase that removes sialic acid from the infected ECs^[156,157]. Collectively, all of these factors compromise the activity of ECs and cause vasculopathy, as demonstrated by vasospasm, focal ischemia, reduction in the blood flow, increased platelet aggregation, and elevated levels of thromboxane A (2) and endothelin-1^[89,158,159]. Studies have shown that *T. cruzi*-infected ECs secrete endothelin-1 and interleukin-1 β (IL-1 β), which activate extracellular signal-regulated kinases 1 and 2 (ERK1/2) and NF- κ B, resulting in the expression of cyclin-D1 in uninfected smooth muscle cells^[93-95]. The activation of these pathways involved in the interaction between *T. cruzi* and the VE are likely to play a major role in the inflammatory responses after vascular injury and endothelial dysfunction and could be potential targets for therapies to control parasite dissemination^[65,85].

It is estimated that 10%-30% of patients infected with *T. cruzi* progress to the chronic stage manifested by cardiomyopathy and prothrombotic/inflammatory status^[160]. Pathophysiological mechanisms such as activation of the endothelium and microvascular alterations occur during the cardiac damage. It is known that thromboxane A₂ increases platelet aggregation and that inhibiting the formation of thromboxane A₂ by aspirin alters the course of Chagas disease in both acute and chronic phases^[161]. Benznidazole, a widely used drug for the treatment of Chagas disease, also acts by preventing endothelial damage caused by *T. cruzi*^[162]. Cholesterol-lowering drugs such as simvastatin have been shown to decrease the endothelial activation and, in combination with benznidazole, improve the pathophysiological condition of chronic Chagas disease patients^[163]. Recent discoveries have provided insights into how *T. cruzi* escapes the BBB and rapidly migrates across the ECs without disrupting the integrity of the monolayer or altering the permeability. Coates *et al.*^[85] identified that this process is facilitated by bradykinin and CCL2, which may be considered in the development of new therapeutic strategies for Chagas disease.

Role of exosomes in Chagas disease

Parasite and host cell exosomes play a role in the pathogenesis in Chagas disease. The release of an elevated number of exosomes is essential for host-parasite interaction, intercellular communication, and enhanced parasite survival^[120]. The infection of *T. cruzi* induces blood cells to release exosomes through a Ca²⁺-dependent manner^[164]. The released exosomes protect extracellular trypomastigotes from complement-mediated lysis by binding to C3 convertase on the *T. cruzi* surface and inhibiting C3 cleavage^[115-117]. Exosomes also aid the parasites to invade the host cells through the expression of TGF- β . The communication between cells takes place through the release of exosomal contents including cytokines, peptides, hormones, microRNA, and numerous bioactive substances, which act as a function of innate immunity^[165,166]. Exosomes released by *T. cruzi* promote cell invasion and parasite survival by modulating the innate immune system and producing several virulence factors including the glycoprotein 85 (gp85), trans-sialidase, phosphatase, and the soluble proteins^[116,119,120,167]. Thus, the exosomes released by the host cells and parasites during infection play a vital role in the invasion of the innate immune system, parasite survival, and the establishment of infection in Chagas disease.

HUMAN AFRICAN TRYPANOSOMIASIS

Human African trypanosomiasis (HAT) is caused by two subspecies of *Trypanosoma brucei*: *T.b. gambiense* that leads to the chronic form of HAT known as West African trypanosomiasis and *T.b. rhodesiense* that leads to the acute form of HAT known as East African trypanosomiasis^[168-170]. A third subspecies of *T. b. brucei* infects cattle and very rarely infects the human host. HAT is transmitted to mammalian hosts by the bite of infected tsetse flies. During a blood meal, the metacyclic trypomastigotes are injected into the skin of the host, eventually entering the lymphatic and blood vessels. As parasites transform into blood trypomastigotes, they are disseminated throughout the body. The life cycle is completed when trypomastigotes infect feeding tsetse flies, wherein they transform and replicated into insect stage parasites^[168,171]. Unlike the other protozoan parasites, the entire life cycle of African trypanosomes consists of extracellular stages, which alternately infect mammalian and insect hosts (CDC.gov, <https://www.cdc.gov/parasites/sleepingsickness/biology.html>). Although *T. brucei* infection occurs through the hemolymphatic stage in the initial systemic stage, the second phase is mainly characterized as a central nervous system disease, due to parasite invasion of brain tissue, leading to the altered sensorium, seizures, coma, and death. These symptoms are the reason that HAT is also referred to as African sleeping sickness.

Role of the VE in HAT

Bloodstream forms of *T. brucei* multiply to high density and eventually invade the central nervous system through the penetration of the VE. Although the mechanism through which the *T. brucei* cross the BBB are yet to be fully understood, it has been shown that *T. brucei* uses a multi-step process using the host derived factors including the cytokines IFN α/β , IFN γ , TNF, ICAM-1, and CXCL10^[97,172]. During infection, the VE cells are activated by the translocation of NF- κ B, due to action of parasite trans-sialidase, to the nucleus and the induction pro-inflammatory cytokines such as TNF- α , IL-6, and IL-8. This process plus the induction of other soluble factors such as the adhesion molecules (ICAM-1, E-selectin, and VCAM-1)^[100] culminates in leukocyte recruitment and transmigration of trypanosomes from the VE to the CNS^[96,98]. Studies have shown that *T. brucei* infection enhances the eNOS protein expression, and enhanced NO production leads to elevated vasodilation and vascular permeability facilitating parasite invasion into the surrounding tissues and the central nervous system^[173].

Parasite phospholipase C, protein kinase, and the parasite cysteine protease brucipain also participate in transmigration of trypanosomes into the CNS^[101,174]. Furthermore, it has been shown that *T. brucei* crosses the VE of cerebral blood vessels of mice through interaction with laminin 8 of the ECs^[97]. The transmigration of *T. brucei* through the vascular endothelium also depends on the calcium and the papain-like cysteine proteases^[101]. Collectively, this shows that interaction with the VE depends on various factors that are essential to penetrate the BBB and infect the CNS.

Role of exosomes in HAT

The progression of HAT is modulated by several factors including macrophage hyper-activation, uncontrolled production of TNF, and the transfer of virulence factors by exosomes^[123,175]. Host-derived exosomes play a major role in host defense and are targeted as vaccine candidates, whereas parasite-derived exosomes transduce signal(s) to the host cells to establish infection^[39,176,177]. A study has shown that a spliced ladder RNA (SL RNA) is present in the exosomes of *T. brucei* that is essential in these parasites for the formation of all mature mRNA. The cells secreting these SL RNA-containing exosomes affect the social motility of these parasites^[178]. The bloodstream form of the parasite is responsible for anemia and tissue damage in the mammalian host^[124,179]. This immunopathological outcome is due to several proteins released from the exosomes that lead to sequential activation of the innate and acquired immune responses^[180]. The parasites secrete several molecules through exosomes to gain access to the host cells. Likewise, *T. brucei* exosomes contain 156 proteins from diverse functional classes^[123]. One study shows that *T. brucei* exosomes fuse with mammalian erythrocytes and causes rapid clearance of erythrocytes and promotes anemia^[123,124].

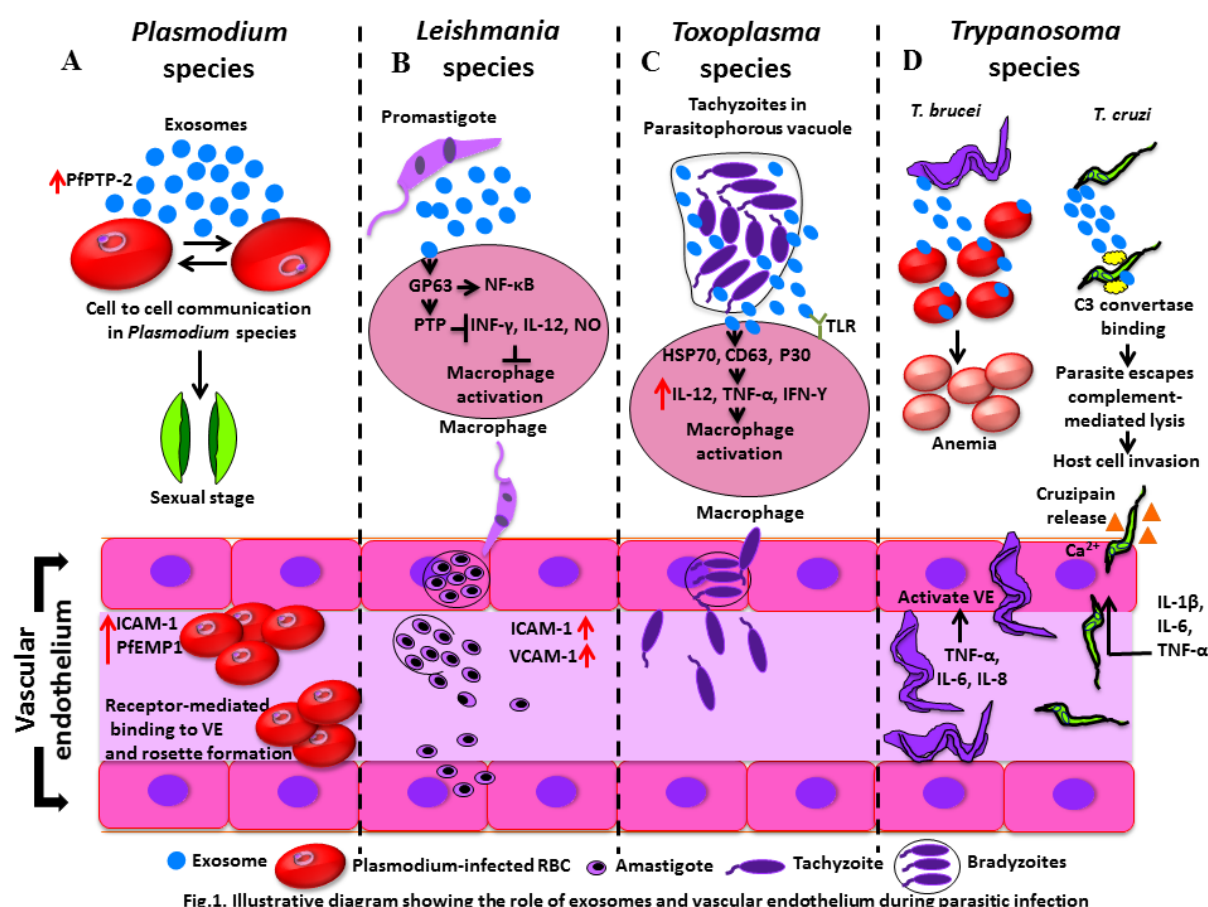


Figure 1. Schematic representation of cellular and molecular mechanisms played by vascular endothelium (VE) and exosomes in *Plasmodium*, *Leishmania*, *Toxoplasma*, and *Trypanosoma* spp. Infection. A: *P. falciparum* protein PfPTP-2 released through the exosomes from infected red blood cells (RBCs) facilitates cell-to-cell communication and promotes the differentiation of sexual forms of the parasites. *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) and intercellular adhesion molecule-1 (ICAM-1) mediate the adhesion of infected erythrocytes to the VE and placental syncytiotrophoblasts; B: *Leishmania* parasites transport glycoproteins such as GP63 into the host cells through exosomes and regulate the protein tyrosine phosphatases (PTPs) and transcription factors such as NF- κ B in macrophages. The PTPs prevent macrophage activation by inhibiting the secretion of IFN- γ , IL-12, and nitric oxide (NO). *Leishmania* infection also increases the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) to initiate an inflammatory response after migrating the mononuclear cells and lymphocytes to the endothelial cells; C: *T. gondii* parasites release exosomes, which contain HSP70 and CD63, as well as the *T. gondii* surface marker P30. These exosomes induce the production of IL-12, TNF- α , and IFN- γ and modulate macrophage activation; D: *T. brucei* releases exosomes that are deposited and fused to RBCs. The virulence factors of exosomes result in RBC membrane alteration and anemia. In addition, *T. brucei* activates the vascular endothelial cells by producing TNF- α , IL-6, and IL-8. The exosomes of *T. cruzi* contain C3 convertase binding protein, which helps the parasites to escape the complement-mediated lysis. *T. cruzi* releases cruzipain and invades vascular endothelium through a Ca^{2+} -dependent mechanism

Thus, the exosomal components (proteases) of the trypanosome could be the promising targets to control sleeping sickness^[181].

CONCLUDING REMARKS

Here, we discuss the important roles played by VE and exosomes in some major protozoan parasitic diseases. Exosomes serve as a carrier of effector molecules that modulate the host immune response in establishing infection. The content of exosomes provides an effectual means to control the protein expression in both parasite and host cells. While parasite-derived exosomes play a key role in establishing infections through intercellular communication and signaling mechanisms, the host-derived exosomes also play a major role in the host-defense mechanism. Understanding the mechanism of the exosomal

component on the host immune system in causing parasitic disease may help in the development of a novel approach of diagnostic tools and treatment. Further research on exosomes is necessary to search for the candidate vaccine and drug development.

The VE provides effective immunological homeostasis that controls the inflammatory response, mainly through the production of cytokines. The VE is an important target of parasite invasion and the parasite interaction on the VE is responsible for the development of clinical manifestations. The trans-endothelial migration of parasites is the major key step in establishing infection. Thus, more experimental studies are needed to provide insights on the interaction of blood parasites and VE, trans-endothelial migration, and the role of endothelial cytokine mediators in parasite dissemination. A better understanding may reveal the way to find more anti-parasitic regimens.

To summarize, both the VE and exosomes regulate the entry of parasites, their multiplication, signaling between the parasite and host cells, and dissemination to the other organs of the hosts. In addition, the VE and exosomes modulate both the innate and adaptive immune responses and maintain the integrity of the inflammatory process (summarized in [Figure 1](#) and [Tables 1 and 2](#)). However, further studies are needed for a thorough understanding of the mechanisms and roles played by the VE and exosomes in parasite survival and disease progression. The novel mechanisms regulated by the VE and exosomes can be considered as potential therapeutic targets to treat and control these human protozoan diseases.

DECLARATIONS

Author's contributions

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Read and approved the final version of the manuscript: Varikuti S, Jha BK, Holcomb EA, McDaniel JC, Karpurapu M, Srivastava N, McGwire BS, Satoskar AR, Parinandi NL

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Contributed expertise in vascular endothelial biology, signaling, and structure and function of exosomes and had the complete oversight of preparing this manuscript: Parinandi NL

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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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Percutaneous mitral valve repair with the MitraClip in patients with handgrip exercise-induced dynamic mitral regurgitation

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Abstract

Aim: To investigate whether patients with symptomatic heart failure and exercise-induced dynamic severe mitral regurgitation (MR) benefit from percutaneous mitral valve repair (PMVR).

Methods: We included patients who underwent PMVR with the MitraClip system in an all-comers observational study. Handgrip echocardiography was performed in patients with a discrepancy between symptoms and echocardiographic findings at rest, giving rise to the suspicion of an exercise-induced increase in MR severity. The primary endpoint of the study was a composite of all-cause mortality or admission for heart failure at 1-year follow-up. The secondary endpoint was the reduction in NYHA functional class.

Results: Two hundred twenty-one patients who underwent MitraClip implantation were included. Ninety-three patients with moderate to severe MR at rest received handgrip echocardiography prior to PMVR. The remaining 128 patients presented with severe MR at rest, making exercise echocardiography unnecessary. Handgrip exercise led to an increase in MR severity in 81% of patients with moderate MR at rest, irrespective of the subtype of MR. Following PMVR, patients with dynamic severe MR experienced comparable clinical improvement as patients



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with severe MR already at rest: During 1-year follow-up, 37 patients died, and 71 patients were re-admitted to the hospital because of heart failure. In this regard, 13 patients (30%) with dynamic severe MR experienced the combined endpoint, while 72 patients (43%) with severe MR at rest did as well ($P = 0.121$). Moreover, the majority of patients with dynamic severe MR similar to patients with severe MR at rest experienced clinical improvement from NYHA class III/IV to I/II (59% vs. 56%; $P = 0.566$).

Conclusion: The data presented provide evidence of a clinical benefit from PMVR using MitraClip in patients with moderate MR at rest who display exercise-induced increases in MR severity during handgrip exercise.

Keywords: Percutaneous mitral valve repair, MitraClip, dynamic mitral regurgitation, exercise echocardiography

INTRODUCTION

In recent years, cardiologists have increasingly recognized the dynamic nature of mitral regurgitation (MR)^[1]. In degenerative MR (DMR) about one-third of patients with moderate to severe MR display notable exercise-induced increases in MR severity [increase in effective regurgitant orifice area (EROA) $> 10 \text{ mm}^2$ and regurgitation volume (RVol) $> 15 \text{ mL}$]^[2]. Owing to these findings, there is a recommendation for exercise echocardiography during the diagnostic work-up of symptomatic patients with DMR in whom there is a discrepancy between symptoms and the severity of MR at rest^[3,4]. In functional MR (FMR) exercise-induced changes in MR severity are also common and may provide prognostic information on the clinical course of heart failure^[5-7]. European Society of Cardiology (ESC) guidelines emphasize the role of exercise echocardiography in unmasking significant dynamic MR in patients with left ventricular systolic dysfunction, since resting MR severity may not correlate with the potentially clinically significant increase in MR during exercise^[4]. Percutaneous mitral valve repair (PMVR) with the MitraClip system has emerged as a treatment option for patients with DMR and FMR, who are inoperable or have a high surgical risk. However, current recommendations do not cover dynamic MR since data on the safety and efficacy of PMVR are lacking in these patients. Likewise, there are only scarce data on exercise echocardiography in patients undergoing PMVR, of whom the majority may be too frail to undergo traditional bicycle exercise testing.

In the current study, we report our experience of PMVR using the MitraClip in patients with moderate MR at rest and dynamic severe MR during handgrip exercise, compared to patients with severe MR already at rest.

METHODS

Study design

We included patients who underwent PMVR with the MitraClip system at our institution from 2012 to 2016 in an all-comers observational study. Patients with moderate to severe MR at rest received handgrip echocardiography prior to MitraClip implantation. Handgrip echocardiography was performed in patients with a discrepancy between symptoms and echocardiographic findings at rest, giving rise to the suspicion of an exercise-induced increase in MR severity. Our interdisciplinary heart team classified all patients as inoperable or at high-risk for surgery. The study was performed in accordance with the Declaration of Helsinki and was part of a registry, which was approved by the local ethics committee of the Heinrich-Heine University and registered at www.clinicaltrials.gov (NCT02033811).

Follow-up

Patients were routinely followed by referring cardiologists and scheduled for a single outpatient visit 12 months after MitraClip implantation in our specialty clinic for structural heart disease. The clinical

course of patients in whom this single follow-up examination in our department was deemed impossible was monitored by telephone interview with referring cardiologists and the patients' primary physicians or the patients themselves. The primary endpoint of the study included a composite of all-cause mortality or hospitalization for heart failure during 12 months follow-up. Hospitalization for heart failure was defined by clinical symptoms suggestive of heart failure (e.g., worsening of dyspnea, edema and fatigue) and the need for i.v. diuretics during re-hospitalization. Secondary endpoint was reduction in New York Heart Association (NYHA) functional class at 12 months following PMVR compared to baseline.

Echocardiographic evaluation

Echocardiographic examinations were performed using a GE Vivid S6/E9 or a Philips iE33. All echocardiographic data were stored on a workstation for offline analysis (Xcelera Cardiology Information Management, Philips). Left ventricular volumes and ejection fraction were calculated using the Simpson biplane method. Systolic pulmonary artery pressure (SPAP) was estimated from the regurgitant jet of tricuspid regurgitation with peak systolic trans-tricuspid pressure gradient. Assessment of MR was performed according to current guidelines^[4]. MR severity was assessed by an integrative approach using semi-quantitative [vena contracta (VC)] and quantitative (EROA, RVol) parameters. The severity of MR was graded mild (1+), moderate (2+) and severe (3+). In patients with secondary MR, the cutoff values for severe MR were: EROA 20 mm² and RVol 30 mL, according to current guidelines^[4]. The radius of the maximal proximal iso-velocity surface area (PISA) was measured using several frames. The largest radius was selected for analysis. RVol and EROA were calculated with standard formulae as previously described. For semi-quantitative assessment, VC width was assessed from the apical four-chamber view. The largest VC diameter was measured for three cardiac cycles and averaged. Patients presenting with moderate MR at rest and severe MR during handgrip exercise were assigned "dynamic severe MR".

Handgrip exercise

Following comprehensive echocardiographic examination at rest, handgrip exercise was performed according to a standardized protocol with a handgrip dynamometer (Jamar® Hydraulic Hand Dynamometer, Sammons Preston Inc.) while the patient lay on his/her left side. After initial recording of blood pressure and heart rate at rest, the patient was asked to squeeze the dynamometer with maximum effort for a short period only. The handgrip exercise was then carried out at half-maximum force for three minutes, while echocardiographic data were obtained focusing on MR and SPAP. Blood pressure and heart rate were recorded every minute. MR was quantified as indicated above, including measurement of PISA, VC, EROA and RVol. In case of non-reproducible results according to the PISA method (e.g., due to eccentric jets in 8 of 93 patients), VC width was used for assessment of MR severity. Medical therapy (including β -blockers) remained unchanged for the exercise test.

Statistical analysis

All analyses were performed using Sigma Plot software (Version 11.0, Systat Software Ltd). Results are expressed as mean \pm standard deviation or percentage unless otherwise specified. Normality distribution was assessed with the Kolmogorov-Smirnov test. A two-tailed paired t-test was used to compare differences between two groups. Fisher's exact test was used to investigate the significance of the association between two kinds of classification. Differences between three groups were assessed by one-way ANOVA with post-hoc Tukey correction for multiple comparisons. The Kaplan-Meier method and the log-rank test were used for presenting the event-free rate. For all analyses, a *P*-value of < 0.05 was considered statistically significant.

RESULTS

We included 221 patients who underwent PMVR with the MitraClip system. Ninety-three patients with moderate to severe MR at rest received handgrip echocardiography prior to MitraClip implantation [Figure 1]. The remaining 128 patients presented with clinically relevant severe MR at rest, making exercise echocardiography unnecessary.

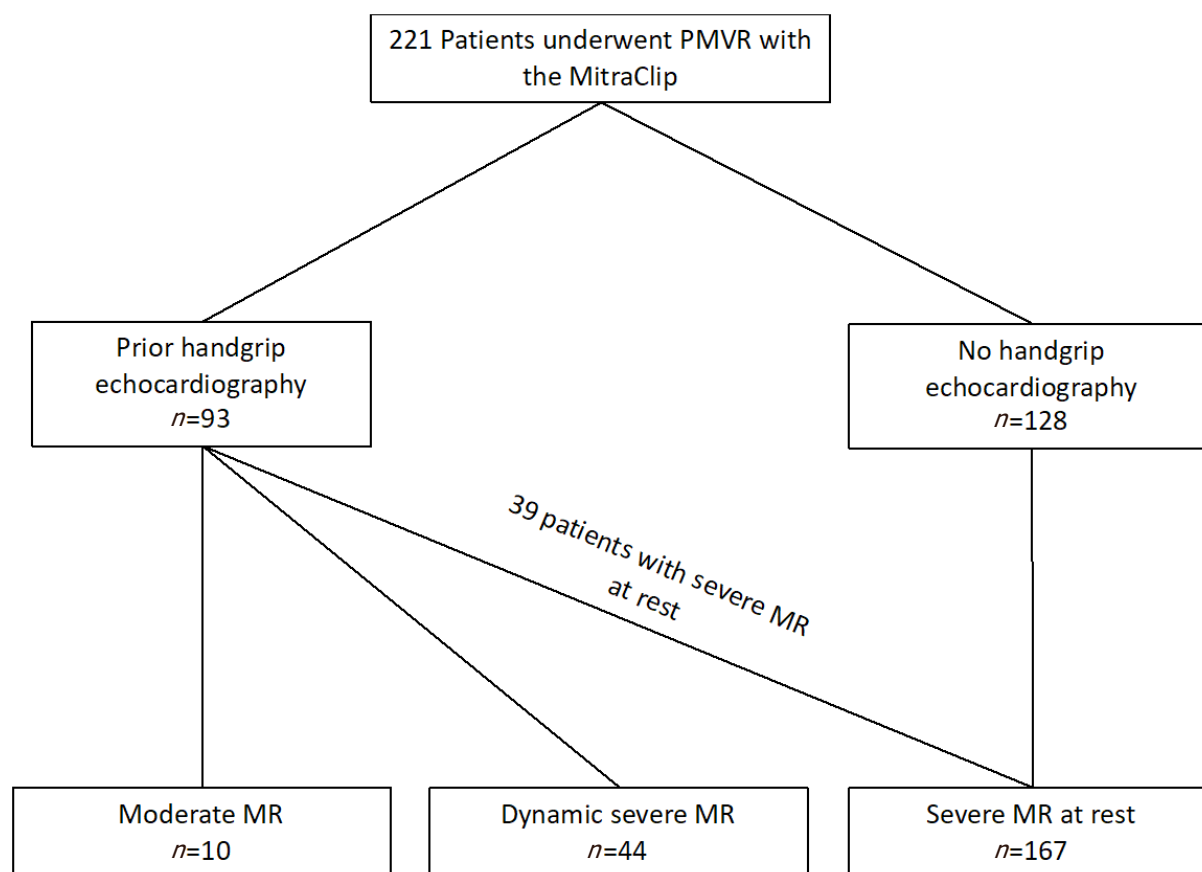


Figure 1. Consort diagram. Two hundred twenty-one patients with complete echocardiographic and follow-up data were included in retrospect. In 93 patients, handgrip echocardiography was performed prior to the procedure. Of those 93 patients, 44 patients presented with moderate MR at rest and dynamic severe MR during handgrip. Thirty-nine patients had severe MR already at rest. However, 10 patients showed moderate MR at rest and during exercise, but nevertheless underwent MitraClip implantation because of persistent severe symptoms. In addition, 128 patients with severe MR at rest were included, who did not undergo handgrip echocardiography. MR: mitral regurgitation

Demographic and clinical data of the cohort are summarized in [Table 1](#). Mean age was 75 ± 10 years, 36% were female. One hundred ninety-six patients (89%) presented with NYHA functional class III or IV. Eighty patients (36%) had DMR, while 141 patients (64%) presented with FMR [[Table 2](#)]. One hundred sixty-seven patients (76%) fulfilled criteria for severe MR at rest according to current recommendations [[Figure 1](#)]^[4]. The remaining patients presented with moderate MR at rest ($n = 54$, 24%). As expected, patients with severe MR at rest displayed more pronounced elevations in NT-proBNP ($P = 0.015$) and had a higher logistic EuroSCORE ($P = 0.037$) compared to patients with moderate MR and dynamic severe MR [[Table 1](#)]. Regarding echocardiographic parameters, LA area was larger in patients with severe MR at rest compared to patients with dynamic MR [[Table 2](#)]. In addition, LVEF tended to be lower in patients with severe MR at rest compared to patients with dynamic MR ($46\% \pm 12\%$ vs. $42\% \pm 13\%$; $P = 0.053$). Furthermore, SPAP was numerically increased in patients with severe MR at rest compared to patients with dynamic MR, however, without reaching statistical significance (47 ± 15 vs. 42 ± 12 mmHg; $P = 0.345$).

Handgrip exercise testing

Handgrip exercise resulted in a meaningful hemodynamic response. Heart rate increased from 67 ± 12 beats/min (bpm) at rest to 78 ± 14 bpm with handgrip exercise ($P < 0.001$). Systolic blood pressure increased from 123 ± 21 mmHg at rest to 138 ± 23 mmHg with exercise ($P < 0.001$), and diastolic blood pressure increased as well from 66 ± 14 mmHg to 73 ± 20 mmHg ($P = 0.005$). Rate pressure product

Table 1. Baseline patient characteristics

	All patients <i>n</i> = 221	Moderate MR <i>n</i> = 10	Dynamic severe MR <i>n</i> = 44	Severe MR at rest <i>n</i> = 167	<i>P</i> -value
Age (years)	75 ± 10	77 ± 8	77 ± 9	75 ± 11	0.696
Gender, female, <i>n</i> (%)	80 (36%)	6 (60%)	21 (48%)	53 (32%)	0.040
Hypertension, <i>n</i> (%)	183 (83%)	9 (90%)	35 (80%)	142 (85%)	0.594
Diabetes, <i>n</i> (%)	57 (26%)	4 (40%)	12 (27%)	41 (25%)	0.541
Previous cardiac history					
ICM, <i>n</i> (%)	151 (68%)	9 (90%)	28 (64%)	114 (68%)	0.290
DCM, <i>n</i> (%)	38 (17%)	1 (10%)	11 (25%)	26 (16%)	0.107
Previous CABG, <i>n</i> (%)	73 (33%)	5 (50%)	12 (27%)	56 (34%)	0.403
Previous VS, <i>n</i> (%)	40 (18%)	4 (40%)	5 (11%)	31 (19%)	0.117
Atrial fibrillation, <i>n</i> (%)	121 (55%)	4 (40%)	27 (61%)	90 (54%)	0.430
Logistic EuroSCORE (%)	22 ± 15	17 ± 7	18 ± 12	24 ± 16*	0.037
NYHA functional class					0.425
NYHA II, <i>n</i> (%)	24 (11%)	1 (10%)	6 (14%)	17 (10%)	
NYHA III, <i>n</i> (%)	156 (71%)	7 (70%)	33 (75%)	116 (69%)	
NYHA IV, <i>n</i> (%)	40 (18%)	2 (20%)	4 (9%)	34 (20%)	
Labaratory assessment					
Hemoglobin (mg/dL)	12.1 ± 1.9	11.2 ± 2.4	12.4 ± 1.8	12.1 ± 1.9	0.185
eGFR (mL/min/1.73 m ²)	51 ± 23	50 ± 17	56 ± 17	51 ± 24	0.853
NT-proBNP (pg/mL)	4499 ± 6499	2077 ± 1917	2161 ± 1555	5158 ± 7048*	0.015

P < 0.05 *vs.* moderate MR; **P* < 0.05 *vs.* dynamic severe MR by *post hoc* analysis. ICM: ischemic cardiomyopathy; DCM: dilated cardiomyopathy; CABG: coronary artery bypass graft; eGFR: estimated glomerular filtration rate; MR: mitral regurgitation; NT-proBNP: N-terminal pro-brain natriuretic peptide; NYHA: New York Heart Association; VS: valve surgery

Table 2. Echocardiographic parameters at rest

	All patients <i>n</i> = 221	Moderate MR <i>n</i> = 10	Dynamic severe MR <i>n</i> = 44	Severe MR at rest <i>n</i> = 167	<i>P</i> -value
DMR (%)	80 (36%)	5 (50%)	18 (41%)	57 (34%)	0.478
FMR (%)	141 (64%)	5 (50%)	26 (59%)	110 (66%)	0.478
LVEDD (mm)	56 ± 11	55 ± 8	55 ± 10	57 ± 10	0.106
LA area (cm ²)	28 ± 9	26 ± 6	25 ± 7	29 ± 10*	0.024
LVEF (%)	43 ± 13	48 ± 13	46 ± 12	42 ± 13	0.053
RV diameter (mm)	30 ± 7	29 ± 3	29 ± 8	31 ± 7	0.548
TAPSE (mm)	18 ± 4	19 ± 4	19 ± 4	18 ± 4	0.888
VC width (mm)	6.4 ± 1.8	5.3 ± 1.2	5.3 ± 1.0	6.7 ± 1.9*	0.002
PISA radius (mm)	7.4 ± 3.1	5.9 ± 0.5	6.4 ± 1.0	7.8 ± 1.8 ^{#,*}	< 0.001
RVol (mL)	43 ± 24	25 ± 5	30 ± 11	48 ± 20 ^{#,*}	0.001
EROA (mm ²)	28 ± 12	19 ± 7	20 ± 8	30 ± 17 ^{#,*}	0.007
MVPG (mmHg)	2.2 ± 1.0	1.5 ± 0.6	1.9 ± 0.8	2.3 ± 1.1	0.041
Moderate/severe TR, <i>n</i> (%)	76 (34%)	5 (50%)	16 (36%)	55 (31%)	0.515
SPAP (mmHg)	45 ± 14	50 ± 16	42 ± 12	47 ± 15	0.345

[#]*P* < 0.05 *vs.* Moderate MR; **P* < 0.05 *vs.* Dynamic severe MR by *post hoc* analysis. LVEF: left ventricular ejection fraction; LVEDD: left ventricular end diastolic diameter; LA: left atrium; DMR: degenerative mitral regurgitation; FMR: functional mitral regurgitation; MVPG: mitral valve pressure gradient; RV diameter: right ventricular diameter; PISA: proximal iso-velocity surface area; EROA: effective orifice regurgitant area; RVol: regurgitation volume; TAPSE: tricuspid annular plane systolic excursion; TR: tricuspid regurgitation; SPAP: systolic pulmonary artery pressure

showed an increase from 8186 ± 2074 to 10735 ± 3043 mmHg*bpm (*P* < 0.001) during handgrip exercise. No patient experienced chest pain, ischemic ECG changes or significant arrhythmias during exercise.

Exercise-induced changes of MR severity and SPAP

Handgrip exercise caused an increase in PISA radius, EROA and RVol (all *P* < 0.01) [Figure 2]. According to established parameters, 44 patients (81%) with moderate MR at rest showed dynamic severe MR during handgrip exercise. Stratified by MR etiology, 18 patients (78%) with moderate DMR at rest showed

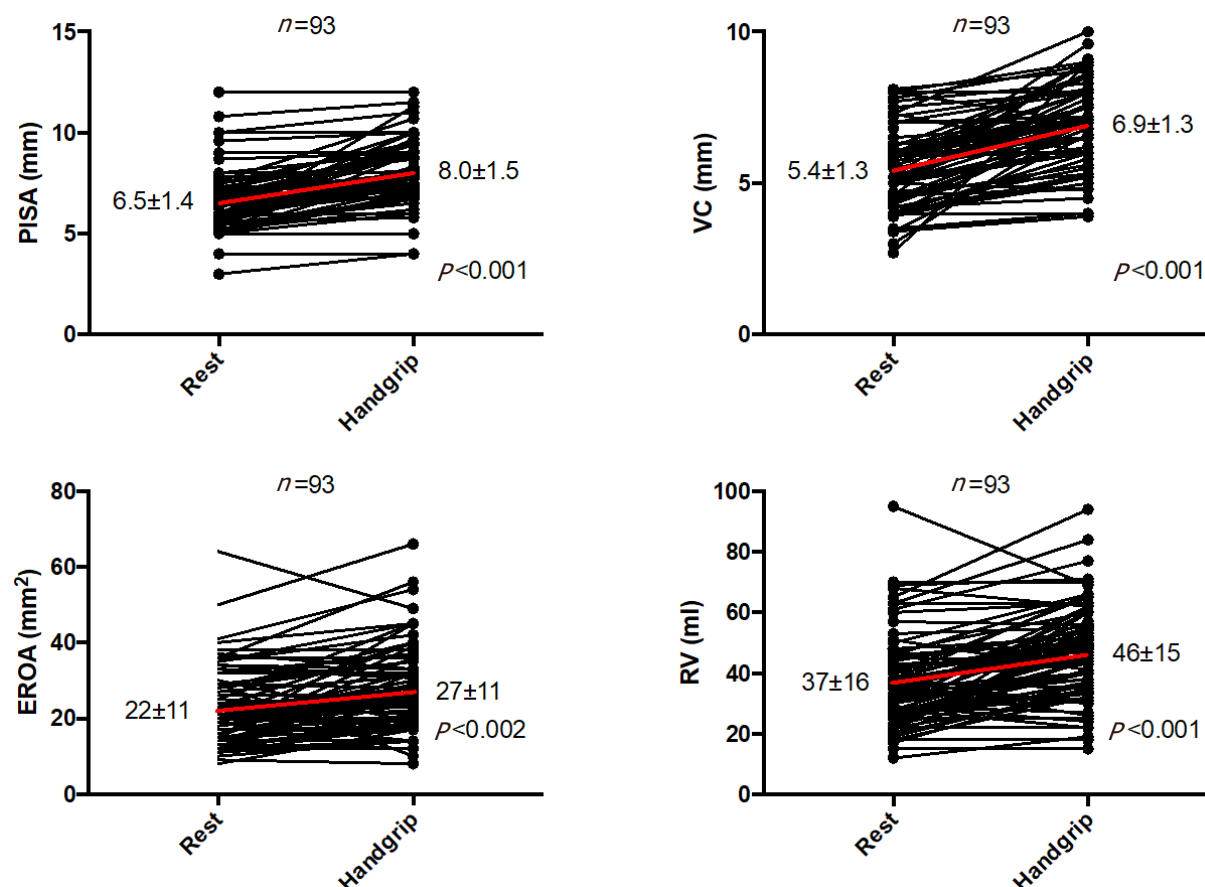


Figure 2. Individual changes in echocardiographic parameters during handgrip exercise ($n = 93$). Exercise-induced changes in PISA, VC, EROA and RVol are shown with mean values (red). During handgrip exercise, we observed a significant increase in different echocardiographic parameters indicating dynamic MR. In case of non-reproducible results according to the PISA method (e.g., due to eccentric jets in 8 of 93 patients), VC width was used for assessment of MR severity. PISA: proximal iso-velocity surface area; EROA: effective orifice regurgitant area; MR: mitral regurgitation; RVol: regurgitation volume; VC: vena contracta

exercise-induced increase in MR severity (dynamic severe MR) [Figure 3]. Similarly, 26 patients (84%) with moderate FMR at rest revealed dynamic severe MR during handgrip exercise. SPAP estimated by peak tricuspid regurgitation jet velocity increased from 42 ± 12 mmHg to 50 ± 13 mmHg ($P < 0.001$). Thirty patients (32%) had pre-existing pulmonary hypertension (PH) at rest and two other patients exhibited PH (> 60 mmHg) during handgrip exercise.

Procedural results and overall clinical outcome

Acute procedural success, defined by a reduction to MR grade $\leq 2+$ was achieved in 215 patients (97%). PMVR with the MitraClip system was equally effective in patients with and without dynamic MR, as indicated by a reduction of MR grade $\leq 1+$ at discharge in 61% of patients with dynamic severe MR and 59% of patients with severe MR at rest ($P > 0.999$), respectively [Figure 4].

Follow-up was complete in all patients (100%). During 12 ± 4 months follow-up, 37 patients (17%) died and 71 patients (32%) were re-admitted to the hospital because of heart failure symptoms. In the whole cohort, 92 patients (42%) experienced at least one event. Furthermore, 70% of patients belonged to NYHA functional class I or II (in contrast to 11% at baseline) (data not shown). In 93% of the surviving patients ($n = 174$), echocardiography after 12 ± 4 months was performed. MR grade improved compared to baseline MR grade $\leq 2+$ in 85% of surviving patients.

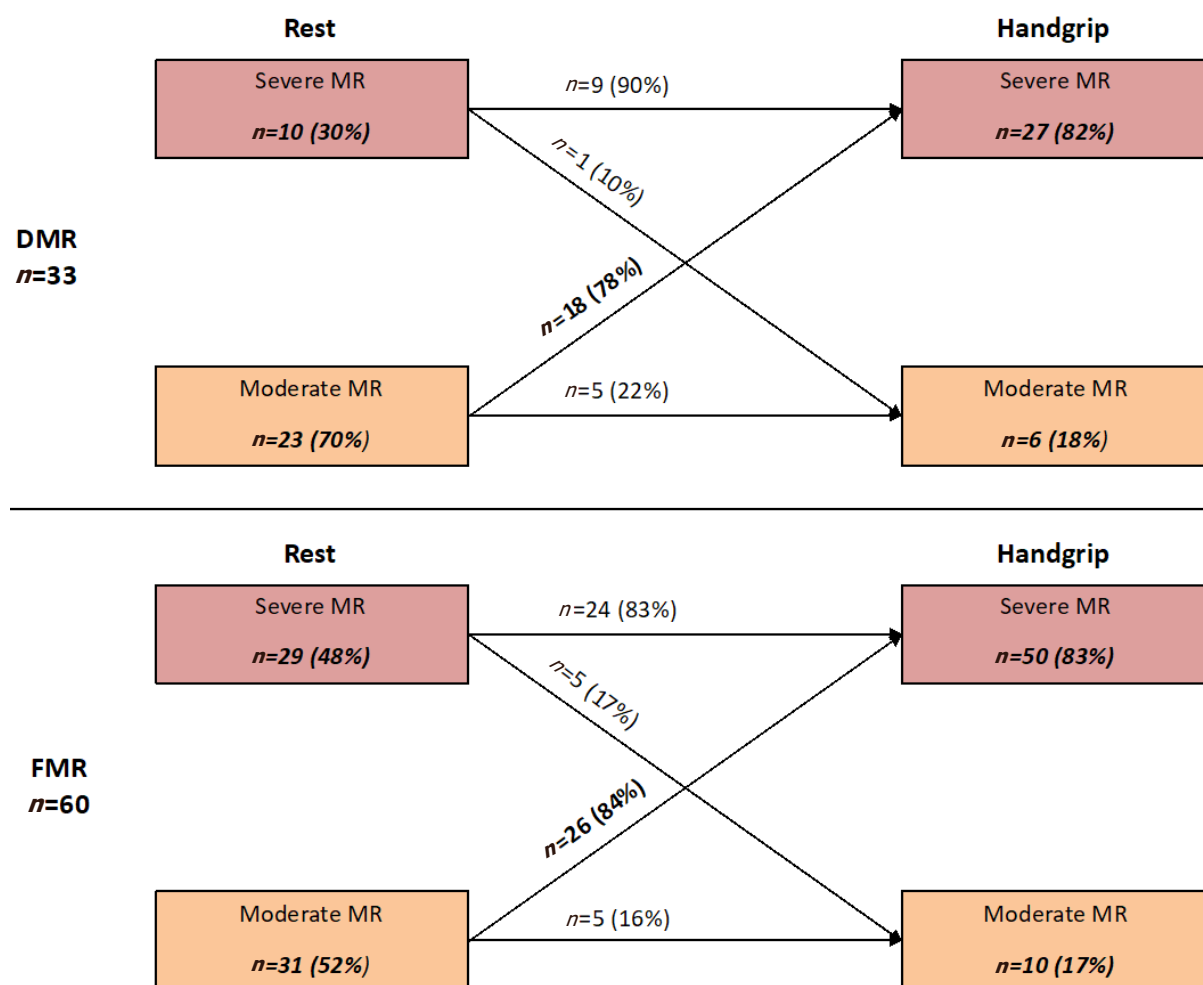


Figure 3. Exercise-induced changes in MR severity during handgrip exercise ($n = 93$). Eighteen patients (78%) with moderate DMR at rest and 26 patients (84%) with moderate FMR at rest revealed exercise-induced increase in echocardiographic parameters representing dynamic severe MR. Note that only patients who underwent PMVR were included and not those who were not selected for PMVR due to remaining mild or moderate MR during exercise, making a selection bias probable. However, 10 patients with moderate MR at rest and during exercise underwent MitraClip implantation due to persistent severe symptoms. MR: mitral regurgitation; DMR: degenerative mitral regurgitation; FMR: functional mitral regurgitation

Comparable clinical benefit in patients with dynamic severe MR and severe MR at rest

Effective reduction of MR following MitraClip implantation was associated with comparable clinical improvements in patients with dynamic severe MR and patients with severe MR at rest. At 12 ± 4 months, in patients with exercise-induced dynamic severe MR, all-cause mortality was half of the patients with severe MR at rest (9% vs. 17%), however, without reaching statistical significance ($P = 0.244$) [Table 3]. Similarly, the rate of heart failure rehospitalizations was numerically lower in patients with dynamic MR compared with those with severe MR at rest (25% vs. 33%), but also not of statistical significance ($P = 0.364$) [Table 3]. Thus, the combined endpoint reached was equally frequent in the two patient cohorts (30% vs. 43%; $P = 0.121$). Respective Kaplan-Meier survival curves are given in Supplementary Figure 1. Four out of 10 patients with moderate MR at rest and during exercise died during follow-up [Table 3]. There was no death and no admission for heart failure in patients with a decrease in MR severity during exercise (severe MR at rest and moderate MR during exercise) (data not shown). Seventy-four per cent of patients with dynamic severe MR and 68% of patients with severe MR at rest belonged to NYHA class I or II [Figure 4]. In this regard, 59% of patients with dynamic severe MR and 56% of patients with severe MR at rest experienced a clinical improvement from NYHA class III/IV to I/II ($P > 0.999$) during one-year follow-up.

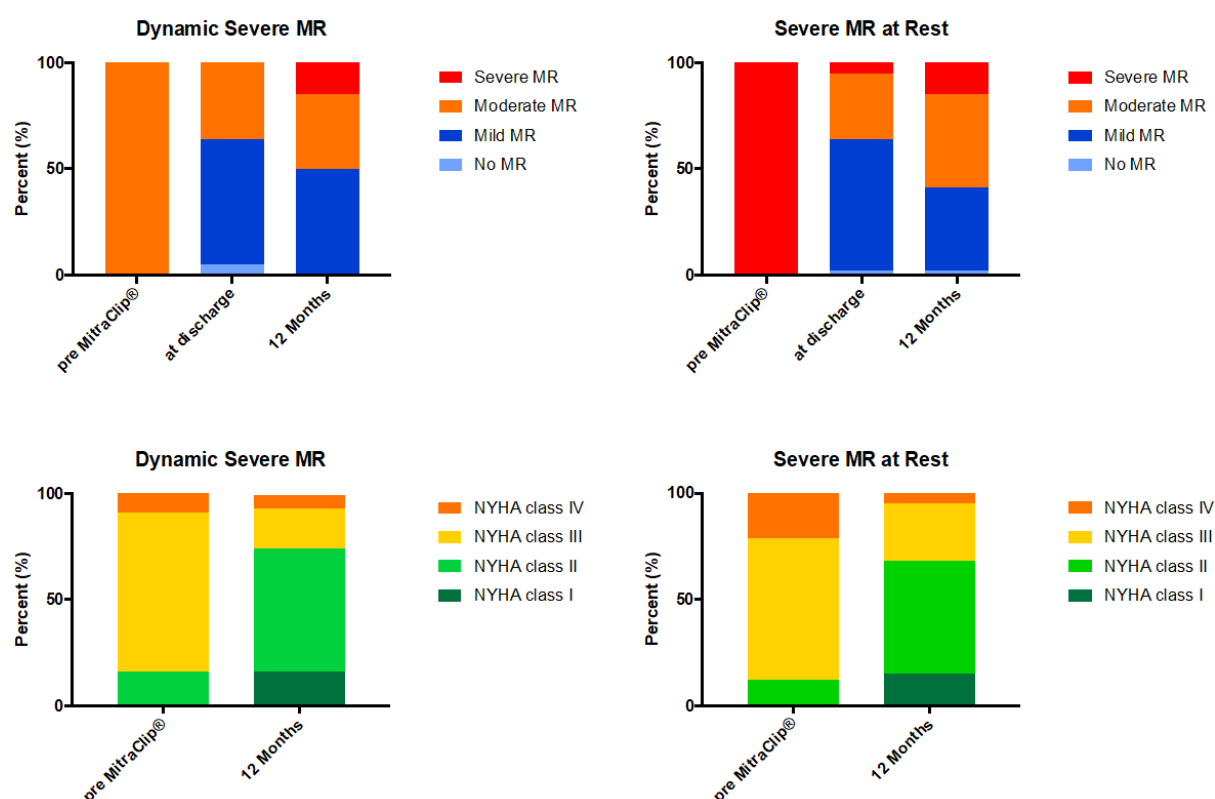


Figure 4. Change in MR severity at rest and NYHA functional class pre-MitraClip implantation, at discharge and at 1-year follow-up in patients with dynamic severe MR ($n = 44$) and severe MR at rest ($n = 167$). MR severity at rest and NYHA functional class improved following MitraClip independent of the presence of dynamic MR. MR: mitral regurgitation; NYHA: New York Heart Association; PMVR: percutaneous mitral valve repair

Table 3. Distribution of post-procedural outcome after 12 ± 4 months according to the grade of MR severity and to the presence of dynamic MR at baseline

	All patients $n = 221$	Moderate MR $n = 10$	Dynamic severe MR $n = 44$	Severe MR at rest $n = 167$	P-value
All-cause mortality, n (%)	37 (17)	4 (40)	4 (9)	29 (17)	0.244
Cardiac death, n (%)	18 (49)	2 (50)	2 (50)	14 (48)	0.532
Non-cardiac death, n (%)	10 (27)	1 (25)	1 (25)	8 (28)	0.689
Unknown, n (%)	9 (24)	1 (25)	1 (25)	7 (24)	0.999
HF-admission, n (%)	71 (32)	5 (50)	11 (25)	55 (33)	0.364
Mortality or HF-admission, n (%)	92 (42)	7 (70)	13 (30)	72 (43)	0.121

P-values represent differences in patients with dynamic severe MR vs. severe MR at rest. HF: heart failure; MR: mitral regurgitation

Eighty-five per cent of patients with dynamic severe MR showed MR grade $\leq 2+$, and 85% of patients with severe MR at rest did as well ($P > 0.999$) [Figure 4].

DISCUSSION

In the current study, we assessed the therapeutic benefit from PMVR with the MitraClip system in patients with dynamic severe MR. We demonstrated that the symptomatic benefit following PMVR in patients with dynamic severe MR, assessed during handgrip exercise, was similar compared to those patients presenting with severe MR already at rest, irrespective of the etiology of MR (degenerative and functional MR).

Effects of MitraClip implantation on dynamic MR

At one-year follow-up, MitraClip implantation was associated with reduction in symptoms as assessed by NYHA functional class and improvement in MR severity as assessed by echocardiography. Our findings are in line with the results from the EVEREST-II trial, supporting MitraClip therapy mainly in primary MR^[8], as well as with the results from real-world registries deciphering clinical improvement in patients with predominately secondary MR^[9]. Notably, there was a similar clinical benefit (regarding all-cause mortality, hospitalization for heart failure and reduction of NYHA functional class) and no difference in reduction of MR severity in patients with dynamic severe MR compared to patients with severe MR already at rest.

Our findings here support the performance of exercise echocardiography in patients displaying moderate MR at rest but reporting high-grade MR from a previous echocardiography or presenting with symptoms suggestive of dynamic MR. Recently, Van de Heyning *et al.*^[10] provided the first clinical evidence of hemodynamic improvements during exercise following PMVR in patients with secondary MR. They compared exercise echocardiography before and six months after MitraClip implantation. In 31 patients, a significant reduction of MR at peak exercise along with increased calculated cardiac output and decreased pulmonary arterial pressures (measured echocardiographically by trans-tricuspid pressure gradient) was documented. Here, we demonstrated in a larger, all-comers cohort that these findings go along with reduction in symptoms as assessed by NYHA functional class and an improvement in MR severity. The findings presented are of clinical relevance because MR resembles a dynamic entity that is sensitive to changes in preload, afterload and ventricular geometry as well^[1]. Even in patients with only mild or moderate MR at rest, Lapu-Bula *et al.*^[11] demonstrated a relevant negative impact on exercise-induced MR deterioration on exercise capacity. This might have been due to the combination of an inhibition of the expected increase in exercise forward stroke volume and a marked increase in pulmonary artery pressure^[11]. Recently, Lancellotti *et al.*^[12] provided clinical evidence to understand the unfavorable consequences of dynamic MR. They described that SPAP during exercise is associated with dynamic increase in EROA in patients with secondary MR and revealed a significant prognostic importance of exercise-induced PH, regarding the occurrence of cardiac death and cardiac events. The prognostic impact of dynamic MR has also been described by others^[2,6,13]. In this regard, mortality in patients with deteriorating secondary MR during exercise managed with optimal medical therapy exhibited increased mortality in the range of patients with severe MR already at rest^[6]. In the present study, we demonstrated a similar benefit in patients with dynamic severe MR and patients with severe MR at rest with regard to outcomes such as all-cause mortality, hospitalization for heart failure and symptomatic improvements. This may be explained by an effective reduction of dynamic MR by PMVR, as optimal reduction of MR seems to be of utmost importance for long-term clinical outcome^[14]. Mechanistically, PMVR with MitraClip implantation effectively increases coaptation area, thus improving closing force efficiency^[15] with an additional decrease in annular dimensions (antero-posterior)^[16]. This may lead to reduction in MR severity not only at rest, but also during exercise as has been recently shown by Van de Heyning *et al.*^[10] and is accompanied by relief from dyspnea.

The role of handgrip echocardiography prior to MitraClip implantation

Our study demonstrates that handgrip exercise serves as a valuable tool to unmask dynamic changes in MR. According to pre-defined parameters [Supplementary Figure 2], 15 patients (45%) with DMR and 24 patients (40%) with FMR revealed a marked increase in MR severity ($EROA > 10 \text{ mm}^2$, $RVol > 15 \text{ mL}$) during handgrip exercise, irrespective of MR severity at rest. These data foster previous results from studies reporting dynamic MR during exercise in one-third of patients with DMR^[17] and in 30%-50% of cases with FMR, both ischemic and non-ischemic etiology^[18-21]. Increasing MR severity during handgrip exercise is exaggerated by the hemodynamic response^[22], which mainly imposes pressure load on the left ventricle due to an increase in systemic vascular resistance, whereas dynamic exercise predominately results in volume overload^[23]. We decided to perform handgrip exercise as we included a frail patients cohort (logistic

EuroSCORE $22\% \pm 15\%$) of whom the majority might have been too unfit to complete bicycle exercise. The increase in blood pressure during isometric exercise may lead to a mismatch between increasing mitral closing force and increased mitral tethering resulting from the impact of the rise in afterload on left ventricular geometry^[24]. Additional mechanisms such as left ventricular dyssynchrony, changed sphericity and papillary muscle dynamics during exercise, may enhance these forces.

Limitations

The present study was retrospective in design so that we did not include a control group with moderate MR at rest and exercise-induced severe MR that was managed conservatively with optimal medical therapy. However, this would be necessary to definitively conclude the clinical benefit of patients with moderate MR at rest and exercise-induced dynamic MR. Moreover, this study was conducted at a single center. Therefore, further validation in a larger prospective multicenter study will be necessary to confirm the present findings. However, this is the largest study including patients with dynamic MR undergoing PMVR, so far. Since exercise echocardiography was performed upon clinical suspicion of dynamic MR and not on regular basis in every patient with MR grade 2+, a selection bias towards patients with relatively advanced disease in MR grade 2+ seems probable. In addition, we only included patients who underwent PMVR and not those who were not selected for PMVR because of remaining mild or moderate MR during exercise. These questions need to be addressed in prospective analyses.

In conclusion, our data provide clinical evidence of symptomatic benefit from PMVR (MitraClip) in patients with dynamic severe MR detected by handgrip echocardiography. These patients equally benefit from MitraClip implantation as patients with severe MR at rest. Further studies comparing an interventional strategy (PMVR) vs. conservative management (optimal medical therapy) are necessary to further evaluate clinical benefit from PMVR in patients with dynamic MR. Handgrip echocardiography may be integrated in the diagnostic workup of symptomatic patients with moderate MR at rest with suspicion of dynamic MR based on discrepancy between symptoms and echocardiographic findings at rest.

DECLARATIONS

Authors' contributions

Designed the study: Spieker M, Hellhammer K, Horn P, Westenfeld R

Responsible for data collection: Spieker M, Hellhammer K

Performed the statistical analysis: Spieker M, Westenfeld R

Involved in data interpretation, critically reviewed and revised the manuscript: Spieker M, Hellhammer K, Spießhoefer J, Zeus T, Horn P, Kelm M, Westenfeld R

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no no conflicts of interest.

Ethical approval and consent to participate

The study was part of a registry, which was approved by the ethics committee of the Heinrich-Heine University Duesseldorf and registered at www.clinicaltrials.gov (NCT02033811). The study was in accordance with the Declaration of Helsinki. All patients gave informed written consent.

Consent for publication

Not applicable.

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Review

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Maintenance with Rituximab in anti-neutrophil cytoplasm antibody-associated vasculitis

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Abstract

Granulomatosis with polyangiitis, microscopic polyangiitis and renal-limited anti-neutrophil cytoplasm antibody (ANCA) associated vasculitis are the main ANCA-associated vasculitides (AAV). Multiple induction therapies for AAV exist and have proven successful in achieving disease remission. Azathioprine and methotrexate have been used to maintain remission of AAV, however, relapse rates and adverse effects with these medications remain high. Rituximab (RTX), a B cell depleting monoclonal antibody, was shown to be safe and effective in maintaining disease remission in AAV in early retrospective reviews. In 2014, the first randomized control trial to compare RTX and azathioprine in maintenance therapy of newly diagnosed AAV (MAINRITSAN trial), revealed that patients who received RTX after cyclophosphamide induction had higher rates of sustained remission, fewer adverse effects and, better overall survival rates as compared to azathioprine. MAINRITSAN 2 revealed that patients receiving tailored regimens of maintenance RTX received fewer infusions but did not have higher rates of relapse than patients who received fixed dose therapy. The RITAZAREM trial conveyed that patients who experienced AAV relapse after induction therapy that received induction and maintenance RTX were significantly less likely to develop a relapse at 24 months *vs.* patients who received maintenance therapy with azathioprine. Overall, these studies suggest that maintenance therapy with RTX represents an exceptional treatment option in patients with AAV in terms of safety and efficacy, resulting in lower relapse rates and less drug toxicity than conventional treatments. As a result, patients have fewer exposures to cytotoxic medications and thus, improved outcomes.

Keywords: Rituximab, ANCA vasculitis, maintenance therapy, B cell depletion, azathioprine, methotrexate



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INTRODUCTION

Anti-neutrophil cytoplasmic antibody associated (ANCA) vasculitis is characterized by infiltration of neutrophils into small blood vessel walls, resulting in autoinflammation and necrosis^[1]. Microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA), and renal-limited ANCA-associated vasculitis are considered the main ANCA associated vasculitides (AAV). Granulomatosis with polyangiitis and MPA present similarly and have significant disease overlap, therefore, they are often considered together in terms of management. Eosinophilic GPA is a process defined by eosinophilic pneumonia and its manifestations are dictated by eosinophilic inflammation; treatment strategies for this disease are therefore focused on this distinct aspect of its pathogenesis and differ from the treatment of the other ANCA associated vasculitides.

These rare diseases mostly affect individuals over the age of 50 and can progress to life threatening, fulminant multisystem disease with complications such as diffuse alveolar hemorrhage (DAH) and necrotizing glomerulonephritis causing respiratory failure and renal failure, leading to significant mortality rates^[2]. Furthermore, despite significant treatment advances in remission induction, preventing disease relapse and successfully maintaining disease remission continues to provide a significant challenge to physicians. Anti-neutrophil cytoplasmic antibody associated vasculitides therefore must be viewed as chronic diseases with complex treatment strategies that require long term immunosuppression.

Given the rarity of these diseases and the complexity of their treatment, significant efforts have been made over the years to investigate safe, effective induction and maintenance therapies for these patients. Prior to these significant advancements, AAV ran a fulminant and fatal course with rapidly progressive multisystem disease and an extremely high mortality rate. Eighty-two percent of patients with untreated GPA would die in 1 year and 90% would die in 2 years^[3]. Initially, induction therapy with glucocorticoids was attempted and improved survival rates to 1 year, however, this strategy was associated with a multitude of side effects and a high rate of disease relapse^[4].

In the early 1970s and 1980s, promising evidence of cyclophosphamide (CYC) use in conjunction with glucocorticoids for induction therapy surfaced. A paramount study in 1985 revealed that 93% of patients with GPA followed over a 21-year period who received induction therapy with CYC and glucocorticoids achieved disease remission^[5]. Unfortunately, however, the cytotoxic side effects of long-term CYC use became apparent including leukopenia, hemorrhagic cystitis, infertility and 31-fold increased risk of bladder cancer^[4]. Despite being an effective agent in inducing disease remission in AAV, the cytotoxicity of CYC provided long term harm to patients and thus, was not an ideal agent for maintaining disease remission. Further research was needed to discover new treatment modalities that would deliver less harmful side effects.

Multiple studies explored alternate induction therapies for AAV, most notably the CYCLOPS trial in 2009 (comparing IV pulse doses of CYC *vs.* daily oral CYC and prednisolone as induction therapy)^[5], the MORAM trial (exploring efficacy of methotrexate (MTX) in inducing remission *vs.* CYC and prednisolone)^[6], and the MEPEX and PEXIVAS trials which studied the use of plasma exchange *vs.* glucocorticoid therapy for patients with AAV and sought to determine whether plasma exchange resulted in any significant difference in renal recovery, progression to ESRD or, overall incidence of death in patients with ANCA associated vasculitis^[7,8]. Although promising, these alternate therapies did not prove to be any more efficacious than CYC while still having high relapse rates and significant drug toxicities.

Two randomized controlled trials exploring induction therapy with RTX *vs.* CYC (RAVE and RITUXVAS trials) found that induction therapy with RTX (administered alone in the RAVE trial and co-administered with CYC in the RITUXVAS trial) was non-inferior to CYC alone in inducing disease remission and that

RTX did not have an increased risk of adverse events as compared to CYC. Additionally, at month 18 in the RAVE trial and at month 12 in the RITUXVAS trial, RTX alone was shown to be as efficacious in preventing disease relapse as CYC followed by azathioprine (AZA) maintenance therapy^[9,10]. The RAVE trial also found that RTX had a better safety profile than CYC^[9]. This was a cornerstone discovery in that, an induction therapy as effective as CYC (especially in severe relapsing disease) had yet to be discovered with the added benefit that RTX had a better safety profile. Despite these promising discoveries for induction therapy, maintenance of AAV remission still poses a significant challenge to physicians. This has prompted further research into alternative maintenance therapies.

Remission maintenance therapies in ANCA associated vasculitis

Cyclophosphamide, an alkylating agent, has been used as the gold standard of induction therapy in AAV for many years. Given its significant toxicity, however, it is not an ideal agent to use in maintaining disease remission in AAV, as long-term exposure significantly increases the risk of adverse effects including hemorrhagic cystitis and bladder cancer. Therefore, research of alternate remission maintenance strategies has been aimed at maintaining disease remission with less toxic immunosuppressants after initial induction therapy with cyclophosphamide.

In 2003, a randomized trial comparing AZA versus CYC in maintaining disease remission in ANCA-associated vasculitis was conducted. The study included patients who had recently been diagnosed with generalized vasculitis that were also found to have renal involvement (with a serum creatinine of 5.7 mg/dL or less). All patients received CYC and glucocorticoids for induction of disease remission. After achieving remission, patients were randomly assigned to receive CYC (1.5 mg/kg) or AZA (2 mg/kg/day) maintenance therapy while both groups continued receiving prednisolone. These patients were followed for 18 months. Relapse rates in both groups were roughly the same (11 patients in the AZA group and 10 patients in the CYC group). The study thus concluded that administering AZA after induction therapy with CYC was effective in maintaining disease remission and that AZA could be used as a feasible and less toxic maintenance therapy than CYC for patients with ANCA-associated vasculitis^[11].

Methotrexate has also been researched for use as a maintenance therapy in AAV. In 2017, a single center, open-label randomized trial studied the use of MTX *vs.* CYC for maintenance therapy in AAV. The study enrolled patients with MPA, GPA or EGPA and administered induction therapy with CYC before randomizing these patients to receive maintenance therapy with CYC or MTX for 12 months. The patients were monitored for relapses for 12 months after receiving induction therapy. The study found that the frequency of relapses and adverse events was the same between two groups. Methotrexate was thus designated a safe and effective alternate option for maintenance therapy in AAV after CYC induction^[12].

Further studies sought to investigate the use of AZA *vs.* MTX in maintenance of disease remission in ANCA-associated vasculitis. The Wegner Granulomatosis-Entretien (WEGENT) trial, an open label, multicenter study, investigated patients with a diagnosis of MPA or GPA who had been given induction therapy with CYC and glucocorticoids and were then randomized 1:1 to receive maintenance AZA (2 mg/kg/day) or MTX (0.3 mg/kg/day increased incrementally to 25 mg/week) for 12 months. The patients were followed for 29 plus or minus 13 months and monitored for relapses. The results showed that similar relapse rates and numbers of adverse events occurred in both the AZA and MTX groups, indicating that the two drugs have similar efficacies in terms of maintenance of disease remission and, similar safety profiles in terms of adverse events^[13].

The above data demonstrates that while AZA and MTX are alternate options for maintenance therapy in patients with AAV after CYC induction and, that they do decrease the overall amount of exposure to CYC, they are still associated with a high risk of relapse and adverse events, with neither of these medications

proving to be safer or more efficacious than the other. This fact prompted further investigation into safer, more efficient remission maintenance therapies such as RTX.

RITUXIMAB IN ANCA ASSOCIATED VASCULITIS

Rituximab is a monoclonal antibody consisting of both human and murine components that specifically targets the B cell CD20 antigen, thus causing removal of B cells from the peripheral circulation^[14]. This is important because B cells have been implicated in the pathogenesis of AAV via their production of ANCAs, activation of the alternative complement pathway, and initiation of inflammatory cascades that induce severe necrotizing vascular inflammation. The ANCA autoimmune response is driven by inhibited suppression of T cells and B cells, and by release of B-cell stimulating factors by activated neutrophils which act to slow the apoptosis of B cells, thus enhancing their proliferation^[15]. Due to the fact that the pathogenesis of AAV is rooted in B cell mediated autoimmunity, it stands to reason that RTX would be of significant therapeutic benefit given its ability to target autoreactive B cells and deplete them from the circulation. As such, RTX provides a promising treatment option for induction and maintenance therapy in AAV because it targets the direct pathogenesis of the disease.

MAINTENANCE THERAPY WITH RITUXIMAB IN AAV

With the advent of promising research demonstrating RTX as a safe and effective induction therapy, investigators set their sights on this drug as a possible maintenance therapy for patients with AAV. Rituximab showed promise as a maintenance therapy for AAV in early case reports and retrospective reviews. In 2001, the use of RTX was described in a man with a history of chronic, relapsing GPA who did not tolerate treatment of disease relapse with CYC due to bone marrow toxicity, which caused significant anemia. Additional immunosuppressive treatments such as AZA and mycophenolate mofetil (MMF) were attempted, however, the patient did not respond to these treatments and had continued relapses with worsening renal failure and a meningeal flare of GPA. The decision was made to initiate therapy with RTX on a compassionate basis given the patient had been refractory to all conventional treatments and MTX was contraindicated. Subsequently, the patient received 4 infusions of 375 mg/m² of RTX and high dose glucocorticoids. The patient entered complete remission after treatment with RTX and it was observed that his cytoplasmic ANCA (cANCA) and B lymphocyte levels had completely disappeared. At 11 months after treatment, the patient's cANCA and B lymphocytes reappeared, yet he did not have any signs or symptoms of disease relapse; he was preemptively treated with RTX at that time and remained in complete remission at 18-month follow up^[16] [Table 1].

In 2005, a case series reviewed 9 patients with a history of GPA or MPA who had either been resistant to treatment with CYC or, had experienced recurrent relapses after cessation of CYC therapy. Two of the patients were myeloperoxidase (MPO) - ANCA positive and 7 were proteinase-3 (PR-3) ANCA positive. These patients were treated with RTX infusions in addition to conventional immunosuppressants (AZA, MMF, or a short course of CYC) in order to prevent the formation of antibodies to RTX. Three patients were treated twice with a once per week infusion and four patients were treated four times with a once per week infusion. Eight out of the 9 patients in the study exhibited a complete response to RTX therapy with one patient having a partial response. Responses included improvement of chest x-ray (four patients), cessation of lower extremity gangrene (one patient), improvement of peripheral neuropathy (one patient), remission of renal vasculitis (two patients), and improvement in severe musculoskeletal pain (one patient)^[17] [Table 1].

A retrospective study of 8 patients with a history of relapsing or refractory GPA who received RTX infusions in conjunction with ongoing immunosuppressive therapy was conducted in 2007. This study investigated the efficacy of RTX in patients with GPA who had either failed treatment with prior

Table 1. Studies regarding maintenance therapy with Rituximab in ANCA-associated vasculitis

Study and year of study	Study design	Objective of study	Results	Study limitations
Response of Wegner's granulomatosis to Anti-CD20 chimeric monoclonal antibody therapy, 2001 ^[16]	Case Report	To describe the successful use of rituximab to treat a patient with chronic, relapsing GPA who did not tolerate cyclophosphamide therapy and was resistant to treatment with glucocorticoids, azathioprine and mycophenolate mofetil	Rituximab was able to successfully induce and maintain disease remission in a patient with chronic, relapsing GPA resistant to other immunosuppressants	The report only describes the response of one individual to rituximab which fails to generalize the results to other patients with ANCA associated vasculitis
Nine patients with anti-neutrophil cytoplasmic antibody-associated vasculitis successfully treated with rituximab, 2005 ^[17]	Case series which included with structured patient follow up	To review the outcomes of 9 patients with MPA and GPA treated with rituximab who were either resistant to or had recurrent relapses after cessation of cyclophosphamide	Rituximab was efficient and safe as an induction and maintenance therapy for patients with MPA and GPA	No control arm Possible selection bias Patients received additional immunosuppressive medications while they were on treatment with rituximab
Adjunction of rituximab to steroids and immunosuppressants for refractory/relapsing Wegner's granulomatosis: a study on 8 patients, 2007 ^[18]	Retrospective study of 8 patients with refractory or relapsing GPA who received rituximab infusions in addition to their ongoing immunosuppressive therapy	To investigate rituximab use in conjunction with ongoing steroid and immunosuppressant therapy as a treatment for relapsing/refractory GPA and to determine the frequency of infusions, time to patient response and, effects on the various manifestations of GPA	Treatment of relapsing/refractory GPA with rituximab in conjunction with steroids/ immunosuppressants resulted in good clinical outcomes There was a dissociation in the time to response of vasculitis manifestations (improved over days to weeks) <i>vs.</i> granulomatous manifestations (improved over several months) to rituximab	Patients were receiving concomitant immunosuppressive therapy so it is difficult to tell if results could be attributed to rituximab alone
A multicenter survey of Rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis, 2009 ^[19]	Standardized, retrospective data collection from 65 patients at 4 centers in the UK with a history of refractory AAV who received rituximab as induction therapy (largest series reported at that time)	To determine if rituximab is a safe and effective option in treating patients with ANCA associated vasculitis	Rituximab was found to be successful as an induction therapy in patients with AAV Additionally, patients who received preemptive retreatment in the absence of any signs of a relapse with a regimen of 1 g rituximab every 6 months had no disease relapse at eleven- month follow up, suggesting rituximab as a viable maintenance therapy	Possibility of positive outcome bias given that the study was a retrospective review
Rituximab as maintenance therapy for anti-neutrophil cytoplasmic antibody-associated vasculitis, 2010 ^[20]	Retrospective review of 39 patients with AAV who received maintenance therapy with rituximab	To determine the efficacy and safety of rituximab infusions as maintenance therapy in patients with ANCA-associated vasculitis who had achieved complete or partial remission	Rituximab was safe and effective in maintaining disease remission in patients with ANCA-associated vasculitis	Comparison with other studies is limited because this cohort had lower disease activity at study onset
Rituximab for remission induction and maintenance in refractory granulomatosis with polyangiitis (Wegner's), 2012: ten year experience at a single center ^[21]	Single-center historical cohort study observing all patients (53 total) with a history of chronic relapsing GPA treated with rituximab therapy from January 1, 2000 to May 31, 2010	To determine the efficacy of rituximab as a therapy for maintenance of remission in patients with a history of chronic relapsing refractory GPA	Rituximab was effective and well tolerated as an induction and maintenance therapy in patients with a history of chronic relapsing GPA	Open-label administration of rituximab Experience of study was only from one center with a predominantly Caucasian population of Scandinavian and Northern European background Except for 1 patient, all patients were PR-3 ANCA positive

Rituximab maintenance therapy for granulomatosis with polyangiitis and microscopic polyangiitis, 2012 ^[22]	Retrospective study of the outcomes and tolerance of patients with MPA and GPA treated with rituximab maintenance therapy	To investigate the efficacy of rituximab as a maintenance therapy in patients with AAV who achieved disease remission with conventional immunosuppressants or rituximab	Rituximab was well tolerated and maintained remission of patients with GPA and MPA however, it did not completely prevent relapses **The preliminary results of this study were to be confirmed by the MAINRITSAN trial which was in progress when the results of this study were published	Half of the patients had received additional immunosuppression concomitantly with rituximab, making it difficult to discern if the low relapse rates were solely attributable to rituximab use Preliminary study which did not allow conclusions about the exact role of rituximab in treating AAV
MAINRITSAN trial, 2014 ^[23]	Nonblinded, randomized controlled trial	To compare rituximab infusions to azathioprine as maintenance therapy in patients with ANCA associated vasculitis (MPA, GPA, and renal-associated ANCA vasculitis)	Rituximab infusions of 500 mg given every 6 months were superior to azathioprine as maintenance therapy in AAV, particularly in patients who are PR-3 ANCA positive	Trial was not blinded Fewer patients with anti-MPO ANCA positive vasculitis, MPA, or renal limited disease
MAINRITSAN2 trial, 2018 ^[24]	Open-label, pragmatic, multicenter randomized controlled trial	To compare rituximab infusions tailored to the appearance of ANCA autoantibodies, increasing ANCA titers and/or the presence of CD19+ B cells in the circulation measured every three months to fixed dose regimens of rituximab in patients with GPA or MPA as maintenance therapy	There was not a significant difference in the number of relapses in the tailored infusion group <i>vs.</i> the fixed dose regimen group; tailored infusion group received fewer infusions while still maintaining a low relapse rate	Open-labeled but all relapses were assessed by an independent Adjudication Committee who was not aware of the treatment arm or the circulating CD19+ B cell count There were 59 centers with testing performed at each individual center (as opposed to all testing being done in the same laboratory), however, all labs for a given patient had to be drawn at the same laboratory
RITAZAREM trial, 2019 ^[25]	International, multi-center, open-labeled, randomized, controlled trial	To compare the efficacy of rituximab <i>vs.</i> oral azathioprine as a maintenance therapy in patients with a history of relapsing AAV who had received induction therapy with rituximab	Rituximab was superior to azathioprine as maintenance therapy in AAV patients with a history of prior relapses	Investigators were given the option to choose the glucocorticoid tapering regimen after induction therapy as opposed to using a blinded, randomly assigned tapering schedule
Prolonged B cell depletion with rituximab is effective in treating refractory pulmonary granulomatous inflammation in granulomatosis with polyangiitis (GPA), 2014 ^[26]	Retrospective case series	To investigate the efficacy of rituximab infusions in treating pulmonary granulomas in patients with GPA who were previously resistant to traditional immunosuppressive treatment	Prolonged B cell depletion following rituximab infusion was effective in reducing both the size and number of pulmonary nodules in these patients for at least 18 months after treatment	Small patient cohort (5 patients) makes results hard to generalize
Rituximab for treatment of severe renal disease in ANCA associated vasculitis, 2016 ^[28]	Retrospective multi-center study	To investigate the efficacy of rituximab and glucocorticoids alone <i>vs.</i> rituximab, glucocorticoids and cyclophosphamide as a treatment for AAV patients with severe renal disease	There was no difference in outcomes between the rituximab and glucocorticoids arm <i>vs.</i> rituximab, glucocorticoids and cyclophosphamide arm	Concomitant glucocorticoid administration with rituximab makes it difficult to discern if the results can be solely attributable to rituximab
Rituximab in the treatment of refractory scleritis in patients with polyangiitis (Wegener's), 2015 ^[29]	Retrospective analysis of interventional case series	To evaluate the efficacy of rituximab in patients with a history of GPA who developed scleritis that was refractory to conventional immunosuppressant therapy	Four weeks after treatment with rituximab, all patients showed improvement of refractory necrotizing anterior scleritis and no further disease progression	Small patient cohort (8 patients) makes results hard to generalize

Rituximab therapy for refractory orbital inflammation: results of a phase 1/2, dose-ranging, randomized controlled trial, 2014 ^[30]	Dose ranging, randomized, double masked phase 1/2 clinical trial	To determine the efficacy of rituximab in treating orbital inflammation	Rituximab was effective in treating orbital inflammation due to GPA that was refractory to previous immunosuppressive treatment	Small patient cohort (10 patients total with orbital inflammation, 2 of these patients had GPA)
Successful treatment of hypertrophic pachymeningitis in refractory Wegener's granulomatosis with rituximab, 2009 ^[31]	Case Report	To describe the use of rituximab to treat a patient with GPA who developed pachymeningitis which was refractory to treatment with cyclophosphamide and pulsed dose methylprednisolone	The patient experienced complete remission after treatment with rituximab	Case report details the response of one individual patient to treatment thus these results cannot be extrapolated to other patients with this condition without further studies
Effectiveness of Rituximab for the otolaryngologic manifestations of granulomatosis with polyangiitis (Wegener's) ^[32]	Retrospective analysis	To determine the efficacy of rituximab in treating the ENT manifestations of GPA	Rituximab was found to be an effective treatment for ENT manifestations of GPA	Comments only on the ENT (granulomatous) manifestations of GPA, does not explore efficacy of rituximab for treatment of GPA manifestations secondary to systemic vasculitis
Diffuse alveolar hemorrhage secondary to antineutrophil cytoplasmic antibody-associated vasculitis: predictors of respiratory failure and clinical outcomes, 2016 ^[33]	Single center historical cohort study	To determine the efficacy of plasma exchange, cyclophosphamide and rituximab in treating diffuse alveolar hemorrhage in patients with AAV	Complete remission was achieved at a higher rate with rituximab than with cyclophosphamide, addition of plasma exchange did not improve outcomes	Study included predominantly patients with GPA rather than MPA

GPA: granulomatosis with polyangiitis; ANCA: anti neutrophil cytoplasmic antibody; MPA: microscopic polyangiitis; AAV: ANCA associated vasculitis; PR-3: proteinase-3; MPO: myeloperoxidase; MAINRITSAN: maintenance of remission using Rituximab in systemic ANCA-associated vasculitis; RITAZAREM: Rituximab *vs.* Azathioprine as therapy for maintenance of remission of anti-Neutrophil cytoplasm antibody-associated vasculitis

immunosuppressive therapy or, had continued to relapse despite treatment. The study found that RTX infusions improved the clinical outcomes of patients with relapsing or refractory GPA when used in conjunction with other immunosuppressants. Additionally, it was found that the granulomatous manifestations of GPA took longer to respond to RTX therapy (several months) as opposed to the vasculitis manifestations of GPA, which responded within weeks to months^[18] [Table 1].

A retrospective data collection two years later in 2009 (the largest data review to be conducted at that time) gathered data from 65 patients across 4 centers in the UK with a history of refractory AAV who received induction therapy with RTX. The study aimed to investigate the efficacy and safety of RTX in patients with refractory AAV as an induction therapy. Complete remission occurred in 49/65 patients (75%), with partial remission occurring in 15/65 patients (23%). Only one patient did not respond to therapy. Furthermore, patients who received preemptive retreatment with 1 g of RTX every 6 months despite not having any symptoms of disease relapse had zero relapses at 11-month follow up, suggesting that RTX was also an effective maintenance therapy in patients with refractory AAV^[19] [Table 1].

A year later in 2010, another retrospective review studied RTX as a maintenance therapy in 39 patients with AAV who had already achieved either complete or partial remission. This study aimed to address the direct role of continuous infusions of RTX as a maintenance therapy in AAV patients. All 39 patients followed up after 1 year and 20 patients followed up after 2 years. The results showed that RTX treatment resulted in good disease control throughout the study. Median disease activity was measured according to the Birmingham Vasculitis Activity Score (BVAS), a comprehensive scoring system of all organ systems possibly affected by vasculitis that contains 59 items divided into 9 groups (i.e., general, cutaneous, mucous,

renal, etc.). A lower score indicates lower disease activity, versus a higher score which indicates higher disease activity. Median disease activity of the patients in the study was 1 at baseline and improved to 0 at 12 and 24-month follow up. Eighty-seven percent of patients were on cytotoxic immunosuppression at the start of the study *vs.* 41% at 12-month follow up and 30% at 24-month follow-up^[20] [Table 1].

A single center historical cohort study in 2012 observed 53 patients with chronic relapsing GPA over 10-year period from January 1, 2000 to May 31, 2010 in whom RTX was used to maintain remission or, to treat disease relapses. The patients in the study received at least 2 courses of RTX (median number of courses of RTX was 4), with 52 of these patients being PR-3 ANCA positive. All patients who were treated for relapses achieved disease remission. Of the 53 patients, all achieved B cell depletion after induction therapy with RTX. Thirty two out of the 53 patients relapsed and, in all cases, relapses were associated with a rise in PR-3 ANCA levels and the reappearance of CD19+ B lymphocytes in the circulation. Remission was maintained successfully in all patients who were treated with preemptive courses of RTX based on re appearance of B lymphocytes and increases in PR-3 ANCA titers. Overall, this study conveyed the effectiveness of RTX as both an induction and maintenance strategy in patients with AAV^[21] [Table 1].

A retrospective study in 2012 examined the outcomes of 28 patients with AAV (4 with MPA and 24 with GPA) treated with RTX for maintenance therapy from 2003-2010. All patients in the study had entered remission with the use of conventional immunosuppressant therapy or RTX and were monitored for relapse rates and tolerance after greater than or equal to 2 RTX infusions used as maintenance therapy. The median range of RTX infusions was 4 with a median follow up time of 38 months from diagnosis or last flare. Out of 28 patients, 2 had pulmonary relapses, 1 patient suffered alveolar hemorrhage 6 months after a RTX infusion and 1 patient developed new lung nodules 11 months after a RTX infusion (this patient was then started on a new induction regimen with RTX which achieved disease remission and was followed by successful RTX maintenance). At final evaluation, 6 patients were in complete remission, 11 patients were in complete remission with irreversible damage and, 9 patients were in partial remission. 7 of these patients had persistent ENT involvement and 2 patients had persistent lung nodules. None of these patients had infusion reactions, 15 patients had hypogammaglobulinemia and 3 patients developed infections. The data of this study suggest that RTX could be used as a safe, effective maintenance therapy in AAV^[22] [Table 1]. Since the safety and efficacy of RTX as a maintenance therapy had been established in this study, the next question posed was the efficacy and safety of RTX as compared to other conventional maintenance therapies like AZA. This laid the framework for the largest randomized controlled trial to compare RTX to AZA as maintenance therapy in AAV titled the Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis (MAINRITSAN) trial.

In 2014, the MAINRITSAN trial investigated the use of RTX *vs.* AZA as a maintenance therapy in patients with AAV. The study was a non-blinded, randomized controlled trial that studied patients with GPA, MPA or renal-limited AAV who had received induction therapy with CYC and glucocorticoids and achieved disease remission. These patients were randomized to receive RTX infusions *vs.* daily AZA for maintenance therapy and were monitored for disease relapse. After 28 months, 5% of the patients in the RTX group had suffered disease relapses *vs.* 29% of the patients in the AZA group. Additionally, adverse event rates were similar between the two groups. This study demonstrated that patients in the RTX group had significantly less relapses and an equal number of adverse events as the AZA group, thus conveying the superiority of RTX to AZA in maintaining disease remission in AAV while maintaining a similar safety profile to AZA^[23] [Table 1]. One interesting point is that the study was comprised mostly of patients who were anti PR-3 ANCA positive; patients who were anti MPO-ANCA positive and patients who had renal associated AAV comprised a smaller patient population in the study. An interesting point of further investigation would be to do additional trials exploring RTX as remission maintenance in patients who have anti MPO ANCA-positive vasculitis to further determine their responsiveness to RTX treatment.

Optimum regimen of rituximab maintenance therapy and reliability of ANCA titers and presence of CD19+ B lymphocytes as predictors of AAV relapse

In light of the evidence that RTX was superior to AZA in remission maintenance in the MAINRITSAN trial, investigators then set out to describe the optimum RTX treatment regimen in order to maintain disease remission in AAV, and to determine whether the reappearance of ANCA autoantibodies, an increase in ANCA titers from baseline or, the reappearance of CD19+ B lymphocytes at follow up could provide a reliable marker of the need for reinfusion of RTX in order to prevent disease relapses.

The study was conducted by comparing the relapse rates of patients receiving individually tailored regimens of RTX infusions which were administered based on the reappearance of CD19+ B lymphocytes, reappearance of ANCA autoantibodies, or a marked increase in ANCA titers from baseline to the relapse rates of patients receiving fixed dose regimens of RTX. This trial was titled Maintenance in Remission using Rituximab in Systemic ANCA-associated Vasculitis-2 (MAINRITSAN2). The study was an open label, pragmatic, multicenter, randomized controlled trial. Patients were randomized in a 1:1 ratio to receive tailored RTX infusions or fixed dose regimens. These therapies were given 1 month after achieving remission with either CYC, MTX, or RTX induction in both groups.

The results of the study showed that 17.3% of the patients in the tailored therapy arm suffered relapse vs. 9.9% of patients in the fixed dose regimen arm. Major relapses occurred in 7.4% of the tailored regimen arm vs. 3.7% of the fixed dose regimen arm. Patients in the tailored infusion arm received 248 infusions vs. patients in the fixed dose regimen arm who received 381 infusions. The results of the MAINRITSAN2 trial demonstrated that individually tailored infusions of RTX based on biomarker activity were associated with low major relapse and relapse rates which did not differ significantly from the fixed dose regimen group, and allowed for administration of fewer RTX infusions. The study also demonstrated that reappearance of ANCA autoantibodies, increasing ANCA titers or, reappearance of CD19+B lymphocytes was not reliable in predicting AAV relapse, particularly because 4 patients in the study had negative ANCA autoantibodies and no circulating B cells at the time of disease relapse^[24] [Table 1].

Rituximab maintenance therapy in patients with relapsing AAV

In 2019, further studies were conducted to determine the efficacy of RTX vs. AZA as a maintenance therapy for patients with a history of relapsing AAV who achieved disease remission with RTX induction. This trial, known as the Rituximab as Therapy to Induce Remission after Relapse in ANCA-associated Vasculitis (RITAZAREM) trial, was an international, multi-center, open labeled, randomized controlled trial. Patients with a history of relapsing AAV were recruited for the study during the time of an active relapse and received glucocorticoids and RTX as induction therapy. The patients were then randomized to receive RTX or AZA maintenance in a 1:1 ratio and followed for 36 months, with 61% of patients having suffered a major relapse at the time of enrollment in the study. At 24 months after treatment, 13% of patients in the RTX group experienced relapses; of the 13% of relapses in the RTX group, 82% were classified as minor and 18% were classified as major. 38% of patients in the AZA group suffered relapses; of the relapses in the AZA group, 62% were classified as minor and 38% were classified as major. The adverse event rate in the RTX group was lower (22%) vs. the AZA group (36%). The RITAZAREM trial successfully demonstrated that RTX maintenance therapy was superior to AZA in patients with a history of relapsing AAV who achieved remission with RTX induction and, that RTX was associated with fewer adverse events^[25] [Table 1].

The role of rituximab in maintenance therapy of lung, renal, and other systemic manifestations of AAV

Anti-neutrophil antibody associated vasculitis is a systemic disease which affects multiple organ systems. Below are studies outlining RTX as maintenance treatment of the various systemic manifestations of the disease.

The role of RTX as a maintenance therapy of pulmonary granulomas in patients with GPA was described in an observational cohort study in 2014. The study looked at 5 patients with a history of PR-3 ANCA positive GPA who had pulmonary granulomas that were previously resistant to traditional immunosuppressive treatments. These patients received RTX infusions in reduced dosing schedules and were monitored for radiographic improvement of their pulmonary granulomas on chest x-ray (CXR) every 6 months for a total of 18-38 months. The results revealed that prolonged B cell depletion following treatment with RTX was effective in reducing both the size and number of pulmonary nodules in these patients for at least 18 months after treatment^[26].

Renal manifestations of AAV include necrotizing and rapidly progressive glomerulonephritis with crescent formation, leading to rapid decompensation of renal function and acute renal failure^[2]. Renal involvement in AAV is an extremely poor prognostic factor, and it is the most significant predictor of mortality in AAV patients^[27]. The renal manifestations of AAV can occur without concomitant systemic vasculitis, an entity known as renal limited AAV. Rituximab has been studied as an induction agent for patients with AAV and severe renal disease. 37 patients with a history of AAV and a GFR < 20/mL/min/1.73 m² were studied in order to determine the safety and efficacy of RTX and glucocorticoids alone vs. RTX, glucocorticoids and CYC as induction therapy in patients with AAV and severe renal disease. The study found that there was no difference in outcomes between the two treatment groups, and that RTX and glucocorticoids alone were as effective as RTX, CYC and glucocorticoids in treating AAV patients with severe renal disease^[28]. The MAINRITSAN trial also included patients with renal associated AAV and displayed the superiority of RTX to AZA in maintenance of disease remission in these patients^[23].

Other manifestations of AAV successfully treated with RTX include refractory scleritis^[29], orbital GPA^[30], hypertrophic pachymeningitis in refractory GPA^[31], ENT manifestations of GPA^[32], and DAH^[33].

ADVERSE EFFECTS OF RITUXIMAB

Hypogammaglobulinemia has been observed in patients receiving maintenance therapy with RTX, which is of particular concern given the increased risk of developing serious infections when immunoglobulins are low^[23,24]. Literature detailing an ideal threshold immunoglobulin level at which to stop RTX in order to prevent development of serious, life threatening infections has not been described. It has been described that antibiotic prophylaxis is effective in preventing infections in patients who develop hypogammaglobulinemia after RTX therapy. These patients should receive influenza and pneumococcal vaccinations prior to the initiation of treatment in order to prevent infections^[34].

Late onset neutropenia has been described in patients with rheumatic diseases who have received RTX therapy, albeit this is a rare side effect. Several mechanisms have been proposed which could potentially contribute to late onset neutropenia in patients with rheumatic diseases receiving RTX including increased production of B lymphocytes which halts production of T cells and, infiltration of bone marrow and peripheral blood by T-large granular lymphocytes^[34,35].

Progressive multifocal leukoencephalopathy (PML) has also been described in patients with GPA and MPA receiving RTX treatment. A cumulative analysis conducted in 2018 which studied patients receiving RTX for GPA or MPA between 2009 and 2015 showed that the confirmed number of cases of reported PML was very low (< 1 case per 10,000), indicating that this is a rather rare adverse effect of the medication. Despite its rarity, physicians should still be aware of the risk of development of PML in AAV patients receiving treatment with RTX, as these patients have often previously received cytotoxic immunosuppressants which are also known risk factors for development of PML^[36].

Continued use of RTX increases the risk of serious infections depending upon the indication for its use and, the dose at which it is being administered. With regard to AAV patients, a retrospective case review demonstrated that severe infections occurred most commonly within the first year of receiving RTX treatment. Old age and lack of ENT involvement was a risk factor for developing severe infection^[37]. Another study found that bronchiectasis and endobronchial involvement were notable risk factors for development of severe respiratory infections, and that antibiotic prophylaxis with Trimethoprim-sulfamethoxazole was effective in preventing infections in patients with AAV who were receiving RTX infusions^[38].

Rituximab as induction and maintenance therapy in EGPA

Due to the fact that the pathogenesis of EGPA is dictated by eosinophil mediated inflammation and eosinophilic pneumonia as opposed to the other ANCA associated vasculitides (which are driven by the pathogenesis of B cell autoimmunity, neutrophil abnormalities and complement activation), the treatment of this specific subset of AAV can differ, which is why EGPA was excluded from most of the studies mentioned above. Induction therapy of life-threatening organ involvement in EGPA is commonly accomplished with CYC and glucocorticoids. A study in 2017 sought to investigate RTX vs. CYC as induction therapy in patients with EGPA who were refractory to prior induction therapy with CYC. This retrospective analysis studied 28 patients with EGPA and measured their treatment response when treated with RTX induction therapy vs. cyclophosphamide. Five of the patients in the RTX arm (36%) achieved disease remission as opposed to four patients in the CYC arm (29%). The remainder of the patients achieved partial remission. There was no difference in response to treatment between the two groups. Rituximab was well tolerated but did result in a decrease in serum immunoglobulin levels^[39]. Further research is yet to be done on effective maintenance therapies for EGPA and would be an interesting subject of continued studies.

EFFICACY OF RITUXIMAB IN TREATING PROTEINASE 3-ANCA VS. MYELOPEROXIDASE-ANCA

During the RAVE trial, patient responses to RTX vs. CYC were analyzed based on ANCA-type (PR-3 vs. MPO). The data concluded that RTX was also superior to CYC in maintaining remission specifically in ANCA PR-3 positive patients vs. patients who were positive for ANCA-MPO^[9]. The MAINRITSAN trial enrolled more patients who were positive for anti-PR-3 ANCA in the study than patients who were positive for anti-MPO ANCA, so the results were more indicative of the response of the anti-PR3 ANCA patient population to RTX [Table 1]. Earlier trials also enrolled greater numbers of PR-3 ANCA positive patients^[17,21]. This poses an interesting question as to whether RTX is more effective in treating and preventing disease relapse in patients who are specifically PR-3-ANCA positive and, would be a good subject of future research.

RISK FACTORS FOR DISEASE RELAPSE IN ANCA ASSOCIATED VASCULITIS

The utility of monitoring the presence of autoreactive ANCA antibodies, ANCA titers and, presence of circulating CD19+B cells as a risk factor for disease relapse in AAV has been a matter of debate due to conflicting data on this subject throughout the years. It is particularly useful to know this information as prevention of relapses is paramount in reducing organ damage and increasing survival in AAV patients. There is conflicting evidence regarding this subject because while ANCA titers do often drastically decrease in patients who have received induction therapy, some patients who have achieved disease remission can remain ANCA positive and certain patients who are negative for ANCA autoantibodies can still relapse. The same question is posed regarding the presence of circulating CD19+ B cells as relapses have occurred regardless of the presence of B cells in AAV patients. The MAINRITSAN 2 trial addresses this fact as the study notes that four patients experienced disease relapses despite being ANCA negative and having no B cells in the circulation.

A single center cohort study conducted in June 2020 sought to investigate the role of the presence of ANCA autoantibodies, ANCA titers and, the appearance of CD19+ B cells in the circulation as predictors of the risk of disease relapse in AAV patients. The study found that ANCA negativity regardless of B cell presence was a strong predictor that disease remission would be maintained. Additionally, PR-3 ANCA positivity or persistence of PR-3 ANCA levels was a strong predictor of relapse. Relapses in the MPO-ANCA population occurred exclusively when B cells were present. This research provides the interesting possibility that a tailored regimen of therapy based on ANCA positivity and presence of B cells could be effective in preventing disease relapse in AAV patients. The question that remains is if this method could lead to overtreatment, as some patients are able to achieve clinical remission despite ANCA positivity and relapses have been known to occur in patients regardless of B cell presence. This will be an interesting topic of further research^[40].

CONCLUSION

Treatment of AAV has always provided a challenge for physicians both because of its severe, life threatening manifestations and, the multitude of side effects caused by the cytotoxic medications needed to induce and maintain disease remission. Despite these therapies, patients with AAV still experienced high rates of relapse. Cyclophosphamide was the gold standard of induction therapy for many years however, given its extensive risk of cytotoxic side effects, it was not an ideal long-term maintenance therapy for patients with AAV and other agents were explored for disease maintenance remission. Among these medications are MTX and AZA, which gained recognition for their ability to induce disease remission and shorten the length of time of CYC exposure, however, these medications had high relapse rates and were associated with numerous adverse effects. The RAVE and RITUXVAS trials demonstrated that RTX was non-inferior to CYC in successfully achieving disease remission in AAV patients and, had a similar safety profile to CYC.

After the discovery that RTX was a promising induction agent for AAV, early retrospective reviews were conducted exploring RTX as a maintenance therapy for AAV. The MAINRITSAN trial provided evidence of the superiority of RTX to AZA in preventing disease relapse in AAV patients, and the MAINRITSAN 2 trial demonstrated that individually tailored regimens of RTX infusions based on the reappearance of ANCA autoantibodies, increase of ANCA titers or, reappearance of CD19+ B lymphocytes from treatment randomization had similarly low relapse rates as fixed dose regimens of RTX and, enabled patients to receive fewer infusions. The RITAZAREM trial demonstrated the superiority of RTX as a maintenance therapy compared to AZA in the prevention of disease relapse in patients with a history of relapsing AAV and, showed that RTX had fewer associated adverse events. Additionally, despite being left out of the above trials due to its distinct pathogenesis, RTX maintenance was also investigated in EGPA as compared to CYC in another study and was deemed an effective and well tolerated treatment option for EGPA.

These studies are monumental in the advancement of therapy for AAV because up until recently, the gold standard induction therapy for this disease was extremely cytotoxic and even though alternate maintenance strategies were aimed at reducing the toxic effects of CYC, they had high rates of relapse and were associated with adverse effects of their own. The above literature reveals that RTX is a safe, effective and well tolerated induction and maintenance therapy in AAV that can be given in a tailored dosing regimen, thus exposing patients to fewer infusions. This has important implications in the treatment of AAV as patients treated with RTX will have better disease control with lower relapse rates, fewer exposures to the medication and, a decreased incidence of adverse events. As a result, patients with AAV receiving RTX therapy will have higher survival rates and longer relapse free periods, leading to an overall enhanced quality of life and improvement in patient outcomes.

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Authors' contributions

Made substantial contributions to conception and design of this review and performed data interpretation: Skopis M, Bag-Ozbek A

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Both authors declared that there are no conflicts of interest.

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Review

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Correlation of carotid artery disease and tinnitus: is it an auditory phantom in vascular surgery practice? A wide evidence-based review

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Abstract

Carotid artery diseases can result in many extracranial manifestations. Tinnitus is a recognised symptom and has long been correlated with significant internal carotid artery stenosis. It has been largely classified into pulsatile and non-pulsatile types with variable management approaches and prognoses. Surgical and endovascular approaches to treat carotid artery stenosis have not only aimed to reduce stroke rates but also to manage such symptoms. The clinical and cognitive evidence of such practices are broad and managed in a combined spectrum with otolaryngologists. This literature review aims to focus on current evidence and practice with vascular surgeons on the importance of dealing with tinnitus in managing carotid artery stenosis.

Keywords: Carotid artery disease, carotid artery stenosis, tinnitus, carotid endarterectomy

INTRODUCTION

Tinnitus is a common symptom affecting 10%-15% of the population and can be generally classified into either pulsatile or non-pulsatile types^[1]. The non-pulsatile form is recognised as the more common presentation of the two and is most often related to episodes of a continuous ringing or whistling sound^[2].



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Pulsatile forms of tinnitus are the rarer manifestation of this condition, where the ringing symptoms experienced are associated with the vascular system^[3]. Carotid stenosis is generally accepted as the most common cause of arterial pulsatile tinnitus^[3].

In current vascular surgery practice, tinnitus is very much correlated with the presence of distorted extracranial or intracranial blood vessels or to the presence of intracranial hypertension. Therefore, precluding an abnormally functioning auditory system. The heartbeat synchronous tinnitus is as we know a predominantly vascular pathology in origin and by in large can be diagnosed by different forms of angiography, including classical intravenous or intra-arterial angiography, computed tomography angiography, or more commonly magnetic resonance angiography.

Pulsatile tinnitus is typically subdivided into arterial heartbeat synchronous or venous “hum-like” pulsatile tinnitus^[4]. It has been referred to as objective tinnitus. These perceived pulsations are most likely transmitted through the cerebrospinal fluid to the cochlea; a mechanism similar to what has been proposed as an explanation for bone conduction^[5,6].

Carotid artery atherosclerotic disease has been widely linked to tinnitus; however, reports emerging on stenotic subclavian, external carotid artery and reversal of blood flow in an aberrant occipital artery have also been implicated in the causality of pulsatile tinnitus^[7-9]. The impact of flow disturbances in the carotid arteries on the inner ear has not yet been investigated thoroughly, and reports are very much limited by single-centre biased reporting. Direct meaning from such experiences has not been sufficient to bridge the gap in evidence in the literature. As the population is ageing, the standard techniques of audiology and oto-neurological testing is also a challenge to conduct in elderly populations, particularly in those with dementia. This coupled with a general absence of public awareness has not led to the introduction of generalised therapeutic interventions. These factors are likely to exacerbate the current health economic turmoil associated with the clinical care of this condition due to the surge of referrals to oto-neurological clinics and vascular surgery practices.

From a vascular surgery perspective and standpoint, carotid artery stenosis as a pre-existing cause for pulsatile tinnitus can be corrected with dilation and stenting or carotid endarterectomy^[10,11]. Such intervention is believed to abolish the turbulent flow and ameliorate the clinical presentation allowing for a reasonable patient-related quality of life. However, it is currently unclear what clinical practice to implement due to the lack of clear evidence of best practice published in the literature. For example, ipsilateral carotid endarterectomy has been shown to effectively reduce if not eliminate pulsatile tinnitus in greater than 90% of patients with demonstrated intracranial carotid artery stenosis^[12]. However, it is imperative to draw a meaningful conclusion from reported experiences and to be able to draw indications on when endarterectomy is to be favoured over the rather quantified approach of stenting. The location of the lesion is no longer sufficient to deduce the best applicable practice and more up to date guidelines are required for treating this disease.

This wide evidence-based literature review aims to describe the current understanding of the natural history and pathology of this condition in respect to carotid artery disease, as well as to compare the current therapeutic, clinical and surgical interventions utilised to allow clinicians and patients to make a well-informed decision in regard to their management plan. We also aim to outline the gap in existing evidence pertaining to this entity and to reflect on potentially different focuses for furthering evidence-based practice in this field.

IS THERE A PHANTOM PERCEPTION BETWEEN CAROTID ARTERY DISEASE AND TINNITUS?

Carotid artery stenosis may be asymptomatic or have subtle neurological presentation albeit of motor or sensory origin. Therefore, identifying a clinically significant carotid artery stenosis can be difficult to

achieve in cases without symptoms or with the most minimal. On a parallel note, a reduction in cerebral blood flow may be a contributory factor and can potentially be the culpable weakest link.

Hence, to date, the standard diagnostic tool for delineating this is the widely available duplex ultrasonography. However, this tool is subjective and dependent on the operator. This raises the issue of potential subjective biases in reporting and utilization of this resource. Consequently, in an attempt to quantify the degree of stenosis present, other imaging modalities can also be implemented. These include classical angiography, computed angiography, and magnetic resonance angiography. However, those modalities raise a prudent question regarding timing and associated timely risk factors. The identification of these risk factors and the unequivocal management of Tinnitus, shown to have a vascular origin, can have an impact on central nervous system symptom manifestation and a natural effect on pulsatile tinnitus. The carotid intima-media thickness as delineated on the aforementioned imaging modalities can be of crucial help when identifiable in formulating the diagnosis and developing the axis of management but cannot surely affect or quantify the pulsatility.

The microvascular remodelling implicated in increased pulsatile tinnitus through the increased resistance in vascular beds and the emergence of microvascular ischemia has large implications on arterial stiffness and crucially on the success of any intervention whether surgical or endovascular.

LITERATURE SEARCH STRATEGY

Electronic searches were performed on a number of databases including PubMed, Scopus, Embase and Cochrane with no limits put in place on dates. Search terms included: natural history, carotid artery disease, and stenosis, lesion size, location, prevalence, and natural history, risk factors, survival rates, medical therapy, surgical intervention, and mortality. Search terms were charted to MeSH terms, combined using Boolean operations and also used as keywords. Papers were selected on the basis of their title and abstract. The reference lists of these selected papers were also reviewed to identify any relevant papers that might be suitable for inclusion for this study. Forty-five publications were eventually identified with dates ranging from 2002-2020. A full breakdown of number of studies identified is detailed in [Figure 1](#).

SELECTION CRITERIA

Research papers were not excluded on the basis of their study design except for case reports. Any comments, editorials or opinions were not included for selection to provide an unbiased view. Papers were selected on the basis of providing primary endpoints of intervention used, the eventuality of the disease intervention and/or information regarding medical therapeutics, endarterectomy and angioplasty. Papers were not excluded on the basis of the age of the patient population.

WHAT IS THE PREVALENCE CHARACTERISTICS AND PATHOPHYSIOLOGY OF TINNITUS IN VASCULAR SURGERY PRACTICE?

To date, there is no prevailing consensus or guidelines that are sufficient to best describe the natural history of this disease and the current literature is lacking in detail regarding the prevalence and characteristics of the condition. Therefore, robust research into the pathophysiological factors, etiological mechanisms and pathogenesis of tinnitus in correlation with factors of carotid disease such as location, lesion size and extent of stenosis of affected vessels among others is required.

Historically, atherosclerosis within the carotids was credited as the main arterial cause of tinnitus. However, arterial stiffness has also become an important parameter that can predict the carotid event and can be correlated with the incidence of stroke^[13].

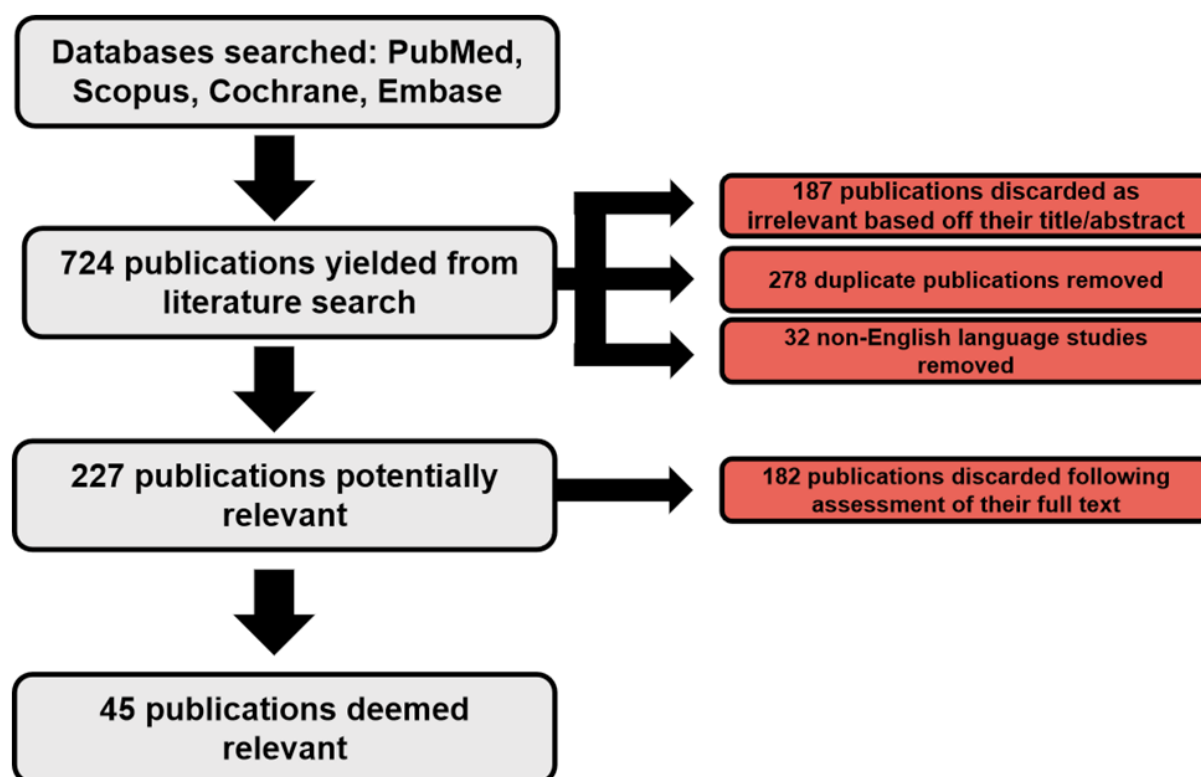


Figure 1. Flow chart detailing steps undertaken in our extensive literature search

Arterial stiffness as a term is inclusive of vascular smooth muscle hyperplasia, an increase in collagen levels, degradation of elastin including fracture of the elastic lamellae, and intima-media thickening, thereby altering vascular smooth muscle cell tone, which over time results in the development of increased stiffness or arteriosclerosis^[14]. Ageing, obesity, diabetes mellitus, and hyperlipidaemia are all factors relating to arterial stiffening^[15]. Arterial stiffness, which can be measured as either aortic or carotid-femoral pulse wave velocity or through augmentation index, is now recognised as an important independent predictor of both future cardiovascular disease and all-cause mortality^[16,17]. Augmentation index measures the central pulse pressure and is typically considered a more complex variable due to a combination of vascular elasticity and peripheral resistance^[18]. An increase in aortic pulse wave velocity of 1 metre/second is correlated to an age, sex and risk factor adjusted increase of 14% for cardiovascular events and 15% for mortality^[16].

The cochlear microcirculation may be impaired by this increased arterial stiffness due to the terminal nature of this circulation. Yet, this emerged evidence did not yield a shred of conclusive pathophysiological evidence as to how it pertains to pulsatile tinnitus. It has been shown that increased arterial stiffness may lead to microvascular damage in the brain and this can manifest itself with a multitude of clinical symptoms and signs including tinnitus^[19]. This had yielded to another entity related to carotid system arterial stiffness implicated in the development and severity of “idiopathic subjective tinnitus”^[8].

Parameters utilized to support this concept from the studies^[8,20] that had recruited participants included serum lipid profile (mg/dL), pure-tone hearing, blood pressure (mmHg), fasting glucose (mg/dL), and body mass index (BMI, kg/m²). Hence, the common carotid artery stiffness index, common carotid intima-media thickness, Young’s elastic modulus, peak systolic velocity, end-diastolic velocity, resistive index, vessel diameter, pulsatility index, mean velocity and volume flow were measured in both the right and left common carotid arteries.

These studies were able to intrinsically shed light on patient characteristics and the severity of the tinnitus. However, this was not uniformly applicable to both carotid artery disease, and independent predictors were unable to be constructed.

Another hypothesized scope outside of vascular surgery practice discussed the abnormal presence of neural activity inside the auditory system or tonotopic reorganization of the auditory cortex due to chronic deafferentation^[21].

In relation to vascular medicine and surgery terms, this was transcribed to the etiological factors that may contribute to vascular hypoxia/ischemia, which have been considered as possible factors in the pathophysiology of tinnitus. However, this inconclusive hypothesis has not revealed any meaning to the cellular significance of vascular-related tinnitus and as such is irrelevant to pinpoint carotid system disease as a culpable factor to the occurrence of tinnitus. Brain-related peptide elements and circulation are far from being understood to conclude as an aforementioned hypothesis for this phenomenon.

Without scientifically led and clinically driven evidence, it is difficult to see how such a hypothesis can determine a correlation between carotid artery disease and tinnitus based on this particular evidence and much further study and exploration is needed to accept or negate this.

OTO-NEUROSURGICAL EVIDENCE: A PROMISING & INNOVATIVE APPROACH

Recent advances in neuroimaging techniques, including positron emission tomography, magnetoencephalography, electroencephalography and functional magnetic resonance imaging, highlight the involvement of the central nervous system in the pathophysiological mechanism for tinnitus^[22]. These neuroimaging investigations have shown that brain regions that are considered to be unconnected to the auditory system, including the anterior cingulate gyrus, amygdala, hippocampus, and parahippocampal regions are strongly indicated in the pathogenesis of subjective tinnitus. Utilizing resting-state functional magnetic resonance imaging (fMRI) in non-auditory brain regions, such as the rectus gyrus, thalamus, cingulate gyrus, inferior temporal gyrus, hippocampus, cerebellar hemisphere, caudate and medial superior frontal gyrus, demonstrated an association with tinnitus-related symptoms including depression, anxiety and loudness^[23].

However, studies surging on this front line were limited not only due to low numbers of control cases but also to a lack of quantification and vague anatomical correlation and orientation. Laureano *et al.*^[24], utilizing single-photon emission computerised tomography study with a sufficient number of control cases ($n = 17$, sound methods of statistics and particularly precise anatomical orientation) were able to demonstrate that the cerebral blood flow in the left parahippocampus was significantly increased in tinnitus with normal hearing. This particular study intended to reconfirm the previous results of other resting-state fMRI studies using a different modality and method with intended benefit to identify ideal therapeutic targets for repetitive transcranial magnetic stimulation^[24].

Furthermore, attempts have been made to identify a different target for tinnitus and its correlation to carotid artery disease. Lesion size or location of carotid artery disease and the ability to combine these factors with arterial stiffness (using methods such as the carotid-femoral pulse wave velocity) and comparisons of local stiffness measurements (performed via Doppler ultrasonography) achieved conceptual accuracy in describing a meaningful mechanism with accurate values for tinnitus occurrence^[12]. However, it is important to remain critical of emerging evidence, and as such, most of those studies had a small number of recruits and were unwarranted in determining the significant difference between the patient and control groups.

CAROTID ENDARTERECTOMY AN OLD APPROACH IN AN ENDOVASCULAR ERA AND THE VASCULAR-NEURO-PSYCHIATRIC AXIS

By default, and without any consensus or attributable guidance, it is well-practiced in various multidisciplinary meetings and known that proximal lesions of carotid artery disease in the neck generally lend themselves to carotid endarterectomy practicing surgeons, while distal lesions get stratified and treated by angioplasty and stenting.

For years, intracranial carotid artery stenosis or carotid stenosis in the skull base have been generally treated using two approaches. One option, an initial balloon occlusion test performed under transcranial doppler and electroencephalogram monitoring can determine whether indeed the ipsilateral carotid artery can be sacrificed. If this is the case, one option is to ligate the symptomatic carotid artery and therefore arrest the turbulent flow. A second further option is to dilate and then stent the intracranial portion of the carotid artery, thereby resulting in a loss of arterial pulsatile tinnitus^[5]. From these practices and reported experiences, more than 70% of patients with carotid stenosis-related tinnitus have immediate amelioration and relief of tinnitus^[5]. Long-term and longitudinal studies correlating survivability and amelioration of symptoms are lagging.

Moreover, functional status and cognitive impact of these approaches are yet to be verified and studied in an unbiased format. As such, interventional as well as non-interventional medical procedures including carotid artery recanalization, appropriate pharmacological therapy and lifestyle modifications that are used to maintain blood flow in cerebral small vessels, are not well documented in any coherent evidence-based approach. This adds to the confounding gap in the literature and strictly limits our practice to surgeon-oriented and not patient-centred care. It is interesting to note that tinnitus attributable to common carotid artery stenosis at its bifurcation has been historically treated by ligation or carotid endarterectomy^[25]. However, curing this entity by endovascular stent angioplasty has received very little attention in the literature. As such, the heterogeneity of reporting and the lack of informal evidence of best medical practice reliability of results are questioned.

The advent but lack of availability of complex magnetic resonance imaging, in particular a combination of dynamic contrast-enhanced magnetic resonance imaging (MRI), dynamic susceptibility contrast MRI and blood oxygen level-dependent imaging MRI should be able to offer wide-ranging information on the effects of cerebral microcirculation on functional brain status and fluctuations of blood-brain barrier permeability. However, those modalities amongst others would not be in a position to add to studies with the concomitant neuropsychological assessment of patients with the carotid disease and post-tinnitus correction.

Recent advances leading to a new generation of stents has prompted clinicians to consider performing stent-assisted angioplasty as an alternative approach to angioplasty alone for intracranial stenosis^[26,27].

Cerebral blood flow, which can undergo assessment during computed tomography perfusion (CTP) examinations, improves after internal carotid artery stenting^[28-30]. However, two further important CTP-derived variables, blood-brain barrier permeability and mean transit time, are far less recognised in both the research and diagnosis of internal carotid stenosis.

Internal carotid artery stenosis-related brain hypoperfusion is generally associated with neurological, psychiatric, psychological and somatic deficits which can manifest with different variants of symptoms and clinical signs, including tinnitus. This can potentiate ill effect on health-related quality of life. To this effect, various studies have demonstrated that internal carotid stenosis without transient ischemic episodes

or strokes, is detrimental to memory and executive functioning^[31-34]. It has also been shown that cognitive function can be improved in symptomatic patients following internal carotid artery recanalization using both endarterectomy and carotid stenting^[35,36]. Grunwald *et al.*^[37] have also shown cognitive improvements in both word fluency and in delayed recall when under neuropsychological testing that was conducted 24 h after internal carotid artery stenting in asymptomatic patients. Results in asymptomatic patients similar to those outlined above have also been published by Wang *et al.*^[30] and Picchetto *et al.*^[38].

To ameliorate the vascular-neuro-psychiatric axis as a causative element for tinnitus amongst other clinical manifestations, Suh *et al.*^[39] demonstrated that balloon expandable intracranial stent placement can be safely utilized in stable symptomatic patients. Adverse effect rates were far lower in the stable patient group (4.1%) in comparison to the unstable patient group (25.9%). Terada *et al.*^[40] further published that stent placement is significantly more effective than percutaneous angioplasty for stenosis in the petrous or cavernous portion of the internal carotid and has low periprocedural morbidity (4.2%). Goessens *et al.*^[41], demonstrated that internal carotid artery stenosis should be considered an independent risk factor for stroke. Rao *et al.*^[42], Mathiesen *et al.*^[43] and Bakker *et al.*^[44] added to this bulk of evidence and stated that this contributes to the pathophysiology of depression and is also actually a risk factor for suicide in these stroke patients^[45].

Therapeutic effects of stenting do exist and it has shown to be superior to that observed by medical prescriptions including selective serotonin reuptake inhibitors in these patient groups. However, in a study by Picchetto *et al.*^[38], it was observed that there was no difference amongst pre- and post-scoring in the neuropsychological evaluation of both depression and anxiety carried out in asymptomatic internal carotid artery stenosis patients who received stenting.

Nonetheless, the approach and its desired effect on selective endovascular stenting for managing the ill effect of the vascular-neuro-psychiatric axis as a culpable cause for tinnitus has been demonstrated to be of promising value and with reliable results.

CONCLUSION

Therapeutic and surgical interventions amongst others such as classical endarterectomy or the surge of endovascular carotid stenting for the management of carotid artery disease-induced tinnitus may ameliorate this clinical condition, but their application is not without complications, compounded with a neurological disability or even death. No culpable evidence exists in the current era on what drives carotid artery stenosis and how this correlates with tinnitus. The exact pathophysiological mechanism is yet to be elucidated. The current reports of intervention are without meaningful and reliable evidence for managing this condition. Further studies of multicentre nature and well-constructed and powered trials are very much needed to build an evidence-based approach in an era of personalized and digital healthcare.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of this study: Abdelhaliem A, Howard C, Bashir M, Elsantawy H, Al-Khaffaf H

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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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Perspective

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Frozen elephant trunk: assets and liabilities of a challenging technique

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Abstract

The development of the frozen elephant trunk (FET) technique for a simplified treatment of complex lesions of the thoracic aorta originated as an evolution of the classic elephant trunk technique, described for the first time by Borst *et al.*^[1] in 1983. Novel technologies and standardization of the surgical approach produced a progressive improvement of early and late outcomes. Most of the time and for specific indications, FET procedure allows physicians to treat lesions involving extensive portions of the thoracic aorta in one single step. Spinal cord injury remains one of the main complications of this procedure, even though spinal protection strategies have led to better results. We hereby report our opinions and recommendations based on our experience started in 2007.

Keywords: Aortic arch, acute aortic dissection, frozen elephant trunk procedure, chronic aneurysm

INTRODUCTION

Complex thoracic aortic lesions represent one of the most relevant challenges in cardiovascular surgery, often requiring more surgical and/or endovascular procedures than other diseases/injuries in the field. Since the introduction of the “Elephant Trunk” (ET) technique, described in 1983 by Borst and colleagues as a two-stage approach^[1], methods and skills have been rapidly evolving with the introduction of innovative materials and more standardized techniques^[2-4]. This progression eventually led to the development of the frozen elephant trunk (FET) technique in 2003, thanks to the introduction of hybrid prostheses made up of a proximal surgical tubular graft and a distal endovascular stent-graft^[5].



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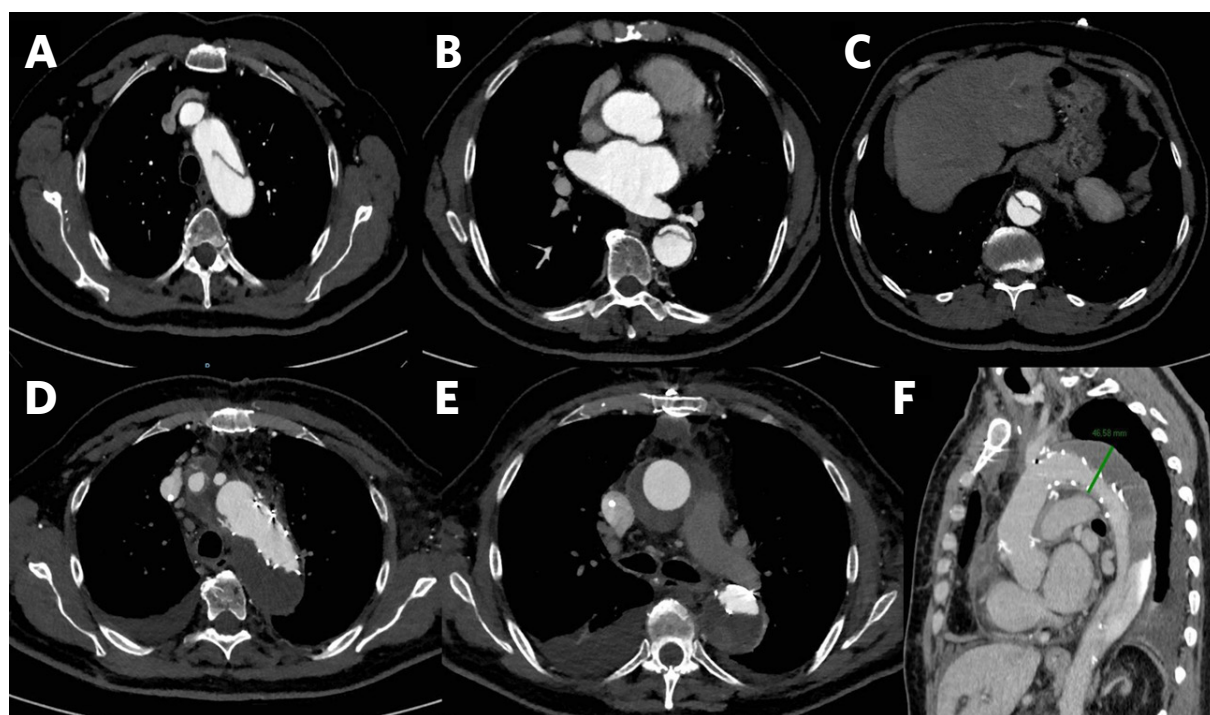


Figure 1. Frozen elephant trunk procedure with Thoraflex hybrid prosthesis in a case of acute type B aortic dissection. Pre-operative computed tomography (CT)-angiogram shows the entry tear downstream of the left subclavian artery and the dissected descending thoracic and thoracoabdominal aorta (A, B, C); two years-follow up CT-angiogram shows the complete peri-stent false lumen thrombosis (D, E, F)

The FET procedure is indicated in chronic thoracic aorta dissection, acute and chronic Stanford type B aortic dissection [Figure 1], when endovascular treatment is not feasible or contraindicated, and chronic degenerative aneurysms of the thoracic aorta and distal arch. Another indication is acute Stanford type A aortic dissection [Figure 2], especially when distal malperfusion occurs and when the intimal tear is located in the distal aortic arch or in the proximal descending thoracic aorta. A recently published position statement of the European Association for Cardio-Thoracic Surgery (EACTS) and the European Society for Vascular Surgery defines specific classes of recommendations to the aforementioned indications: in particular, class IIA is given to the cited cases of acute type A and type B aortic dissection and to aortic disease involving distal thoracic and thoraco-abdominal aorta, that is likely to require, at a later stage, either surgical or endovascular treatment. On the other hand, class IIB recommendation is given to type A aortic dissection to prevent aneurysms development in the downstream aorta^[6].

There are two available hybrid stent grafts for FET procedure in Europe with CE (Conformité Européenne) mark approval: the E-Vita Open and E-Vita Open Plus (Jotec GmbH, Hechingen, Germany) and the Thoraflex (Vascutek, Terumo, Inchinnan, Scotland, UK) [Figure 3]. These two grafts both are composed of a proximal gel-coated woven polyester tubular graft and a distal self-expanding stent graft made of polyester and nitinol ring stents. The two grafts differ from each other by the presence of three side branches for the arch vessels in the Vascutek Thoraflex prosthesis; on the other hand, the E-Vita Open Plus is a single tubular graft and the arch vessels are implanted on it with the “island” technique.

Our experience with FET procedure started in 2007 with a total of 318 procedures: 173 were carried out using the E-Vita Open hybrid prosthesis and 145 using the Thoraflex. The indications for FET procedure were chronic degenerative aortic aneurysms in 82 patients, acute type A aortic dissection in 44 patients, residual dissection in operated acute type A aortic dissection in 119 patients, chronic type A aortic

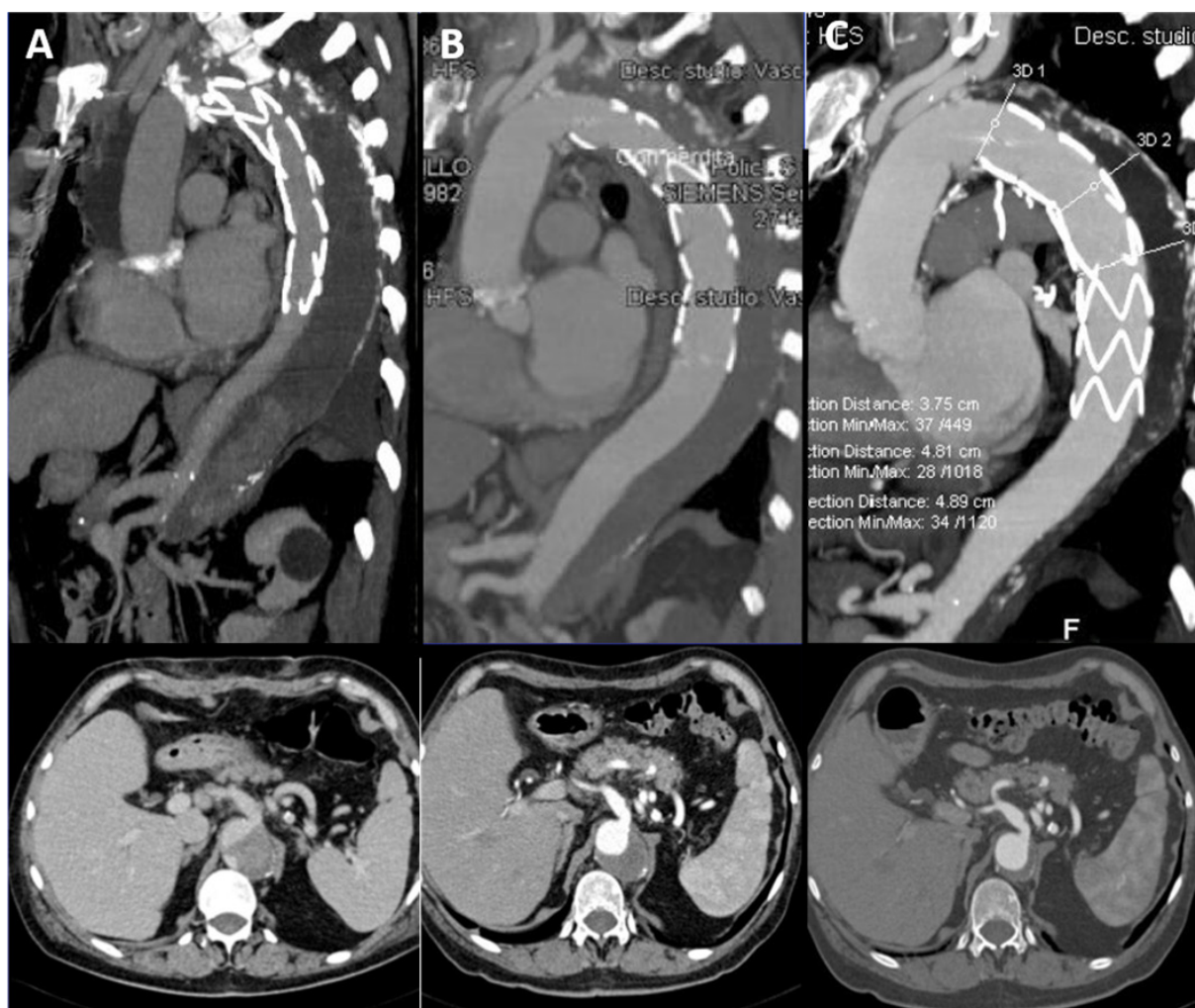


Figure 2. Frozen elephant trunk procedure with E-Vita Open plus prosthesis in a case of acute type A aortic dissection with the progressive thrombosis and shrinkage of the false lumen after 1 week (A), after 3 months (B) and after 2 years with almost complete aortic remodeling (C)

dissection (not undergone surgery) in 25 patients, and acute and chronic type B aortic dissection in 13 and 35 patients, respectively. Combined procedures were 128 (40.3%). With a mean follow up of 5 years, endoprosthetic extensions were performed in 85 patients (45 in E-Vita Open group and 40 in the Thoraflex group). In the vast majority of cases, the indication for extension was the incomplete thrombosis of the false lumen, whilst in very few cases, it was the inadequate distal sealing.

Some crucial recommendations we learnt from our experience are: accurate pre-operative assessment of the entire aortic anatomy, with identification of intimal tear and re-entry tears and visceral arteries origin from the true or false lumen in aortic dissections, careful evaluation of aortic diameters for appropriate graft sizing, and employment of safe and reliable organ protection and surgical strategies. In acute aortic dissection, oversizing is contraindicated since it can produce stent-induced new entry tears. Conversely, in chronic degenerative aortic aneurysms, oversizing allows an optimal distal graft sealing and it is therefore indicated.

Surgical technique

At our institution, the surgical technique for FET procedure is standardized to allow reproducibility and better outcomes. Following a full median sternotomy and administration of heparin, a stiff guide-wire is

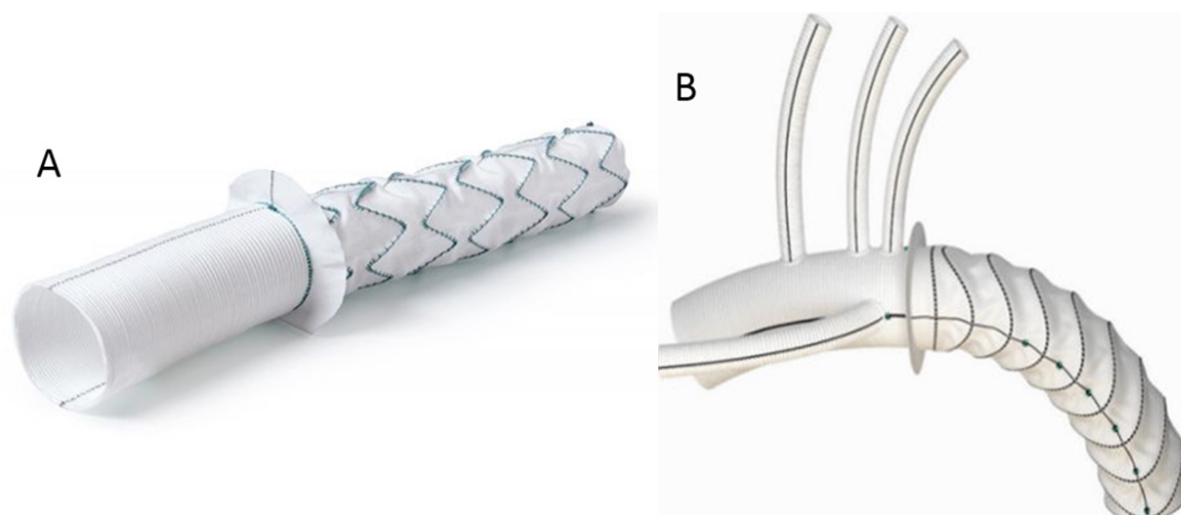


Figure 3. The E-Vita Open Plus (A) and the Thoraflex hybrid prosthesis (B)

inserted in the true lumen of the aorta up to the descending thoracic portion via the femoral artery, under transesophageal echocardiographic guidance. Cardiopulmonary bypass is then established, preferably via the right axillary artery or the right carotid artery (this can be done with interposition of an 8 mm Dacron graft), or directly through the brachiocephalic artery. For the venous cannulation, the chosen sites are usually the right atrium or the femoral vein, for example in case of reinterventions. A left ventricular venting cannula is inserted into the right superior pulmonary vein. Cerebral perfusion is monitored in all the cases with near infrared spectroscopy. Circulatory arrest is achieved at a nasopharyngeal temperature of 25 °C. Cold crystalloid cardioplegia is administered; specifically, we use the modified Bretschneider solution (Custodiol, Koehler Chemie, Alsbach-Haenlein, Germany). Following complete resection of the arch, we use the Kazui's Antegrade Selective Cerebral Perfusion (ASCP)^[7], specifically inserting cannulas into the left carotid and subclavian arteries, under moderate hypothermia^[8,9]. The next step is the preparation of the proximal descending aorta with addition of an external Teflon strip and four internal pledgeted U-stitches. In the case of aortic dissections, we carry out a surgical obliteration of the distal stump false lumen. The prosthesis (E-Vita Open or Thoraflex hybrid device) is introduced antegradely in the descending aorta, with the guidance of the previously inserted guide-wire, and then released.

In the case of Thoraflex implantation, following release, distal anastomosis between the prosthesis collar and aorta is performed. The cardiopulmonary bypass is restarted and the systemic perfusion restored via the designated graft side branch. Thereafter, separate reimplantation of the arch vessels is performed, starting with the left subclavian artery. The proximal anastomosis is commonly carried out following the reimplantation of the left subclavian artery, with the aim to reduce cardiac ischemic time. The distal anastomosis sites are usually arch zone 3 of Ishimaru (beyond left subclavian artery), arch zone 2 (between left subclavian and left carotid artery), or more proximal sites. More proximal anastomoses are, in fact, easier to perform and carry a lower risk of left recurrent nerve damage.

In the case of E-Vita Open hybrid prosthesis implantation, following release over the guide-wire, the Dacron graft is retracted and the collar anastomosed to the distal stump. Usually the lower body is reperfused for ten minutes through the graft; thereafter the arch vessels "island" is prepared and implanted on it. Distal flow is eventually restored, and proximal anastomosis performed.

We routinely use cerebrospinal fluid (CSF) drainage as a method of spinal cord protection, positioning the lumbar catheter one day before the procedure.

We prefer to use the Thoraflex graft when the arch vessels originate widely separately from each other or when they are severely dissected.

As already suggested by Tsagakis^[10], we routinely use angioscopy both to analyze the anatomy of the aorta before graft deployment, and to verify afterwards the correct positioning and expansion of the stent-graft.

DISCUSSION

FET technique is an elaborate procedure aimed to simplify the treatment of complex thoracic aortic lesions, which combines the classic elephant trunk technique features with the endovascular stent technology. The evolution from the classic ET to FET started at the end of the 1990s, with the “open stent-grafting technique”, combining antegrade endovascular stenting with classic arch repair^[2-4]. This technique was then modified by the Hannover group, with the development of a custom-made hybrid prosthesis, giving birth to the “Frozen Elephant Trunk” technique^[5]. Over the last few years, hybrid prosthesis technology has evolved, until the latest introduction of branched grafts in 2012.

Outcomes of FET procedure are variably reported in the literature. In the previous EACTS 2015 position statement on the use of FET technique, analyzing data extracted from 97 focused publications available in the literature, in-hospital mortality rates of 1.8% to 17.2% have been described^[11]. Similarly, Ma and colleagues showed an early mortality rate of 6.4% to 15.8% in a review article^[12].

Mortality and complication rates of FET procedure are comparable to those of classic aortic arch surgery, except for spinal cord injury (SCI), with variable rates from 0% to 21% as reported in the EACTS 2015 position statement. The most recent single-centre experience report by Shrestha and colleagues showed an in-hospital mortality of 11% (12% in acute type A aortic dissection) and a 2% incidence of SCI^[13]. Higher rates of SCI are found in patients who underwent FET procedure due to chronic aortic dissection^[11]. Presumably, the mechanisms involved in the pathophysiology of SCI are coverage of descending thoracic aorta beyond T7-T8 level, longer spinal cord ischemia times, and thromboembolic events. We therefore assume that the incidence of SCI could be reduced with a shorter descending aorta coverage and spinal cord ischemia time, in addition to the validated use of CSF drainage, which we strongly recommend.

In acute and chronic aortic dissection, FET technique promotes flow restoration in the true lumen, coverage of the proximal entry and re-entry tears and thrombosis of the false lumen, either partial or complete. On this regard, the Essen group reported rates of false lumen thrombosis of 90% in acute aortic dissection and 78% in chronic dissection^[13]. This induces positive aortic remodeling, with a drastic reduction of the risk of aneurysmal dilatation and rupture. Nonetheless, complete thrombosis of the false lumen could be the trigger for visceral ischemia when the visceral arteries originate from the false lumen and there are no re-entry tears located in the distal aorta. Therefore, our opinion is that FET procedure should be contraindicated in absence of re-entries in the distal thoracic, thoracoabdominal, and abdominal aorta when the visceral vessels originate from the false lumen.

In conclusion, FET technique represents a safe and effective procedure for the treatment of complex descending thoracic aortic lesions, allowing a one-stage repair and, when necessary, endovascular extension with a secure proximal landing zone. Early and late outcomes have been improving thanks to novel technologies and standardization of the surgical approach.

OUR “IDEA” ABOUT FET

Our experience with the FET technique started with the use of Jotec E-vita open prosthesis, but some years later, in 2014, we added the Thoraflex hybrid prosthesis to our “armamentarium”. With the introduction of

this new hybrid prosthesis, we expanded the indications for the FET technique also to type A and B acute aortic dissections. In this field, with the increased complexity of the cases, we began to “proximalize” the open distal anastomosis to arch zone 2 with a sensible reduction of the median visceral ischaemia time (42 min in zone 2 *vs.* 54 min in zone 3). In our opinion, this could depend on the fact that in the zone 2 group we used more frequently the Thoraflex hybrid prosthesis, allowing an earlier antegrade reperfusion through its side branch. In addition, zone 2 makes the distal anastomosis easier and faster, especially in the case of reoperations or emergency cases, so we recommend it. Furthermore, the presence of the side branches allows to perform an individual arch vessel reconstruction instead of the island technique and to restart the reperfusion in an antegrade fashion. In fact, we found that achieving an antegrade reperfusion when possible is preferable, especially in the case of chronic aneurysm. For this reason, when we use E-vita open, we create a fenestration on the vascular Dacron portion of the graft in order to antegradely reperfuse through a “Y” line for at least 10 min at the end of the distal anastomosis before reimplanting the left subclavian artery, which can be performed following discontinuation of the circulatory arrest, during the reperfusion-phase. In our experience, the Thoraflex hybrid prosthesis was used more frequently in arch zone 2 for acute dissections. In fact, we believe that a stent graft length of approximately 100 mm is sufficient to stabilize the intimal flap and to favour expansion of the true lumen in the down-stream aorta, also because a large intimal tear is often located near the left subclavian artery and can be easily excluded. The use of a shorter stent graft is also another key factor in order to reduce the rate of spinal cord injuries, especially in acute aortic dissection. This is because a shorter stent graft covers a shorter segment of the aorta and therefore fewer intercostal arteries are closed. On the other hand, to achieve a single-stage treatment in case of chronic aneurysm of the thoracic aorta, the use of a shorter stent graft, such as with Thoraflex hybrid prosthesis, is not always possible because the distance from zone 2 to the distal end of the aneurysm does not allow a perfect sealing of the stent. Therefore, in such cases, we suggest to perform the distal anastomosis in zone 3 or to use a longer stent such as the E-vita open prosthesis (130-160 mm).

DECLARATION

Authors' contributions

Substantial contribution to the concept and design of the study, data analysis and interpretation: Di Marco L, Votano D, Pacini D

Data collection, administrative, technical, and material support: Leone A

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflict 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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Risk of aortic dissection in patients with ascending aorta aneurysm: a new biological, morphological, and biomechanical network behind the aortic diameter

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Abstract

Thoracic aortic aneurysm represents a deadly condition, particularly when it evolves into rupture and dissection. Proper surgical timing is the key to positively influencing the survival of patients with this pathology. According to the most recent guidelines, ascending aorta size ≥ 55 mm and a rate of growth ≥ 0.5 cm per year are the most important factors for surgical indication. Nevertheless, a lot of evidence show that aortic ruptures and dissections might occur also in small size ascending aorta. In this review, we sought to analyze a new biological and morphological network behind the aortic diameter that need to be considered in order to identify the portion of patients with thoracic aortic aneurysm who are at increased risk of aortic complications, despite current aortic guidelines not advising surgical intervention in this group.

Keywords: Ascending aorta aneurysm; ascending aorta size; aortic dissection; genetic risk factors; morphological aspects; surgical indication for aortic repair



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INTRODUCTION

The two most widespread diseases of the thoracic aorta are aneurysms (TAA) and dissections (TAD)^[1]. In the United States, TAA is the 18th most common cause of death. TAA has an incidence rate of 10 cases per 100,000 patients per year and a prevalence of 0.16% to 0.34% in the general population^[2,3]. Men are more like to have TAA compared to women; however, women tend to develop worse clinical outcomes and have an increased risk of TAD^[4]. It is important to closely monitor TAA patients. At the same time, optimal surgical timing is crucial to improve survival. Cardiac surgery aims to prevent TAD or rupture of the aneurysm^[5]. As a predictor of adverse aneurysmal outcomes, aortic diameter is still the most used criteria^[6-9]. However, several studies have found that in a particular group of patients, complications may occur at smaller aortic sizes than we would predict^[10-12]. In our opinion, it is necessary to investigate other parameters that better identify these high risk TAA patients for which earlier surgical intervention is necessary and at smaller aortic size^[13]. The aim of this review is to analyze the biological, morphological, and biomechanical network as a potential useful tool to detect TAA subjects at higher risk of complications behind the diameter.

Role of the ascending aorta diameter in predicting acute aortic dissection

The in-hospital mortality rate of TAD is nearly 30%^[14]. Until now, the only prevention is monitoring of the ascending aorta dilation and performing prophylactic surgical replacement. Although hypertension and specific genetic syndromes are well known risk factors of TAD, it is still difficult to predict this deadly condition with accuracy^[15,16]. Current guidelines recommended surgery when the ascending aorta size reaches 5.5 cm for non-syndromic patients and 4.5 cm in syndromic patients^[17]. However, data from the International Registry of Acute Aortic Dissections^[18] showed that aortas could dissect at smaller sizes than that advocated in the guidelines. Among 591 type A TAD, 59% occurred at sizes less than 5.5 cm and 40% occurred at < 5.0 cm. These data correspond with our center's experience. Among 326 patients treated for Type A TAD in our Cardiac Surgery Department from April 2005 to March 2018, 212 patients had a maximal diameter less than 5.5 cm^[19]. Svensson *et al.*^[20] showed that 12.5% of 40 bicuspid aortic valve (BAV) patients with TAD had aortic sizes < 5 cm at the time of surgery. The same aortic diameter has been detected in Marfan population. In addition, several studies have showed that the aortic diameter before TAD is much smaller than after TAD. In experimental studies of human and porcine cadaver specimens, Williams *et al.*^[21] showed that the onset of TAD caused a significant increasing of the aortic diameter (140%) in relationship to the hydrostatic pressure and to the percentage of the dissected aortic wall. Neri *et al.*^[22] calculated pre-dissection aortic size from surgical specimens withdrawn from 220 individuals who underwent surgery for acute type A TAD. Using a specific explant technique, they performed cylinders of fresh aortic tissue and measured the inner layer of the true aortic lumen in the absence of perfusion pressure. The median ascending aorta size was 41.4 mm for the entire cohort. These authors concluded that that only 10% of the study population had aneurysms before TAD onset. It is very important to remember that looking only at the number of people operated for TAD with small diameter is not sufficient to determine the relative risk of TAD at sizes < 5.5 cm. That number has to be put into context by knowing how many people at those smaller diameters exist so that an actual risk can be determined. Accordingly, Paruchuri *et al.*^[23] calculated the relative risk of TAD at sizes < 5.5 cm by analyzing both the number of occurring dissections (numerator) and the population at risk at each aortic size (denominator). They found that in the general population a large percentage of subjects (79.2%) had an aortic diameter < 3.5 cm and only the 0.22% of subjects had an aortic diameter ≥ 4.5 cm. Yet, while the majority of TAD may occur at aortic diameters below the surgical threshold, it is also true that the vast majority of aortas within this population are considerably smaller than this threshold. Thus, the true statistical risk of TAD at small aortic diameters may well be negligible given the anticipated enormous patient pool in the small aortic size range. However, there is a group of patients in which TAD may occur at smaller aortic sizes than the guidelines predicted. This questions the true prognostic value of the absolute aortic diameter and emphasizes the need for optimal timing of surgical intervention, especially in those patients under surveillance who do not meet

established size criteria for surgery but may still be at significant risk of TAD. Accordingly, Davies *et al.*^[24] showed in 2006 that indexing absolute aortic diameter to anthropometric measurements provides individualized risk classification in patients with TAA. These authors introduced the concept of aortic size index (ASI), defined as aortic size/body surface area, as a predictor of aortic dissection, rupture, and death. In particular, they termed low risk patients as those with an $ASI \leq 2.05 \text{ cm/m}^2$. Moreover, weight fluctuates throughout the lifespan and can be deliberately influenced. Unlike weight, height does not change during adult life. Therefore, height-based relative aortic measures may be a more reliable long-term predictor of risk. For this reason, Zafar *et al.*^[25] in 2018 introduced the concept of aortic height index (AHI), defined as aortic size/height; and they assessed that AHI is as good as the ASI for risk stratification. They defined low risk patients those with an $AHI \leq 2.43 \text{ cm/m}$. In addition, Acharya *et al.*^[26] introduced the concept of *aortic area/height ratio* (IAAs) that was calculated indexing the aortic area ($\pi \times \text{aortic radius}^2$) to the patient height and correlating it with the absolute aortic diameter. According these authors, a IAAs $> 10 \text{ cm}^2/\text{m}$ could be considered the limit for early and proactive surgery to prevent TAD.

New evidences behind the diameter

Beside the aortic diameter, there is need to analyze other aspect of TAA that could better identify patients in which aortic complications might occur at smaller aortic sizes than guidelines predict. In our opinion, there are specific biological, morphological, and biomechanical markers of early rupture and dissection that must be investigated in order to prevent deadly complication. In particular, in this review we focused our attention on: (1) specific gene mutations that confer an increased risk for adverse outcomes, even at small or normal aortic size; (2) histomorphological change and the quality of the aortic wall at the time of the operation; (3) morphological markers of rupture and dissection in aortic root and ascending aorta; and (4) flow abnormalities and the aortic wall shear stress.

Genetic features of thoracic aorta aneurysms

Recent progress in the understanding the pathophysiology of TAA have produced evidence suggesting different molecular pathways and their genetic variants as potential biomarkers of TAD, which might be applied into TAA clinical management in order to prevent deadly complications^[27-29]. These specific gene mutations are reported to induce an increased risk for adverse outcomes, even at small or normal aortic size^[30,31]. The most interesting aspect is that this genetic risk is characteristic not only of syndromic patients but also of non-syndromic patients [Figure 1]. The three main genetic syndrome associated with TAA are: Marfan syndrome^[32] (mutations in the fibrillin-1 gene) [Figure 2]; Ehlers-Danlos syndrome^[33] (mutations in COL3A1), and Loeys-Dietz syndrome (mutations in TGF β R1 or TGF β R2)^[34]. It has been recognized that aortic dissection in Marfan syndrome patients can occur also at smaller sizes, therefore we recommend early intervention. The non-syndromic TAA are divided into sporadic TAA and familial TAA. In familial TAA, one or more family members are affected by TAA. Sporadic TAA is characterized by sudden onset and no family history of aneurysm. On the other hands, many genes have been associated to familial TAA^[35] [Figure 3]. Interestingly, recent evidences showed that the immune system and inflammatory related genes have an important role in the onset and progression of sporadic TAAs even at small aortic sizes. Among these inflammatory mediators, the Toll-like receptor 4 (TLR-4) is one of the most important player^[36-38]. The activation of TLR-4-mediated signaling pathway, both on endothelial cells (ED) and vascular smooth muscle cells (VSMCs)^[39,40], could determine the deregulation of angiotensin converting enzyme (ACE)^[41-43], nitric oxide (NO)^[44], metalloproteinases (MMP)^[45-48] associated with endothelium dysfunction, extracellular matrix remodeling, and chronic inflammation causing medial degeneration in sporadic TAA [Figure 4]. Evans *et al.*^[49] discovered that the interaction between TLR-4 and NO is one of the most important mechanisms by which aorta-derived mesenchymal progenitor cells activate the immune and inflammatory cells. The increasing inflammation induces sporadic TAA onset and progression. Li *et al.*^[50] reported the importance of TLR-4-mediated signaling pathway in regulating the metalloproteinases-9 (MMP-9) expression in human aortic smooth muscle cells. Increased MMP-2 and

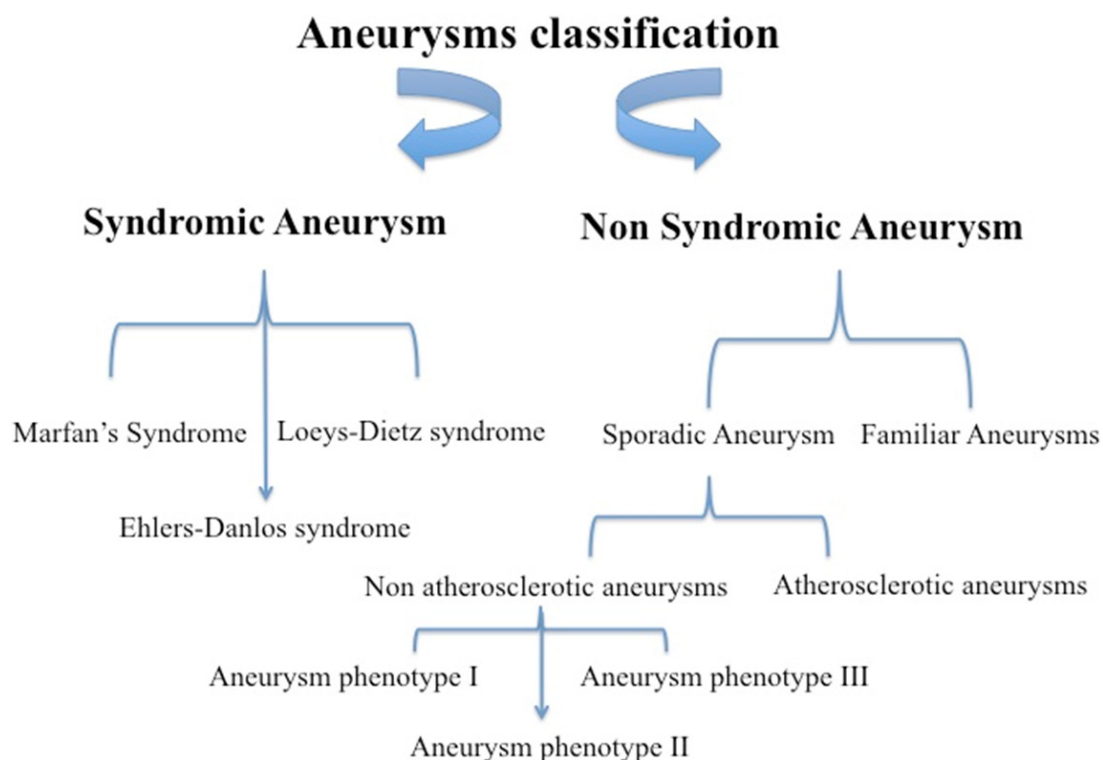


Figure 1. Aneurysm classification

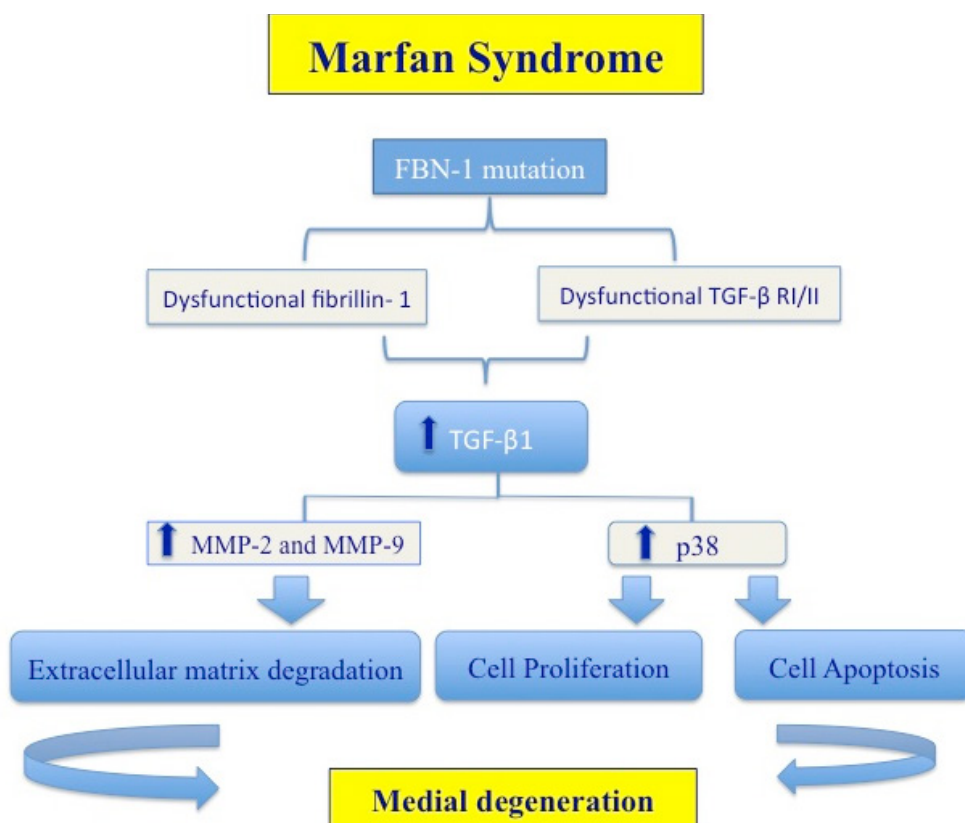


Figure 2. Marfan syndrome pathogenesis and related genes

Principal Genes involved in Familial Aneurysms

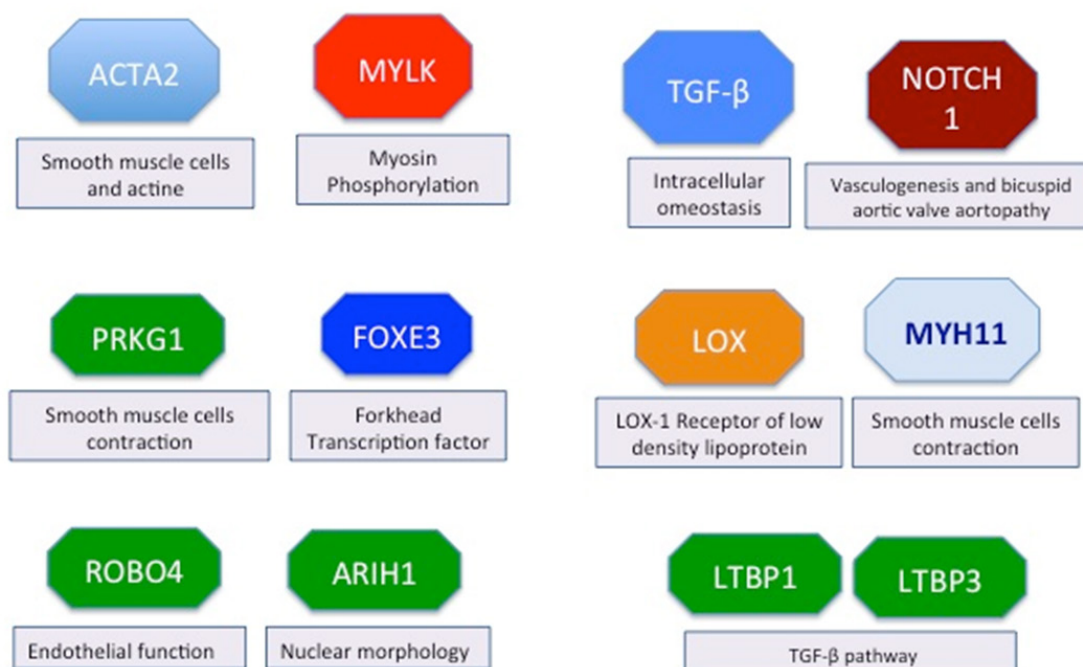


Figure 3. Principal genes involved in familial aneurysms

Pathogenesis of Sporadic Ascending Aneurysms

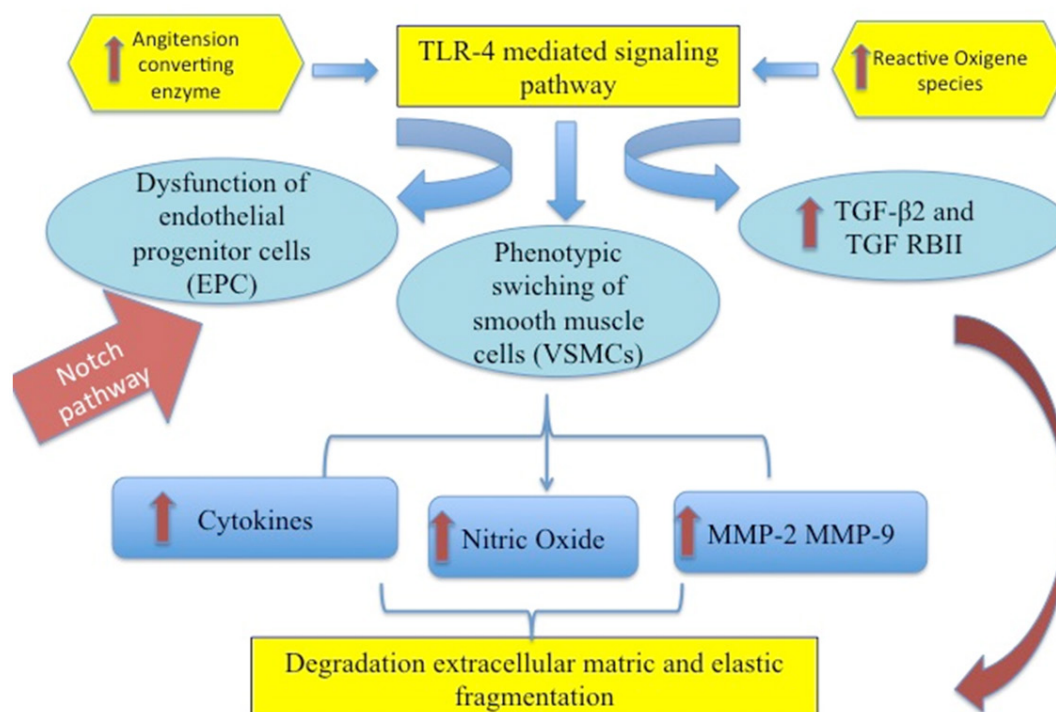


Figure 4. Sporadic ascending aneurysm pathogenesis and related genes

MMP-9 expression induced an increase in proteolysis in TAA and TAD as compared to the normal aorta. Finally, the TLR-4-mediated pathways seems to influence the activity of two important genes involved in the onset and progression of TAA: transforming growth factor- β (TGF- β)^[51] and Notch^[52,53]. Different roles of TGF- β pathways in tissue remodeling mechanisms have been reported in both syndromic and sporadic TAA. The activation of TGF- β results in an increase in extracellular matrix degradation through MMPs activation and multiple cytokines upregulation including interleukin-10. Additionally, a loss of function of the TGF- β receptors (TGFBR1 and TGFBR2) has been associated with both familial syndromic and non-syndromic TAAs. Furthermore, mutations in Notch gene homolog 1 (Notch1) and Notch1 pathway, typically associated to TAA patients with BAV, seem to regulate TGF- β cascade. In the aorta, the Notch pathway appears to regulate the differentiation of vascular smooth muscle cells, the most representative cells involved in aneurysmatic pathology. Finally, a very recent study^[54] revealed the important role of the phosphodiesterase 5A (PDE5) gene mutation in human aorta and thoracic aortic aneurysms. Affected aortas showed lower levels of all the PDE5A isoforms compared to control aortas. Because PDE5 is expressed early during human aorta development, the study revealed an association between PDE5 gene mutation and anomalous aortic development.

Impact of histopathological changes in thoracic aortic diseases and genetic biomarkers of risk

One of the most important aspect to consider in order to optimize surgical indications in TAD is the severity of medial degeneration of the aortic media and, consequently, the fragility of the aortic wall. Previously, we observed that the severity of aortic media degeneration in TAD and TAAs are not related to the diameter of the aneurysm^[55-58]. In atherosclerotic degenerative aneurysms (ADA), the grade of medial degenerative lesions was balanced to the grade of substitutive medial fibrosis. In contrast, in non-atherosclerotic degenerative aneurysm (NADA) and TAD, medial fibrosis was absent or of grade I. The relative absence of restorative fibrosis should predispose patients to aortic rupture. In particular, our study showed that TAD has the same histological and immunohistochemical features as NADA phenotype III: elevated medial cystic degeneration without replacement fibrosis, with plurifocal medial apoptosis, and strong collagenase concentration. Additionally, NADA phenotype III showed a very fragile aortic wall at the time of the operation. The morphological identity of the medial lesions observed both in NADA phenotype III and in the samples of patients with TAD, could be considered the precursor - and consequently the optimal biomarker - of the dissection, regardless of the diameter of the aneurysm or valve disorder. This evidence agree with recent studies that showed that up-regulation of metalloproteinases, related to inflammatory processes or genetic aspects, might affect the formation of TAA and TADs^[59].

In order to identify patients with small to moderate sized aneurysms and at high risk of developing TAD, Balistreri *et al.*^[55] investigated the genetic biomarkers specific for TAA phenotype III. Investigations were made into the potential role of 10 common and functional single nucleotide polymorphisms (SNPs) of the following genes: CCR5 (C-C chemokine receptor 5), TLR4 (toll like receptor 4), MMP-9 (metalloproteinase-9), MMP-2 (metalloproteinase-2), ACE (angiotensin-converting enzyme), and eNOS (endothelial nitric oxide synthase). Indeed, highly significant associations were observed between -786T/C eNOS, D/I ACE, and -735C/T MMP-2 SNPs and the risk of TAD. The presence of these genotypes may induce the development of this disease through different mechanisms [Figure 5]. A relationship between ACE gene SNPs and arterial hypertension has been demonstrated in different populations. At the same time, chronic systemic hypertension is considered to be the most common predisposing factor for TAD, with unfavorable effects on the vascular system such as cellular apoptosis, the production of reactive oxygen species, and vascular matrix MMP synthesis (particularly of MMP-2 and -9). In addition, it is known that molecules such as NO are involved in several pathways for the maintenance and regulation of a healthy intimal endothelium. Different SNPs of the eNOS enzyme have been found to vary in the expression and tissue levels of NO. In particular, -786 T/C eNOS reduced the transcriptional activity by around 50%, leading to a reduction in eNOS tissue endothelium levels that may result in reduced NO

Prolapse and asymmetry of the sinus of Valsalva

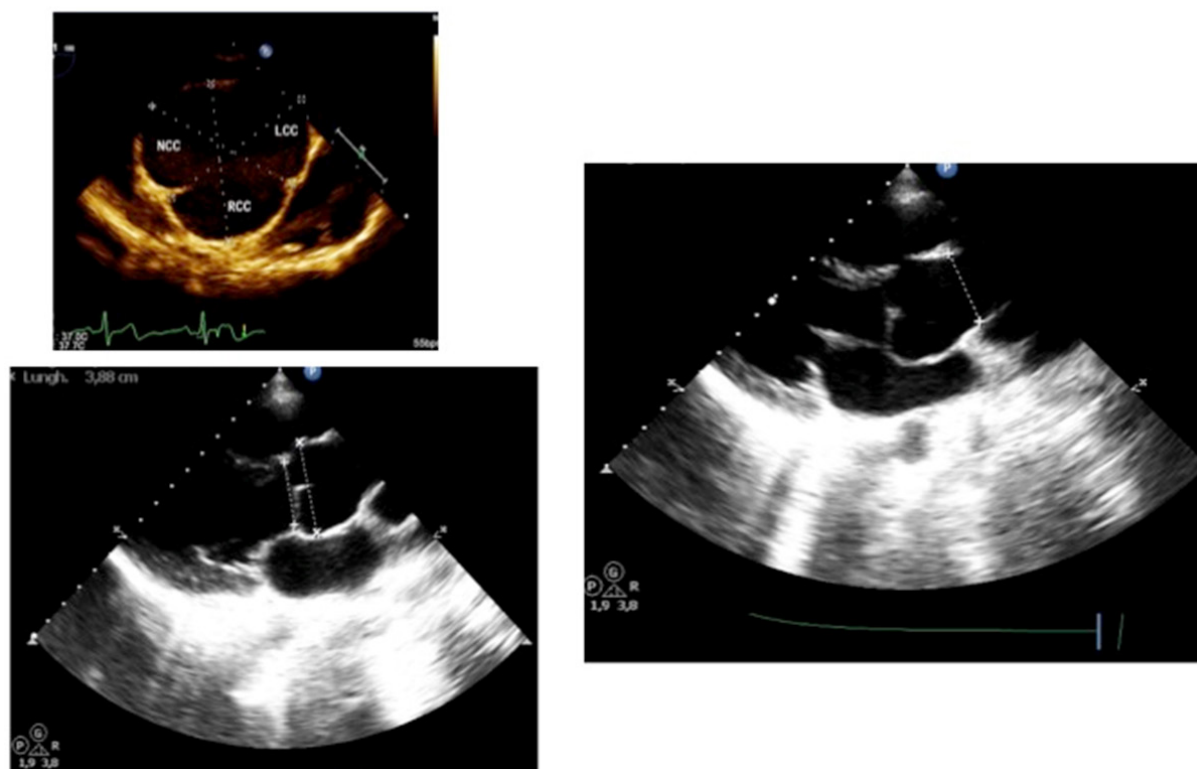


Figure 5. Prolapse and asymmetry of the sinus of Valsalva

production and consequently endothelial dysfunction and activation of the stretch pathway with the release of molecules, such as MMPs. Furthermore, a strong relationship between hypertension and increased and altered activity of MMPs (particularly MMP-2 and -9) and aortic wall remodeling has been reported. Among these, -735C7T MMP-2 SNP is associated with a threefold increase in MMP-2 levels and seem to be associated with hypertension, aortic remodeling, and aortic fragility, and consequently with aortic diseases such as aneurysm and dissection. Hence, the determination of D/D ACE, -735 T/T MMP-2, and -786 T/T eNOS genotypes might contribute to a prediction of the development of TAD in patients with S-TAA, independent of the aneurysm size.

Morphological markers of rupture and dissection in ascending aorta aneurysm

Beside the genetic and morphological aspect, in our opinion, there are specific morphological markers of rupture and dissection in TAA that is necessary to consider for surgical indication beyond the diameter. This reflection arises from our single operator surgical experience. From December 2003 to January 2020, a surgeon in our Cardiac Unit performed 320 Bentall de Bono operations (254 isolated procedure; 66 cases Bentall procedure associated with other cardiac surgery). We treated both sporadic aneurysms (287 patients) and syndromic aneurysms (33 patients). The in-hospital mortality for isolated procedure was 1% (from 2003 to 2014) and 0.8% (from 2015 to 2020). The in-hospital mortality for combined procedure was 3% (from 2003 to 2014) and 2.8% (from 2015 to 2020). In all these cases, our surgical indication was based not only on the diameter (≥ 5.0 cm for sporadic TAA and ≥ 4.5 for syndromic TAA) but also on certain morphological aspects such as: prolapse and asymmetry of sinus of Valsalva (mostly the non-coronary sinus) [Figure 5]; asymmetric ascending aorta dilatation [Figure 6]; aortic-ventricle disjunction [Figure 7]; arising of the epiaortic vessels from the convexity of the ascending aorta; ascending aorta length^[60]; aortic volume.

Asymmetric ascending aorta dilatation

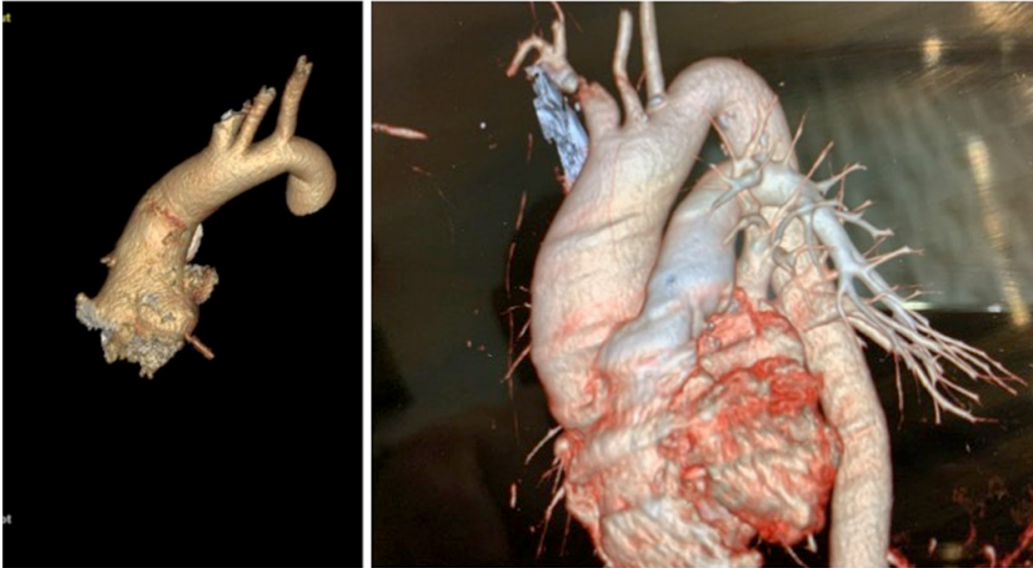


Figure 6. Asymmetric ascending aorta dilatation

Aortic-ventricle disjunction

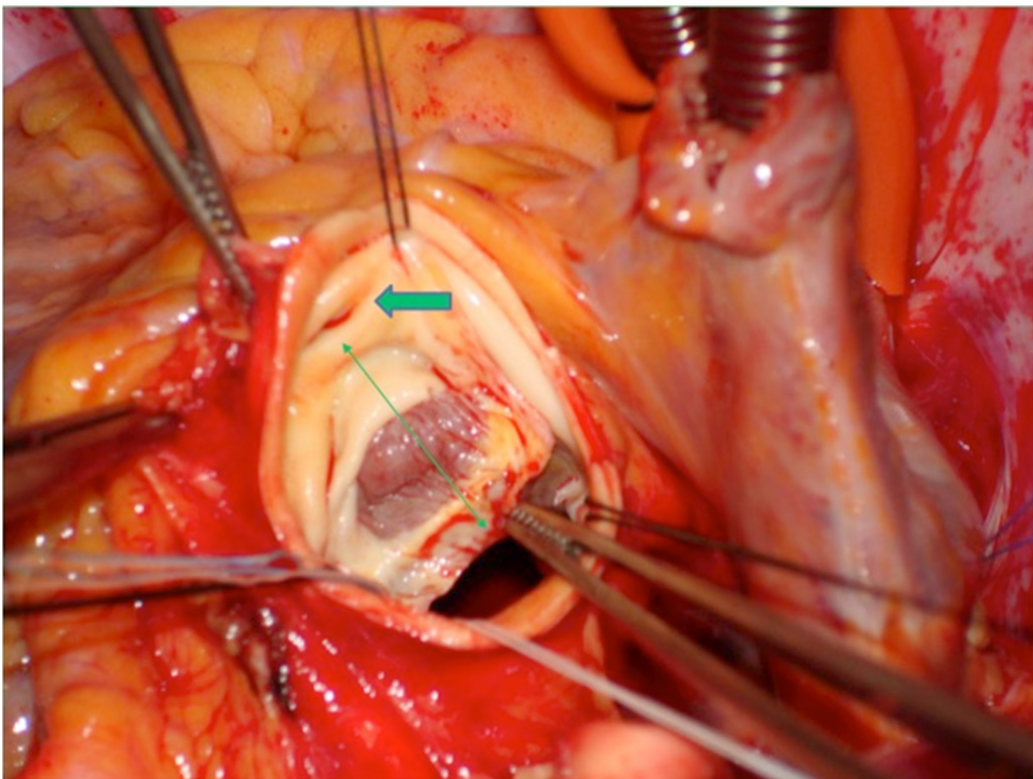


Figure 7. Aortic-ventricle disjunction

In a recent and interesting paper, Wu *et al.*^[61] focused the attention on the longitudinal changes of the TAA. They measured the *ascending aortic length* (AAL) from the aortic annulus to the origin of the innominate artery using CT scan images. Interestingly, an AAL of ≥ 13 cm was associated with almost 5-fold higher average of aortic adverse events. In addition, Heuts *et al.*^[62] assessed that measurements of aortic volume and length have superior diagnostic accuracy compared with the maximal diameter and could improve the timely identification of patients at risk for TAD.

However, we are aware that to validate our opinion and to confirm the importance of these morphological parameter for surgical indication, a multicentric study is needed.

Flow abnormalities and shear stress

Finally, other important aspects to consider are flow abnormalities and wall shear stress (WSS) in TAA. Beside the genetic aspects, hemodynamic factors play a crucial role in TAA onset and progression through the endothelial dysfunction^[63]. Endothelial cells, in fact, line the lumen of blood vessels and they are at the interface between hemodynamic forces and vascular wall biology. Endothelial cells transduce mechanical and biological signals from blood flow into intracellular signals cascades through a process called mechanotransduction^[64] which leads to inflammation and pathological conditions such as aneurysm and dissection. The endothelial dysfunction induces a switch in phenotype of smooth muscle cells and fibroblasts. These cells start to synthesize metalloproteinases and inflammatory pathways involved in the elastic fragmentation and medial degeneration causing aneurysm and finally dissection.

Several studies have been focused on WSS related to BAV patients with aortopathy. Barker *et al.*^[65] found that WSS in the ascending aorta of patients with BAV was significantly elevated compared to healthy volunteers. Different phenotypes of BAV have been described according the cusps fusion (right-left; non-coronary left; non-coronary right) associated with different grade of WSS. In particular, BAV with fusion of the right and non-coronary cusps (non-coronary right phenotype) seems to have to higher WSS and a greater risk of TAD^[66]. Additionally, it is evident that the WSS distribution is different according the BAV phenotype. Mahadevia *et al.*^[67] described elevated WSS in the right-anterior wall of the ascending aorta for right-left BAV phenotype, and right-posterior wall for non-coronary right BAV phenotype. In all cases of BAV associated with aortopathy, the WSS is higher at the greater curvature of the ascending aorta. Accordingly, Della Corte *et al.*^[68] found that medial degeneration was more severe in this region. Furthermore, Guzzardi *et al.*^[69] has shown a direct association between WSS and histological alteration of the aortic wall in TAA patients. BAV patients undergoing ascending aorta replacement had pre-operative WSS mapping. In particular, they showed high levels of TGF β -1, MMP-1, MMP-2, and MMP-3 in high WSS regions causing severe elastic fiber degeneration and extracellular matrix degradation, two important mechanisms underlying TAA progression and TAD onset^[70]. This may be the explanation why some patients with aortic size below current intervention criteria develop acute aortic complications.

CLINICAL PRACTICE

Many different options are available to be used as criteria for determining when to operate on patients with aortic aneurysm, but it remains to be seen which ones will be most predictive of TAD. The identification of TAA patients with a high risk of TAD is very difficult in clinical practice. In the evaluation of TAA patient, we thought that the quantification of the absolute aortic diameter is not enough to decide the optimal surgical timing. It is necessary to perform specific and multiple evaluations. First of all, the absolute aortic diameter to anthropometric measurements are needed to calculate the ASI, AHI, and IAAs. At the same time, it is necessary to perform an imaging analysis of the TAA to identify markers of rupture and dissection in the aortic root and ascending aorta (e.g., prolapse and asymmetry of sinus of Valsalva, asymmetric ascending aorta dilatation, aortic-ventricle disjunction, and arising of the epiaortic vessels from the convexity of the ascending aorta). Yet, it is necessary to evaluate the aortic length and the aortic

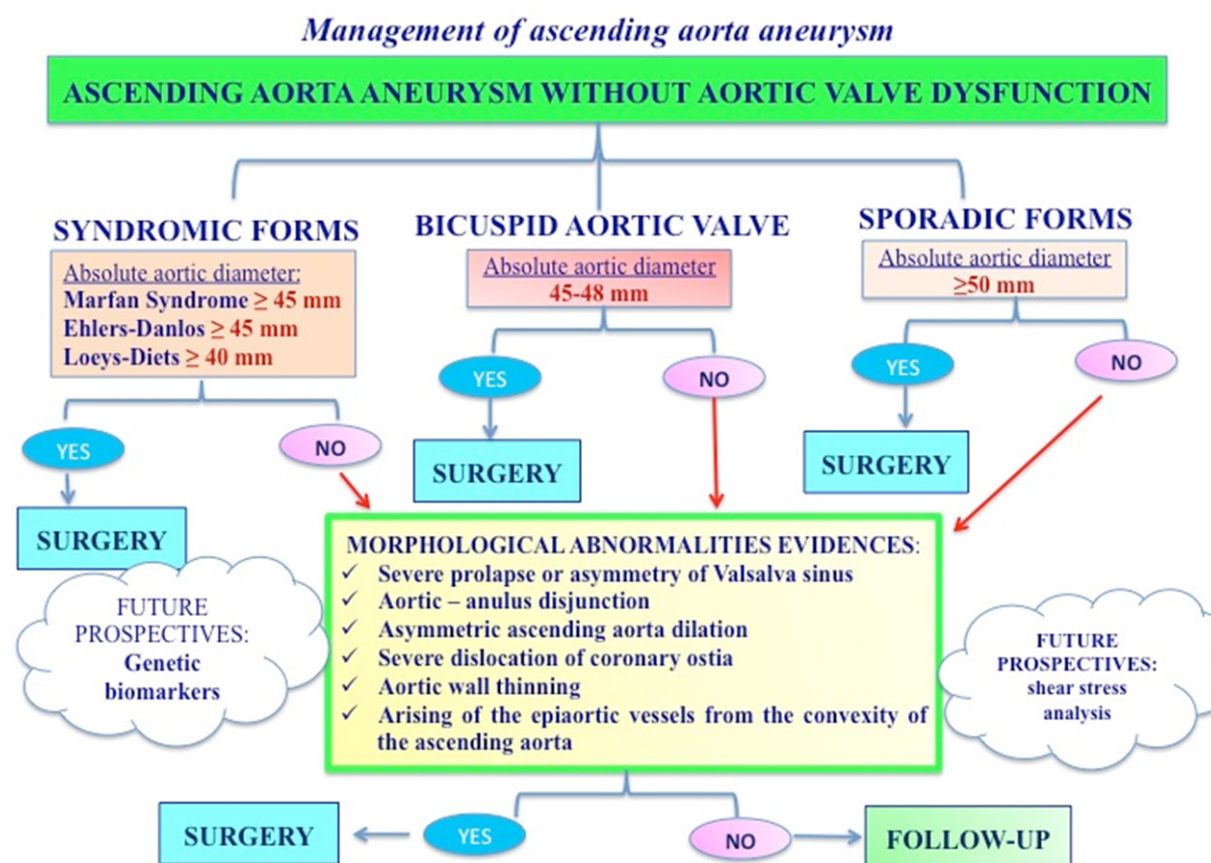


Figure 8. Management of patient with ascending aorta aneurysm without aortic valve dysfunction (Flow Chart). If surgery is indicated for the aortic valve disease, ascending aorta/ aortic root replacement must be performed according with morphological abnormalities despite the aortic size

volume. The morphological analysis could be integrated with a biomechanical evaluation using MRI or positron emission tomography. Finally, the patient evaluation must be completed performing a blood test in order to identify a particular genetic risk profile (*D/D ACE*, *-735 T/T MMP-2*, or *-786 T/T eNOS*) that could confer a particular phenotype of aneurysm (phenotype III) to non-syndromic patients and that phenotype evolves earlier to rupture or dissection despite the small diameter of the aorta. In these cases, surgeons should consider operating earlier and at smaller diameter [Figure 8]. Further studies comparing the predictive value of these many parameters would be necessary to help us decide which ones should be used in regular clinical practice.

CONCLUSION

The decision-making process of treatment in thoracic aortic aneurysms of the ascending aorta is complex, both as regards to the timing of the intervention and the treatment strategy. From the clinician's point of view, it is important to balance the risks of vigilant waiting with respect to preventive surgery and choosing a surgical treatment strategy that translates into the least number of early and late events. Preventive surgery of the aorta on the basis of the aortic size alone remains controversial among the patient population without known risk factors for dissection. Other markers, including histopathological phenotypes, genetic factors, morphological aspects, and flow abnormalities should be used as an appropriate surgical indication to prevent catastrophic complications.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Pisano C, Balistreri CR, Ruvolo G

Perfomed data acquisition, as well as provided administrative, technical, and material support: Nardi P, Altieri C, Bertoldo F, Buioni D, Ferrante MS, Asta L, Trombetti D

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Review

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Congenital arch malformation: a review of morphology and surgical management of circumflex aortic arch

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Abstract

Circumflex aorta is an unusual form of congenital arch malformation with a retroesophageal arch segment. Circumflex aorta crosses the midline behind the oesophagus and above the level of carina to become continuous with the proximal descending aorta on the opposite side to form a true vascular ring with the arterial ligament. The term is often used to refer to the more common variant of circumflex aorta with a retroesophageal right-sided aortic arch with a left-sided descending aorta and left-sided ligamentum. Its mirror-form with a retroesophageal left-sided aortic arch is much rarer. Although originally described without obstruction, it may occur in association with aortic arch hypoplasia, adding to its clinical burden. This article describes the morphology of circumflex aorta and its clinical presentation, and provides review of surgical management of circumflex aortic arch.

Keywords: Congenital, arch malformation, morphology, circumflex aorta, surgery

INTRODUCTION

Circumflex aorta is an unusual form of congenital aortic arch anomaly with a retroesophageal arch segment. The term is most commonly used to refer to an aorta that passes to the right of the trachea (i.e.,



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a right aortic arch), which crosses the midline behind the oesophagus above the airway carina, to become continuous with the proximal descending thoracic aorta on the opposite side^[1,2]. In the majority of cases an associated Kommerell's diverticulum with an aberrant left subclavian artery also gives rise to a left-sided arterial duct, which forms a complete vascular ring. Simple vascular ring division may not fully address all the deleterious effects of circumflex aorta, and a major aortic arch uncrossing procedure may be required^[1-4]. The aortic arch is often unobstructed or presents with variable degrees of obstruction from hypoplasia to near-interruption requiring major arch repair^[5-7]. When there is also obstruction of the proximal aberrant subclavian, it can be confused clinically with aortic dissection in adult patients^[8]. The mirror image of the usual form of circumflex aorta, with left aortic arch and right descending aorta, is very rare, and may require a different surgical approach^[7,9].

MORPHOLOGY AND EMBRYOGENESIS

Although the term circumflex aorta was used in the literature as early as 1960, morphological arch descriptions similar to circumflex aorta can be traced back to late 19th century^[10-14]. Circumflex aorta with a right aortic arch is much more common than with a left aortic arch^[13,15].

Right aortic arch

A circumflex aorta with a right aortic arch refers to a retroesophageal right aortic arch, a left-sided descending thoracic aorta, and a left-sided ligamentum arteriosum. This aortic arch passes over the right main bronchus to the right of trachea and oesophagus. It then crosses the midline behind the oesophagus, and anterior to the spine in the upper mediastinum or above the level of the carina, to join the proximal end of left sided descending aorta. Usually the distal portion of the embryological left dorsal aortic arch forms a Kommerell's diverticulum which gives rise to an aberrant left subclavian artery. A left-sided arterial duct or ligament connects the base of the aberrant subclavian artery and the proximal left pulmonary artery, thus forming a complete vascular ring^[10,12]. In usual circumflex aortic arch, the order of origin of the arch vessels is: (1) left common carotid artery; (2) right common carotid; (3) right subclavian; (4) from the proximal descending aorta, an aberrant left subclavian artery [Figure 1].

Left aortic arch

A circumflex aorta with a left aortic arch is a rarer variant of this anomaly and was first reported by Paul (1948). It mirrors the circumflex aorta with right aortic arch: left-sided aortic arch, right-sided descending aorta and right-sided arterial duct or ligament^[7,11,15-22]. The retroesophageal transverse arch will cross the midline from left to right.

The head and neck arteries arise sequentially as follows: (1) right common carotid; (2) left common carotid; (3) left subclavian, and from proximal descending aorta; (4) an aberrant right subclavian artery [Figure 2].

Retroesophageal transverse arch

A retroesophageal transverse arch, which crosses the spine at T2-T3 level above the tracheal carina is the hallmark of circumflex aorta. The proximal descending aorta is located contralateral to the aortic arch, giving rise to the characteristic course of a circumflex arch. The thoracic descending aorta can course back to the other side of the spine before it descends via the aortic hiatus at the diaphragm to form the abdominal aorta^[7,10,12].

Aberrant subclavian artery and Kommerell diverticulum

A circumflex aortic arch is associated with an aberrant subclavian artery where a bulbous dilatation can often be found at its origin. This subclavian artery arises aberrantly from the proximal descending aorta, rather than the aortic arch^[23]. It is at this base of aberrant subclavian where the arterial duct is connected to the pulmonary artery. After involution of ductus arteriosus, the arterial ligament remains connected

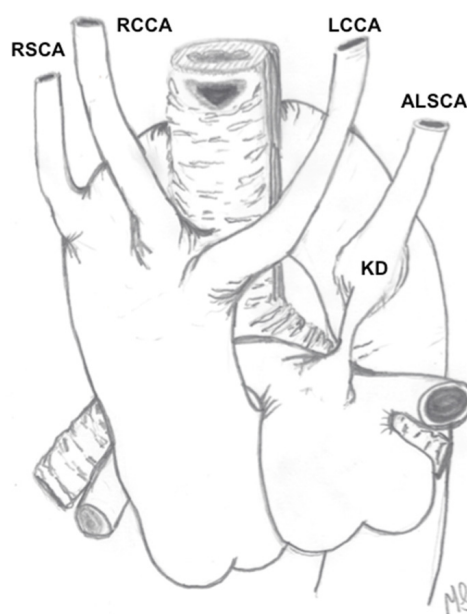


Figure 1. Circumflex aorta with retroesophageal right aortic arch, left sided descending aorta and left ductal ligament with the following branches: left common carotid artery (LCCA), right common carotid artery (RCCA), right subclavian artery (RSCA), and aberrant left subclavian artery (ALSCA) arising from Kommerell's diverticulum (KD)

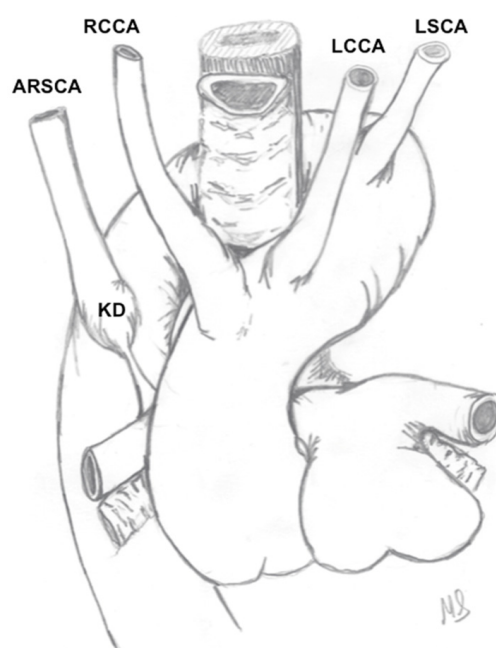


Figure 2. Circumflex aorta with retroesophageal left-sided aortic arch, right sided descending aorta and left-sided ductal ligament with the following branches: right common carotid artery (RCCA), left common carotid artery (LCCA), left subclavian artery (LSCA) and aberrant right subclavian artery (ARSCA) arising from Kommerell's diverticulum (KD)

to the base of this subclavian artery, forming a vascular ring. Named after Kommerell who described the anomaly as an incidental radiographic finding in 1936, this bulbous dilatation is a remnant of fourth dorsal aortic arch^[24-26]. A significant Kommerell's diverticulum is one which is at least 1.5 times the size of distal subclavian artery^[4]. Abnormal histology with cystic medial necrosis in the aortic diverticulum has been described in young children, which explains predilection to form an aneurysm with risk of rupture and dissection later in life^[24,27].

A Kommerell's diverticulum or aberrant subclavian artery is not a constant feature in all reported circumflex aortic arch. Instead of bulbous dilatation of the base of aberrant subclavian, stenosis and obstruction at its origin has also been described in circumflex aortic arch^[8]. However, this may represent a ductal tissue constriction that results in "coarctation" of the junction between the Kommerell's diverticulum and the origin of the embryological left subclavian artery. A subclavian with usual origin from the innominate trunk has also been described in circumflex aortic arch^[12,15].

Concomitant lesions

Aortic arch obstruction with a hypoplastic retroesophageal segment is the most important associated lesion. The retroesophageal arch segment is usually of adequate caliber, but significant hypoplasia can occur particularly with concomitant ventricular septal defect, which can mimic interruption^[6,28,29]. When arch hypoplasia is present, it can be tubular with a long segment, or tortuous, extending from the proximal to distal transverse arch as it courses around the side of the trachea and back of the oesophagus^[5-7,28]. A long hypoplastic retroesophageal segment can be mistaken as an Abbott artery which is an anomalous artery arising from the posterior wall of the aortic arch or others in coarctation of the aorta^[30-32].

Concomitant intra-cardiac lesions, commonly VSD, can also be present with circumflex arch^[6,14,33]. Other reported cardiac lesions include atrial septal defect and bicuspid aortic valve^[14,33]. Less commonly, Tetralogy of Fallot or double outlet RV, a lesion with a higher frequency of right aortic arch than in the normal population, had also been reported in circumflex aorta^[15]; as well as left heart hypoplasia^[5,34].

Vascular ring variants resembling circumflex aorta

Following repair of double aortic arch where the smaller left arch is divided [Figure 3A-C], the preserved retroesophageal arch segment will resemble a circumflex arch^[35]. A completely atretic segment [Figure 3D] can be present between the left common carotid and subclavian arteries in the setting of a double aortic arch with a dominant right^[36-38]. They are strictly speaking, by morphology criteria, a double aortic arch, but functionally resemble a circumflex arch with a high retroesophageal segment above the tracheal carina^[36]. In true circumflex arch, there is no connecting segment between these two neck vessels [Figure 3A]. Cross-sectional imaging does not differentiate between them.

A common variant of vascular ring is a right aortic arch with aberrant subclavian artery and Kommerell diverticulum and left sided arterial ligament (Neuhauser anomaly) [Figure 4]. It shares the same vascular arrangement, in terms of neck vessel origins, with a circumflex aorta, and the latter may be considered as an extreme form of this common variant of right aortic arch. However, there are some important differences as follows: (1) the retroesophageal component is the Kommerell's diverticulum; whereas in circumflex aorta, this aortic diverticulum is to the left of the oesophagus; and (2) the proximal descending aorta is on the right of the spine, i.e., the same side as the arch. Therefore, its transverse arch does not cross behind oesophagus. Both are closely related, but this common variant of right aortic arch is not functionally a circumflex aorta and is likely to have different embryogenesis.

Embryology

The developmental aetiology of the circumflex arch is unclear. Genetic associations with right arch include a higher prevalence of chromosome 22q11 microdeletions 3,8 and the syndromes that are now known to be associated with this genetic defect, such as DiGeorge, velo-cardio-facial syndrome and conotruncal anomaly face syndrome. The Rathke diagram [Figure 5], based on work of the renowned embryologist, Martin Heinrich Rathke (1793-1860) is classically used to understand the normal development of aortic arch and its branches^[39]. During the development of the normal aortic arch, there is involution of the right fourth and sixth aortic arches and of the right dorsal aorta (dorsal aortic root segment 8) distal to the right subclavian artery. In normal development, the dorsal aorta that persists is the one on the same side of the

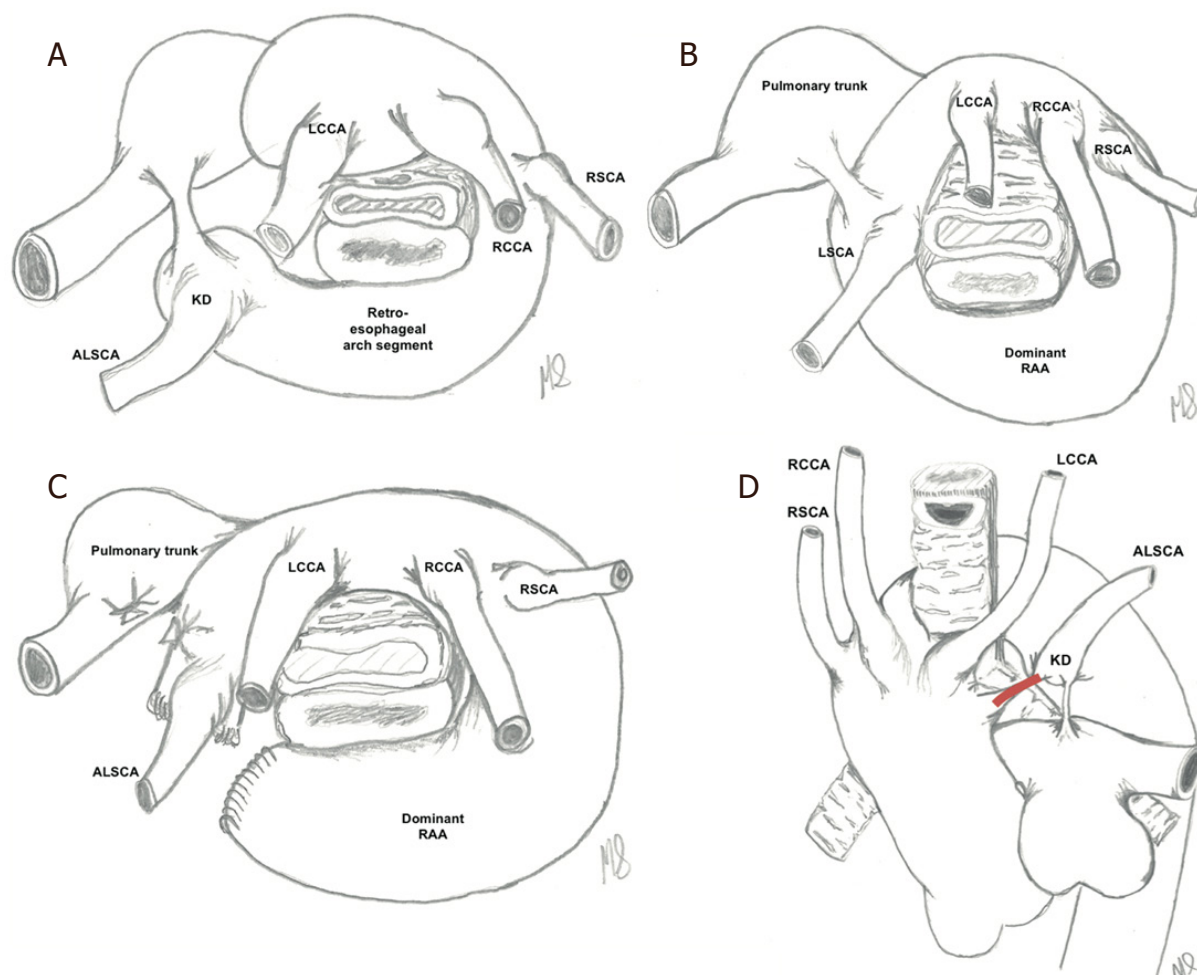


Figure 3. Comparison between A: circumflex aorta with right aortic arch; B: double aortic arch with dominant right aortic arch (dominant RAA) with right common carotid (RCCA) right subclavian artery (RSCA), left common carotid artery (LCCA) and the left subclavian artery (LSCA); C: post-surgical division of the non-dominant distal left aortic arch in a patient with double aortic arch; D: double aortic arch with atretic left segment marked in red with the aberrant left subclavian (ALSCA) arising from Kommerell's diverticulum (KD)

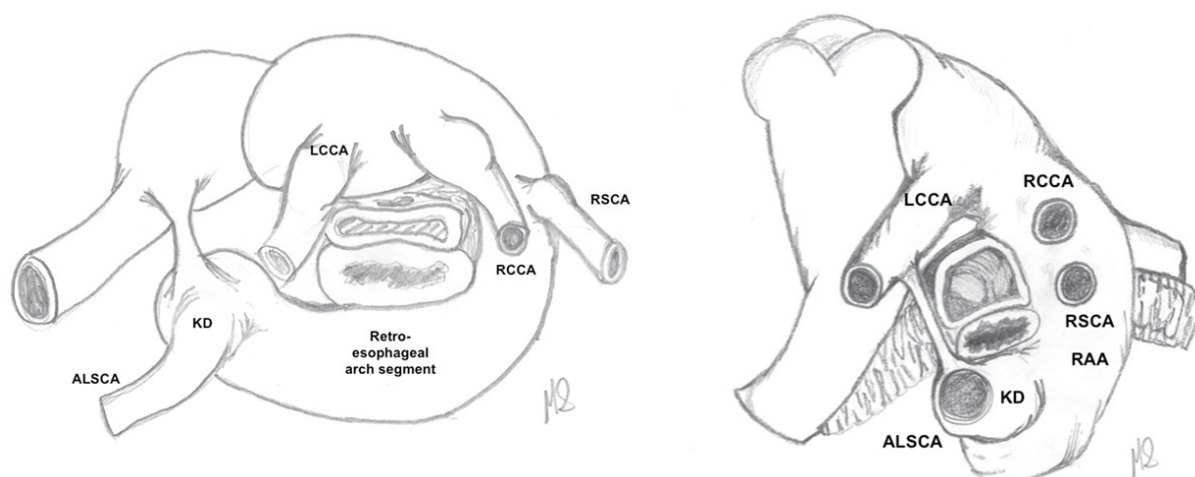


Figure 4. Comparison between circumflex right aortic arch and common variant of right aortic arch with retroesophageal aberrant left subclavian artery (ALSCA) arising from Kommerell diverticulum (KD). Head and neck vessels are left common carotid (LCCA), right common carotid artery (RCCA), right subclavian artery (RSCA), and the ALSCA

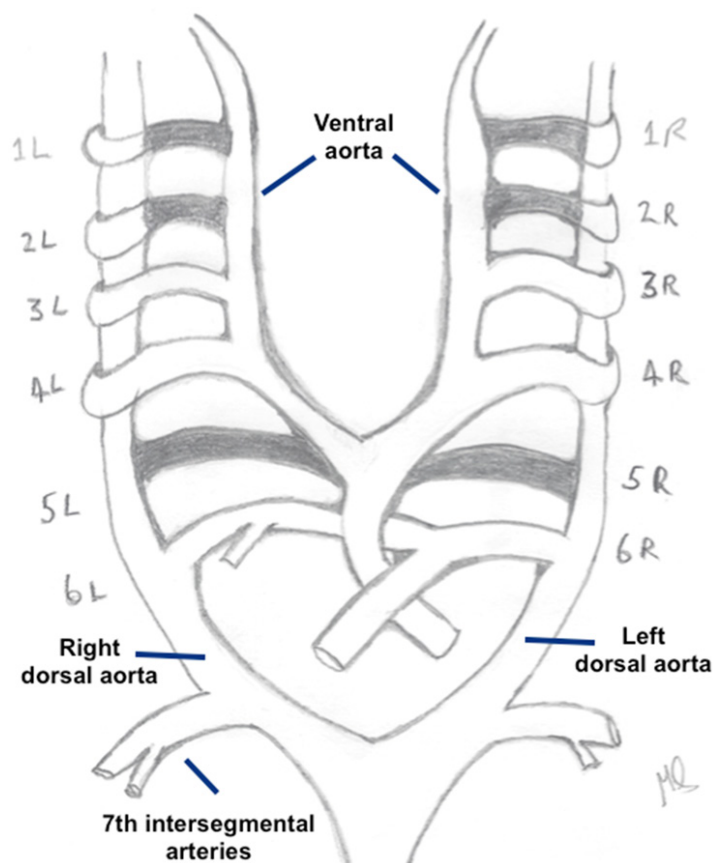


Figure 5. Classic Rathke's diagram representing embryological development of aortic arches

aortic arch; in circumflex aorta, the dorsal aorta contralateral to the aortic arch is the one that persists in life^[40]. Therefore, based on this hypothetical model, involutions that occur in circumflex aorta with right aortic arch are (1) the right sixth aortic arch; and (2) the left fourth aortic arch with persistence of the left sixth aortic arch and left dorsal aorta (dorsal aortic root segment 8). Alternatively, it may also share a common pathway with embryological formation of a cervical aortic arch or a double aortic arch^[5,12,41]. In the latter, if obliteration between the left common carotid and subclavian arteries occurs early in-utero, the segment may completely involute giving rise to a true form of circumflex arch at birth^[12].

As with other types of aortic obstruction, hemodynamic mechanisms resulting in reduced flow in the ascending aorta during fetal life may contribute to the development of arch hypoplasia^[5]. In addition, compression of the retroesophageal component between oesophagus and spine during fetal development may also contribute. The aetiology of hypoplasia, when it occurs, is probably not genetic but rather acquired as a consequence of changes in fetal flow patterns.

TERMINOLOGY

The adjective 'circumflex' describes a structure that is curved or bends around another structure, in this case, the aortic arch bending posteriorly around the oesophagus. The term circumflex aorta and circumflex aortic arch are also used interchangeably and both terms have been used in the surgical literature to refer to the much more common form of circumflex aorta with right aortic arch.

The term circumflex aorta or circumflex aortic arch should be used as a generic term to refer to a congenital arch anomaly with high retroesophageal arch segment above the tracheal carina with both the

proximal descending aorta and ductal ligamentum on the opposite side to the arch, to form a vascular ring. It only refers to the proximal segment of the descending aorta that is on the contralateral side of the aortic arch, and the subsequent descending aorta may or may not cross back to the other side of the spine. Not all retroesophageal arch is morphologically a circumflex arch, such as double aortic arch. A circumflex aorta can be described as a constellation of abnormal lie and development of both the aortic arch as well as the descending aorta, keeping in mind that the course of the descending aorta may have implications on therapeutic management strategies.

In describing circumflex aorta, the following need to be specified further: (1) the laterality of the arch - either “circumflex aorta with right aortic arch” or “circumflex aorta with left aortic arch” - (2) the presence of any aberrant subclavian artery; (3) the caliber of the arch - unobstructed, or degree of hypoplasia, e.g., “Circumflex aorta with right aortic arch with aberrant left subclavian artery - unobstructed”; “Circumflex aorta with left aortic arch with aberrant right subclavian artery - critical hypoplasia”. Such a morphological description will summarize the potential deleterious physiological effects of this anomaly on the patient: (1) a vascular ring that causes tracheal or esophageal compression; and (2) any degree of arch obstruction if present, which causes haemodynamic compromise.

PATHOPHYSIOLOGY AND CLINICAL PRESENTATION

The clinical manifestation of circumflex aortic arch is associated with pathophysiology that results from vascular compression; Kommerell's diverticulum; associated arch hypoplasia and intra-cardiac lesions. The constellation of this pathophysiology, as well as ductal patency, will therefore dictate the severity and timing of clinical presentation.

The hallmark of circumflex aortic arch is a high retroesophageal arch segment. This crowds the mediastinal space posteriorly, and may result in aortic compression of the oesophagus and the trachea. Older children or adults can report long-standing, intermittent noisy breathing. Although severe airway symptoms are uncommon in young children, a sudden presentation with acute respiratory distress has been reported in infancy without major preceding symptoms^[18]. The mechanism of acute deterioration is not clear but we postulate that this could be due to closure of arterial duct and subsequent tightening of the vascular ring with ductal ligamentum. When compression is severe, the underlying tracheobronchomalacia could result in severe residual airway obstruction in the infant despite relief of vascular ring, which may persist despite full anatomical correction of circumflex aortic arch^[7].

Paediatric patients, who need intervention, usually present with significant airway symptoms^[35]. Dysphagia is an uncommon presentation in children but likely to be under reported^[19]. In the authors' experience, parents may describe intermittent “choking” when the infant starts to take solid food and some toddlers with a vascular ring develop an aversion to solid food.

Severe arch hypoplasia is uncommon, but could result in cardiovascular collapse during neonatal period or early infancy^[5,29]. As opposed to the usual situation with coarctation of the aorta, the timing and mechanism of obstructive symptoms is much more akin to that of interruption of the aortic arch, as for type B aortic interruption, and the two may be very easily confused on echocardiographic assessment^[5]. The hypoplastic distal circumflex aortic arch does not exhibit the dynamic obstruction that occurs with classical coarctation; however, treatment with prostaglandin to open and maintain ductal patency to the descending aorta is just as important in cases of severe arch hypoplasia presenting with signs of heart failure or shock and variably diminished pulses. Stenosis and hypoplasia in different sites or even multiple sites in patients with circumflex arch has been reported. When the base of aberrant subclavian is stenosed or obstructed, there will be differentiation of blood pressure between two arms; when presented without chest pain and diminished pulse in one arm, it simulates an asymptomatic chronic dissecting aortic aneurysm^[42].

A small proportion of patients with circumflex aortic arch may remain asymptomatic or do not present until much later in life^[11,16,43]. Asymptomatic circumflex arch may also present as an incidental finding with intracardiac lesion such as VSD^[33]. However, the presence of a retroesophageal arch segment may result in a more severe form of vascular ring and symptoms may be more common in those with an unobstructive arch than in those with hypoplastic arch.

IMAGING

When presented incidentally, mediastinal widening on plain chest x-ray in circumflex aortic arch can be mistaken as a mediastinal mass or an aortic aneurysm^[8]. The proximal and distal ends of the retroesophageal transverse arch may form bilateral “aortic knobs” on chest x-ray resulting in a symmetrical superior mediastinal widening.

Barium swallow is useful to provide a clear demonstration of posterior oesophageal compression from the circumflex arch^[17,22]. In severe oesophageal compression, there could reflux of the contrast materials into the nasopharynx^[17]. Prior to the advent of CT, suspicious findings on barium study would trigger cardiac catheterization, leading to the diagnosis of circumflex aorta^[17].

Transthoracic echocardiography is the most common initial diagnostic test, which leads to the suspicion of congenital arch malformation. Echocardiography also further evaluates for any arch obstruction and intracardiac lesions. As a hypoplastic retroesophageal segment is not easily demonstrated on echocardiography, a circumflex aorta with right aortic arch, aberrant left subclavian artery, and critical arch hypoplasia can be easily mistaken for type B aortic arch interruption^[5].

CT angiogram with 3D reconstruction is the best imaging modality to demonstrate the presence of retroesophageal transverse arch and confirms the definitive diagnosis of circumflex aortic arch. A CT scan will also identify compression of the trachea and oesophagus [Figures 6 and 7].

In patients presenting with respiratory symptoms, bronchoscopy is often performed first by the otolaryngology team. The presence of pulsatile compression will then trigger cross-sectional imaging and establish the diagnosis of a circumflex arch. The diagnosis can be missed in the absence of pulsatile compression, which may not be easily evident in cases where there is complete occlusion of the main stem bronchus^[9]. CT is, therefore, recommended to exclude vascular compression. When the diagnosis of circumflex aortic arch is established, intra-operative bronchoscopy may help to confirm adequate relief of vascular compression at the time of surgical repair.

SURGICAL MANAGEMENT

Surgery is indicated for significant symptoms and for any associated significant aortic arch hypoplasia. The surgical approach and timing of surgery are likely dictated by the severity of symptoms and arch obstruction. Complications associated with Kommerrell diverticulum, such as progressive aneurysm or dissection may also trigger for surgical intervention. Surgical management varies in literature, from simple division of the arterial ligament alone to full anatomical correction such as an aortic uncrossing procedure.

Division of arterial ligament

Without arch hypoplasia or obstruction, division of arterial ligament alone has been used to relieve the symptoms from vascular ring. Symptomatic improvement had been reported following division, although long-term data are lacking^[18]. Nevertheless, this approach may offer a simple surgical solution associated with low surgical morbidity, without needing cardiopulmonary bypass or extensive posterior mediastinal dissection. Surgery can be approached via a standard posterolateral thoracotomy, or less invasive procedure

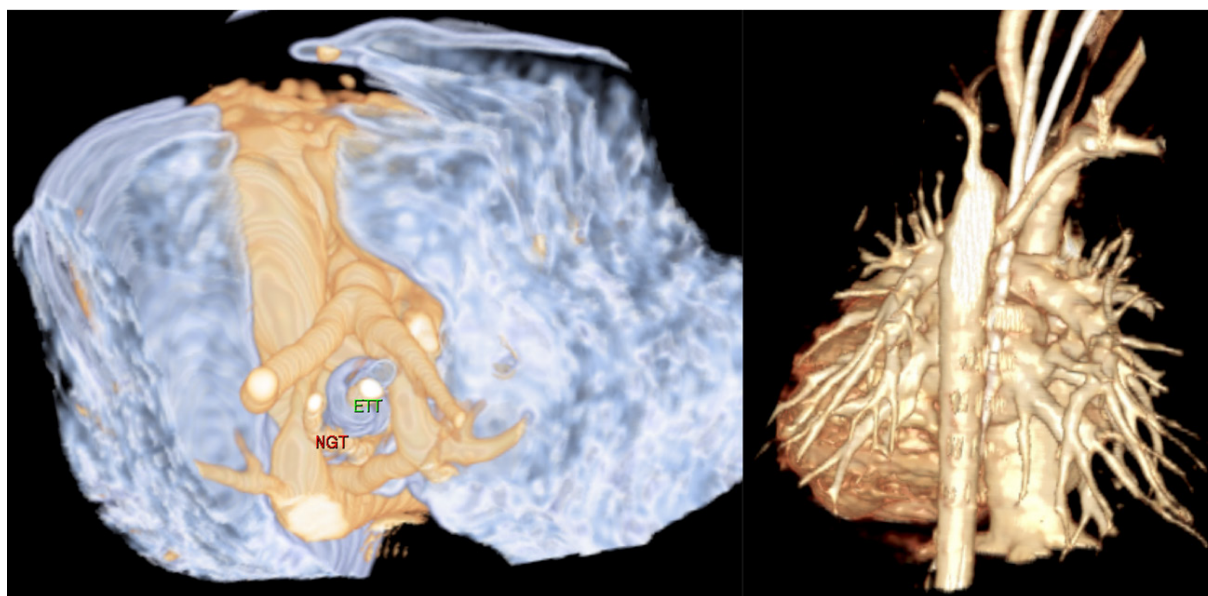


Figure 6. Computed tomography scan demonstrating (left) cross-section aerial view of a circumflex aorta with right aortic arch, encircling the airway [endotracheal tube (ETT)] and oesophagus [nasogastric tube (NGT)]; and (right) posterior view of the same patient demonstrating the hypoplastic retroesophageal aortic arch segment

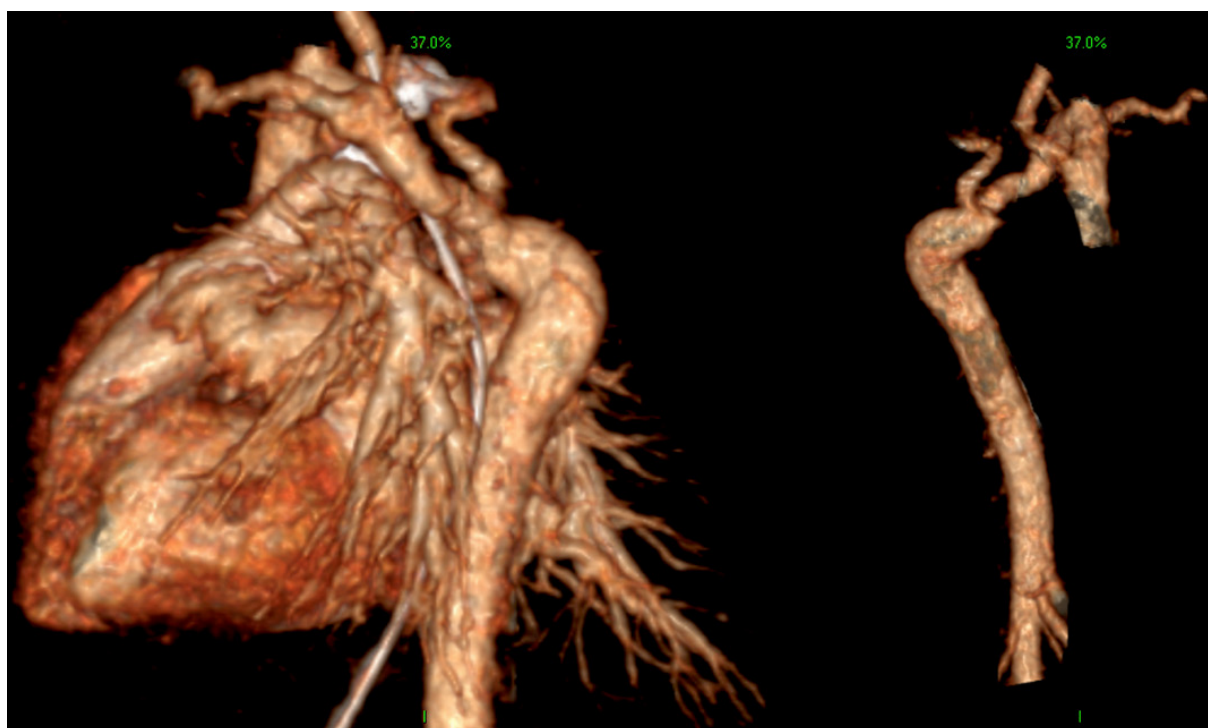


Figure 7. Computed tomography scan demonstrating (left) the left lateral view demonstrating a circumflex aorta with left aortic arch; and (right) anterior view of the same patient showing a circumflex aorta with left aortic arch and hypoplastic arch segment

such as posterior mini-thoracotomy, or video assisted thoracoscopic procedure. The arterial ligament is a structure that cannot be visualized in pre-operative imaging but its presence is presumed at the base of aberrant subclavian artery.

Division of the arterial ligament alone, however, does not address compression of the mediastinal organs from the retroesophageal arch segment. In the absence of aortic arch hypoplasia, this approach may be justifiable in patients with mild symptoms or asymptomatic patients who underwent other cardiac or thoracic procedures^[33].

Aortic arch uncrossing procedure

Optimal symptomatic relief may not be achieved by dividing the arterial ligament alone due to persistent retroesophageal aortic arch compression. Therefore, the aortic uncrossing procedure was described by the French group in 1984 in three infants with circumflex aortic arch - all of whom had previously undergone ligamentum division alone but remained ventilator dependent^[1,2]. These 3 patients were extubated after undergoing aortic uncrossing procedure; and became the subjects on three French reports of circumflex arch^[1,2,14].

The aortic uncrossing procedure is performed through a median sternotomy with cardiopulmonary bypass, hypothermia, and a short period of circulatory arrest. Deep hypothermic total circulatory arrest is used in the original and subsequent series^[1,2,4]. The aorta is transected distal to the origin of the right subclavian artery and the proximal stump is over-sewn. The ligamentum arteriosum is ligated and divided. The retroesophageal aortic arch is dissected from its posterior attachments and brought anteriorly to the left side of the ascending aorta. An arteriotomy is performed on the side of the ascending aorta below the left carotid artery. An end to side anastomosis is performed. The right subclavian artery can be divided to facilitate mobilization of the arch anteriorly, although this is not mandatory^[4,14]. Selective antegrade cerebral perfusion with continuous cardiac perfusion and moderate hypothermia - avoiding total body circulatory arrest - is an alternative cardiopulmonary bypass strategy [Figure 8]^[6].

Posterior aortic translocation

The surgical approach to address circumflex aorta with left aortic arch is less well described^[7,9]. The aortic arch is on the left side, and therefore does not need to be uncrossed to the “normal” side. In our own experience and of past reports, the proximal descending aorta is on the right but later courses back to the left^[7]. Therefore, instead of “uncrossing” the arch, posterior aortic translocation is required. The presence of the right-sided location of the proximal descending aorta is the main issue, which requires translocation into the left chest. The division of the ligamentum relieves the ring and the translocation procedure removes the posterior airway compression. This procedure requires extensive mobilization of the entire descending thoracic aorta. In the presence of unobstructed arch, posterior aortopexy is performed. The procedure can be approached via left thoracotomy without cardiopulmonary bypass. In the presence of the arch hypoplasia, the retroesophageal segment can be resected and an extended end-to-end anastomosis can be performed [Figure 9]^[7].

Aortic arch hypoplasia or obstruction

Several surgical approaches have been reported for the management of circumflex aorta with hypoplastic arch. These include addressing the arch obstruction alone without correcting the circumflex anatomy - resection of the coarctation, relieving the obstruction either using a patch or placing an extra anatomic graft. However, this approach will leave a complex vascular ring in situ which could be a problematic issue in the future with persistent oesophageal and airway compression.

Full anatomical correction of circumflex aorta will therefore require the following procedures: circumflex aorta with hypoplastic right aortic arch: resection of the hypoplastic segment, aortic arch uncrossing procedure and end-to-side anastomosis to the aorta anteriorly. Cardiopulmonary bypass with deep hypothermic total circulatory arrest can be used; but we preferred the strategy of selective antegrade cerebral perfusion via the innominate artery perfused via a 3 mm to 3.5 mm Goretex shunt^[6]. Aortic

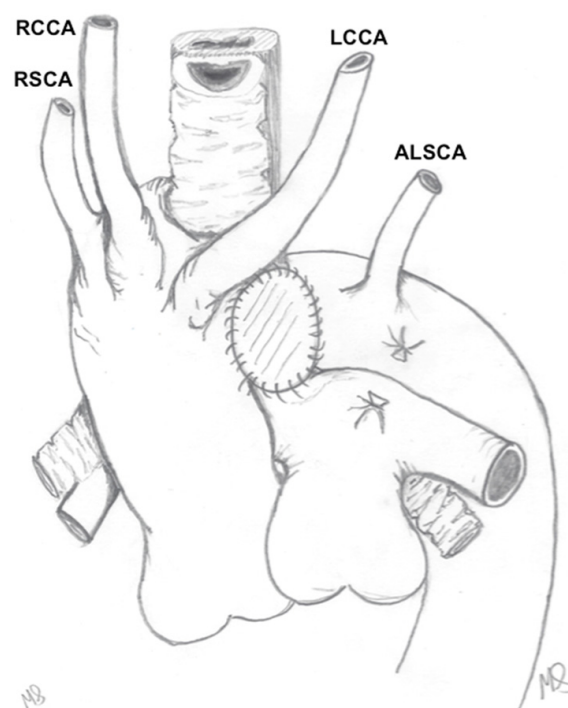


Figure 8. Sketch demonstrating the aortic uncrossing procedure with reimplantation (with patch augmentation) of the retroesophageal arch anteriorly on the left side of the ascending aorta. The head and neck vessels are: left common carotid artery (LCCA), right common carotid artery (RCCA), right subclavian artery (RSCA) and the aberrant left subclavian artery (ALSCA)

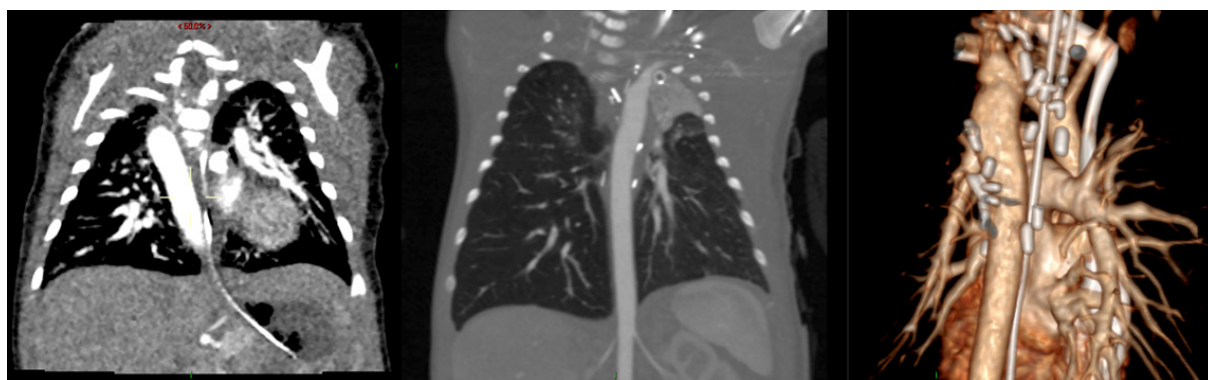


Figure 9. Computed tomography scan of a neonate with circumflex aorta with left aortic arch and critical arch hypoplasia: (left) descending aorta location to the right side of the spine; (middle) normalization of the course of the descending aorta following posterior aortic translocation; (right) 3D reconstruction of the aortic arch following surgery

uncrossing procedure has also been used in single ventricular physiology with aortic arch obstruction requiring a concomitant Norwood procedure^[34].

Circumflex aorta with hypoplastic left arch: a rare variant of obstructed circumflex aorta. Resection of a long hypoplastic segment, posterior aortic translocation, division of the arterial ligament, and extended end to end repair of the arch has been described^[7]. These surgical strategies addressed all the associated pathophysiological mechanisms of circumflex aorta with hypoplastic arch: (1) relief of vascular ring; (2) removal of posterior arch compression on the trachea; and (3) correction of any arch obstruction. If the proximal arch (between innominate and left common carotid artery) is adequate, the procedure can be performed off-pump from the left chest.

Post-operative issues

Due to its proximity, recurrent laryngeal nerve injury is a reported post-operative complication especially when a more extensive procedure such as aortic uncrossing surgery is required^[4,14]. We had not encountered this complication in our reported surgical experience in both circumflex right and left aortic arch^[6,7]. Other complications associated with arch surgery include phrenic nerve injury and chylothorax.

Persistent respiratory symptoms can be due to (1) residual compression from retroesophageal arch if only arterial ligament division alone was performed; and/or (2) underlying tracheobronchomalacia. In children who presented early in the neonatal period or infancy, underlying tracheomalacia could be severe and persistent even after total anatomical correction^[7]. Failure to extubate will require tracheostomy and a home ventilator may be required. Posterior tracheobronchopexy has been used following repair of hypoplastic circumflex aortic arch to facilitate extubation and avoid tracheostomy and long-term ventilation^[7]. Concomitant circumflex aortic arch repair with posterior tracheopexy has been reported recently by the Boston group^[35].

CONCLUSION

The role of surgical intervention in the management of circumflex aorta is evolving. The prevalence of functionally circumflex aorta and persistent symptoms following double aortic arch repair remains to be defined. The aortic arch uncrossing procedure provides full anatomical correction for circumflex aorta with right aortic arch, but requires hypothermic circulatory arrest, and therefore considerable debate still exists if this is always required in the absence of arch obstruction. Undoubtedly, the aortic arch uncrossing procedure has an important role as a rescue strategy in patients with significant residual symptoms post-operatively but its role as a primary strategy is also emerging for severely symptomatic patients, especially with improvised technique to minimize operative morbidities. Posterior aortic translocation also provides full anatomical correction in circumflex variants with appropriate anatomy, without the need of arch uncrossing. When arch obstruction is present, full anatomical repair with aortic uncrossing or posterior aortic translocation should always be advocated to relieve arch obstruction as well as to correct the pathological effects of circumflex aorta.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study, performed literature review and preparation of the manuscript: Bader V, Peng E

Made substantial contributions to conception and design of the study and manuscript review: Knight WB, Danton MH

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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

Patient consents were sought for publication of cross-section images used as figures in this manuscript.

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Systematic Review

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Insufficient evidence regarding benefits from sodium-glucose cotransporter-2 inhibitors in heart failure with preserved ejection fraction

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Abstract

Aim: Sodium-glucose cotransporter-2 (SGLT2)-inhibitors improve survival in adults with reduced ejection fraction. Clinical outcomes in adults with heart failure (HF) with preserved ejection fraction (HFpEF) have not been systematically reviewed.

Methods: We conducted a systematic rapid literature review and appraised the quality of evidence using the Grading of Recommendations Assessment, Development and Evaluation methodology.

Results: We identified *post-hoc* subgroup analyses of four randomized controlled clinical trials (RCTs) and unpublished results from 2 RCTs. In 2 RCTs *vs.* placebo, Canagliflozin reduced the risk of fatal or hospitalized HF in adults with HF and documented or assumed left ventricular ejection fraction (LVEF) $\geq 50\%$ (hazard rate ratio, HR = 0.71, 95%CI: 0.52-0.97) but had no effect in a subpopulation with documented LVEF $\geq 50\%$ (HR = 0.83, 95%CI: 0.55-1.25). Dapagliflozin or ertugliflozin did not improve all-cause or cardiovascular death or hospitalization for HF in adults with HF and LVEF $> 45\%$ in two pivotal RCTs *vs.* placebo. Empagliflozin did not improve exercise ability, patient-reported outcomes or congestion, diuretic use and all-cause healthcare resource utilization in unpublished RCT *vs.* placebo. Various definitions of HFpEF, *post-hoc* interaction analyses suggesting outcome improvement



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regardless of heart failure type, small number of events, and probable publication bias hampered the quality of evidence.

Conclusion: Existing evidence is insufficient to support definitive clinical recommendations for use of SGLT2-inhibitors in adults with HFpEF. Future research should employ consistent definitions of HFpEF and examine the effects from SGLT2- Inhibitors in patients with various HFpEF phenotypes and underlying causes.

Keywords: Sodium-glucose cotransporter-2 - inhibitors, heart failure with preserved ejection fraction, cardiovascular mortality, heart failure hospitalization, systematic literature review, grading of recommendations assessment, development and evaluation methodology

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) presents a significant and growing clinical and economic burden in aging populations, specifically with prevalent arterial hypertension and diabetes^[1-4]. Estimated 1-year all-cause mortality rates of 33% and all cause readmission rates of 67% in patients with HFpEF have not improve over the last decade in the US^[3]. Diabetes is a widely recognized risk factor for cardiovascular morbidity and mortality^[5,6]. Although emerging treatments improved cardiovascular outcomes in people with diabetes^[7,8], no treatments have been proven to improve survival and reduce health care utilization in people with HFpEF^[9-14]. Sodium-glucose cotransporter-2 (SGLT2)- inhibitors are found to improve survival in heart failure with reduce ejection fraction and reduce the risk of major cardiovascular events including heart failure hospitalizations in adults with type 2 diabetes^[15-19]. Empagliflozin and canagliflozin have been approved by the US Food and Drug Administration (FDA) to reduce the risk of cardiovascular death in adults with type 2 diabetes and established cardiovascular disease while dapagliflozin has also been approved to reduce the risk of cardiovascular death and hospitalization for heart failure in adults with reduced left ventricular ejection fraction (LVEF \leq 40%)^[20-25]. Recent evidence-based guidelines recommend SGLT2- inhibitors for the improvement in cardiovascular outcomes in adults with type 2 diabetes^[26-29]. However, the evidence regarding the benefits from SGLT2- inhibitors in adults with HFpEF has not been systematically reviewed and appraised. We conducted a systematic rapid literature review of all completed and ongoing clinical studies aimed at patient outcomes in adults with HFpEF.

METHODS

We conducted our review according to the developed priori protocol^[30-32]. We hypothesized that SGLT2-inhibitors improve cardiovascular mortality, morbidity and hospitalizations in adults with HFpEF, with or without diabetes^[33-38].

Eligible interventions included SGLT2- inhibitors regardless of country' approval [Supplementary Table 1] focusing on the availability in the US, for example dapagliflozin, canagliflozin, empagliflozin and ertugliflozin [Supplementary Table 2]. We included studies that compared SGLT2- inhibitors with antidiabetic medications or placebo. We abstracted reported number of events or rates of all-cause and cardiovascular mortality, incident or progressing of heart failure, and hospitalizations for heart failure^[14,39]. We also looked at the reported intermediate outcomes, e.g., exercise tolerability and the quality of life or other patient reported outcomes as defined in the primary studies^[40-45].

We conducted a comprehensive search with MeSH terms and key words in PubMed, Scopus, the Cochrane Library, www.clinicaltrials.gov, the World Health organization International Clinical Trials Registry Platform, Health Technology Assessment databases, and regulatory agencies up to October 2020 to find

systematic reviews, published and unpublished randomized controlled clinical trials (RCTs), and real-world evidence from the high quality nationally representative controlled observational studies^[30,31]. All of the authors looked at the retrieved publications as well as the evidence-based guidelines that provided definitions of HFpEF and recommend treatments for HFpEF. We documented the eligibility of studies in a reference database.

We planned a quantitative direct meta-analysis of similar interventions and outcomes using random effects models in compliance with recommended meta-analytic methods^[46]. We intended to calculate pooled relative risk, absolute risk difference, number needed to treat and number of attributable events per 1000 treated with 95% confidence intervals (CI). We proposed to examine inconsistency in treatment effects with recommended I² statistics (if I² was > 50%)^[30]. We planned pooled analyses regardless of statistically significant heterogeneity^[46]. Instead, we proposed exploring heterogeneity with a priori defined patient characteristics, e.g., definitions of HFpEF, outcomes, and study quality^[46].

Since *post hoc* analyses of statistical power is not recommended^[47-50], we downgraded the quality of evidence for imprecision based on an estimated priori optimal information size in an adequately powered RCT (e.g., ≥ 250 patients with the event)^[51].

We concluded statistical significance at a 95% confidence level using Statistics/Data Analysis, STATA software (StataCorp LP, College Station, Texas).

We judged the risk of bias in primary studies with the Cochrane risk of bias tool^[52-54]. We judged the quality of evidence according to the recommendations by the grading of recommendations assessment, development and evaluation (GRADE) methodology^[55]. We downgraded the quality of evidence from RCTs according to the domains of the risk of bias in the body of evidence, directness of comparisons, precision and consistency in treatment effects, and the probability of the reporting bias^[55]. We assigned low quality of evidence to all nonrandomized studies, upgrading the quality for the evidence of a strong or dose-response association^[56]. We concluded insufficient evidence when valid information about treatment effects was not identified.

RESULTS

We excluded the majority of clinical studies of SGLT2- inhibitors because they did not report patient outcomes in adults with HFpEF (search strings are available in the appendix and the list of excluded publications and registered studies is available by the request from the authors). We identified *post hoc* subgroup individual patient data meta-analysis of the CANVAS (Canagliflozin Cardiovascular Assessment Study) Program that examined canagliflozin when compared with placebo in patients with HFpEF [Table 1]^[57]. We identified *post-hoc* subgroup analysis of the pivotal DECLARE-TIMI 58 (Dapagliflozin Effect on Cardiovascular Events-Thrombolysis in Myocardial Infarction 58) RCT that examined dapagliflozin when compared with placebo in patients with HFpEF [Table 1]^[58]. We also identified unpublished results from pivotal EMPERIAL trials that examined empagliflozin when compared with placebo in patients with HFpEF^[59-61]. We identified *post-hoc* subgroup analysis of the pivotal VERTIS CV RCT (Evaluation of Ertugliflozin Efficacy and Safety Cardiovascular Outcomes Trial) that examined ertugliflozin when compared with placebo in patients with HFpEF [Table 1]^[62].

We did not identify observational studies that reported patient outcomes after SGLT2- inhibitors in patients with HFpEF and concluded probable publication bias because several completed registered studies remain unpublished. We downgraded the quality of evidence for high risk of bias in *post-hoc* subgroup analyses, imprecision in treatment effects due to small number of events, and probable publication bias. We concluded that the evidence is insufficient for definitive clinical recommendation to use SGLT2- inhibitors

Table 1. Sodium-glucose cotransporter 2 inhibitors in adults with heart failure with preserved ejection fraction, the results from *post-hoc* subgroup analyses of the randomized controlled clinical trials

Population Definition	Outcome	Treatment effect
Canagliflozin vs. Placebo the CANVAS Program ^[57] * ClinicalTrials.gov/NCT01032629/NCT01989754		
Heart failure event with documented EF of $\geq 50\%$ at the HF admission	Fatal or hospitalized heart failure	HR 0.83 (0.55-1.25)
Heart failure event with documented EF of $\geq 50\%$ or assumed to be $\geq 50\%$	Fatal or hospitalized heart failure	HR 0.71 (0.52-0.97) ¹
Dapagliflozin, 10 mg vs. Placebo DECLARE-TIMI 58 (Dapagliflozin Effect on Cardiovascular Events-Thrombolysis in Myocardial Infarction 58) ^[58] ** ClinicalTrials.gov/NCT01730534		
Heart failure with EF of $\geq 45\%$ or without known reduced ejection fraction	Cardiovascular death or hospitalization for heart failure	HR 0.88 (0.66-1.17)
	Hospitalization for heart failure	HR 0.72 (0.5-1.04)
	Cardiovascular death	HR 1.41 (0.93-2.13)
	All-cause mortality	HR 1.02 (0.75-1.38)
Heart failure with EF of $\geq 45\%$	Cardiovascular death or hospitalization for heart failure	HR 0.79 (0.56-1.13)
	Hospitalization for heart failure	HR 0.74 (0.48-1.14)
	Cardiovascular death	HR 1.44 (0.83-2.49)
	All-cause mortality	HR 1.06 (0.71-1.59)
Heart failure with EF 45-< 55%	Cardiovascular death or hospitalization for heart failure	HR 0.83 (0.58-1.2)
	Hospitalization for heart failure	HR 0.76 (0.48-1.19)
	Cardiovascular death	HR 1.18 (0.69-2.01)
	All-cause mortality	HR 0.98 (0.66-1.46)
Ertugliflozin, 5 mg, 15 mg vs. Placebo (Evaluation of Ertugliflozin Efficacy and Safety Cardiovascular Outcomes Trial) ^[62] *** ClinicalTrials.gov/NCT01986881		
Heart Failure with EF > 45%	Cardiovascular death or hospitalization for heart failure	HR 0.92 (0.61-1.39)
	Cardiovascular death	HR 1.08 (0.64-1.8)
	All-cause mortality	HR 1.01 (0.66-1.56)
	Hospitalization for heart failure	HR 0.70 (0.39-1.26)

¹Statistically significant differences at 95% confidence level. *Ejection fraction was assessed during retrospective secondary review of the medical record data by one of the members of the original adjudication committee who was blinded to individual participant treatment assignment; **Prospective baseline assessment of ejection fraction was conducted in all participants; ***Ejection fraction was assessed from medical records when available. EF: ejection fraction; HR: hazard rate ratio

for the reduction in cardiovascular mortality, morbidity or heart failure hospitalizations in patients with HFpEF.

Canagliflozin

Canagliflozin did not reduce the risk of fatal or hospitalized heart failure when compared with placebo in adults with type 2 diabetes and heart failure with documented LVEF of $\geq 50\%$ [Table 1]^[57]. Canagliflozin reduced the risk of fatal or hospitalized heart failure in a subpopulation with heart failure and documented LVEF of $\geq 50\%$ [Table 1]^[57].

The CANVAS RCTs did not examine LVEF at baseline in enrolled adults of ≥ 30 years of age with a history of symptomatic atherosclerotic cardiovascular disease or aged ≥ 50 years with 2 or more risk factors for cardiovascular disease^[44,63]. *Post hoc* subgroup analysis was based on retrospective secondary review of the medical hospitalization record data by one of the members of the original adjudication committee to identify patients with HFpEF defined as heart failure with documented LVEF of $\geq 50\%$ (101 patients)^[57]. The authors conducted a sensitivity analysis assuming that patients with unknown LVEF had HFpEF (61 patients) and found a significant protective effects from canagliflozin in this combined subpopulation^[57].

Based on *post hoc* interaction model and protective effects from canagliflozin in heart failure with reduced ejection fraction (LVEF < 50%), the authors concluded similar canagliflozin benefits in the overall trial population^[57].

Canagliflozin improved diastolic function in patients with type 2 diabetes in two Japanese non-randomized controlled clinical trials^[64,65]. One trial of canagliflozin in outpatients with chronic heart failure and diabetes (CANOSSA trial: prospective, open-label, add-on trial of canagliflozin for diabetes mellitus and stable chronic heart failure) enrolled 94% of patients with HFpEF (exact definition was not provided)^[65]. Canagliflozin improved echocardiographic parameters of diastolic function at 6 and 12 month ($P < 0.001$)^[65]. The second pilot study reported improved left ventricular diastolic function after 3 months of canagliflozin treatment although it did not specify baseline HFpEF^[64].

Dapagliflozin

Dapagliflozin did not improve all-cause or cardiovascular death or hospitalization for heart failure in adults with type 2 diabetes and HFpEF (LVEF $\geq 45\%$) [Table 1]^[58].

DECLARE-TIMI 58 investigators conducted a prospective baseline assessment of ejection fraction in all enrolled patients with established atherosclerotic cardiovascular disease (ASCVD) or multiple risk factors for ASCVD and with a creatinine clearance ≥ 60 mL/min^[58]. The authors acknowledged the absence of universally accepted definitions of HFpEF and reported outcomes in subpopulations with various baseline LVEF thresholds ($< 45\%$, $\geq 45\%$, and $45\%-55\%$). Based on post hoc interaction model and protective effects from dapagliflozin in heart failure with reduced ejection fraction, the authors concluded similar dapagliflozin benefits in overall trial population^[58].

We identified two RCTs that examined the effects from dapagliflozin on diastolic function in adults with type 2 diabetes^[66,67]. The RCT enrolling patients with heart failure reported that dapagliflozin significantly improved diastolic function in those with baseline LVEF $\geq 45\%$ ^[66]. The second RCT that enrolled patients without prior history of heart failure, reported that dapagliflozin had no effect on diastolic function when compared with placebo^[67].

Ongoing registered studies reported different definitions of HFpEF, exclusion of adults with various thresholds of reduced LVEF (e.g., $< 45\%$ or $< 50\%$) and various definitions of primary and secondary outcomes [Table 2]. Available protocols did not provide details on estimated statistical power and required sample size to detect statistically significant differences in primary outcomes.

Empagliflozin

Empagliflozin did not improve exercise tolerance, patient-reported outcomes related to the quality of life and patient satisfaction, congestion, diuretic use and all-cause healthcare resource utilization in adults with HFpEF enrolled in the pivotal EMPERIAL trials^[59-61]. Trials enrolled adults with heart failure with or without diabetes^[45]. The authors defined HFpEF as symptomatic heart failure with LVEF $> 40\%$ and elevated N-Terminal Pro-Brain Natriuretic Peptide [Table 3]. The unpublished results have been presented in the meeting of the European Society of Cardiology in June 2020^[59,60]. Some positive trends in improving congestion after empagliflozin in HFpEF did not achieve statistical significance, possibly due to insufficient statistical power^[59].

The pivotal Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME Trial) did not examine baseline ejection fraction but reported improvement in diastolic dysfunction as a possible mechanism in the observed reduced cardiovascular mortality and morbidity^[68].

Ongoing registered studies reported different definitions of HFpEF (e.g., LVEF ≥ 40 or $\geq 50\%$), exclusion of adults with various thresholds of the reduced LVEF (e.g., $< 30\%$ or $< 40\%$) and various definitions of primary and secondary outcomes [Table 3]. Available protocols did not provide details on estimated statistical power and required sample size to detect statistically significant differences in primary outcomes.

Table 2. Ongoing registered clinical trials of dapagliflozin in adults with heart failure with preserved ejection fraction

NCT number phase enrollment	Title acronym	Inclusion criteria defining HFpEF	Exclusion by LVEF	Outcome measures
NCT03030235 Phase: Phase 4 Sample: 320	Dapagliflozin in PRESERVED Ejection Fraction Heart Failure PRESERVED-HF	Symptomatic heart failure (NYHA class II-IV) Left Ventricular Ejection Fraction (LVEF) \geq 45% Elevated NT-proBNP (\geq 225 pg/mL) or BNP (\geq 75 pg/mL). For patients with permanent atrial fibrillation (AF) BNP \geq 100 pg/mL or NTproBNP \geq 375 pg/mL	Previous LVEF < 45%	Change from baseline in: NTproBNP and BNP, heart failure related quality of life using the Kansas City Cardiomyopathy Questionnaire (KCCQ) overall summary score, 6-min walk test (6MWD)
NCT02751398 Phase: Phase 4 Sample: 60	Impact of dapagliflozin on diastolic dysfunction in type 2 diabetic patients	\geq grade 1 diastolic function (relaxation abnormality) at resting echocardiography	LV ejection fraction < 50%	Subclinical diastolic dysfunction assessed by diastolic stress echocardiography
NCT03619213 JPRN- JapicCTI-184157 EUCTR2018-000802-46-CZ PER-026-18 Phase: Phase 3 Sample: 6100	Dapagliflozin evaluation to improve the LVEs of patients with preserved ejection fraction heart failure. DELIVER	Symptomatic heart failure (NYHA class II-IV) LVEF > 40% and evidence of structural heart disease Elevated NT-pro BNP levels	NR	The first occurrence of any of the components of this composite: (1) CV death; (2) Hospitalization for HF; (3) Urgent HF visit Total number of hospitalizations for HF and CV death; Change from baseline in KCCQ-TSS; All-cause mortality
NCT03877224 EUCTR2018-003441-42-DK JPRN- JapicCTI-194724 Phase: Phase 3 Sample: 500	DETERMINE-preserved - Dapagliflozin Effect on Exercise Capacity using a 6-min walk test in patients with heart failure with preserved ejection fraction	Symptomatic heart failure (NYHA functional class II-IV) LVEF > 40% and evidence of structural heart disease Elevated NT-proBNP levels 6MWD \geq 100 meters and M 425 meters	NR	Change from baseline in: 6MWD, KCCQ-TSS, movement intensity during walking
NCT03794518 Phase: Phase 3 Sample: 648	Effect of Dapagliflozin plus low dose pioglitazone on hospitalization rate in patients with hF and HFpEF	Hospitalized for HFpEF (hospitalization require intravenous diuresis) in the 6 months preceding recruitment. LVEF > 50% Presence of LV diastolic dysfunction in echocardiography	LVEF < 50%	Time to first hospitalization for heart failure after starting intervention; All-cause mortality
JPRN- UMIN000038380 Sample: NR	Yokohama add-on inhibitory efficacy of dapagliflozin on left ventricular filling pressure in patients with acute heart failure with preserved ejection fraction complicated with type 2 diabetes study	Acute hear failure with LVEF \geq 40% and stable hemodynamically	NR	Change from baseline in: diastolic parameters of echocardiography, BNP; CVD events, not specified

BNP: brain natriuretic peptide; NT-proBNP: N-terminal pro b-type natriuretic peptide

Ertugliflozin

The ongoing evaluation of ERTugliflozin efficacy and Safety CardioVascular outcomes trial (VERTIS-CV) enrolled adults with type 2 diabetes and established atherosclerotic cardiovascular disease, did not specify subgroup analysis depending on baseline ejection fraction obtained from medical records but reported that 80.6% of 8,238 randomized patients had HFpEF (LVEF > 40%)^[69]. This RCT was designed to determine non-inferiority of ertugliflozin when compared with placebo on major adverse CV events including death, nonfatal myocardial infarction, or nonfatal stroke^[69]. Preliminary publications defined HFpEF as LVEF > 45% and reported no reduction in patient outcomes in this subpopulation after comparing ertugliflozin vs. placebo [Table 1]. Based on the post hoc interaction model and protective effects from ertugliflozin in heart failure with reduced ejection fraction, the authors concluded similar ertugliflozin benefits in the overall trial population^[62,70].

Table 3. Ongoing registered clinical trials of empagliflozin in adults with heart failure with preserved ejection fraction

NCT number phase enrollment	Title acronym	Inclusion criteria defining HFpEF	Exclusion by LVEF	Outcome measures
NCT02932436 Phase: Phase 4 Sample: 158	Effects of Empagliflozin on left ventricular diastolic function compared to usual care in type 2 diabetics EmDia	Diastolic cardiac dysfunction E/E' ratio ≥ 8	NYHA classification III - IV	Change from baseline in: E/E' ratio, Left end-diastolic volume (LEDV)
NCT03057951 EUCTR2016-002278-11-DE Phase: Phase 3 Sample: 5750	Empagliflozin outcome trial in patients with chronic heart failure with preserved ejection fraction (EMPEROR-Preserved)	Symptomatic heart failure (NYHA class II-IV) LVEF $> 40\%$ NT-proBNP > 300 pg/ml for patients without AF, OR > 900 pg/ml for patients with AF	NR	Composite primary endpoint: CV death or hospitalization for heart failure failure); All hospitalizations for heart failure; All-cause mortality; Change from baseline in KCCQ; All-cause hospitalizations
NCT03448406 Phase: phase 3 sample: 315	Empagliflozin in Patients with chronic heart failure with preserved ejection fraction (HFpEF) EMPERIAL-preserved	6MWT ≥ 350 m Symptomatic heart failure (NYHA class II-IV) LVEF $> 40\%$ NT-proBNP > 300 pg/ml for patients without AF, OR > 600 pg/ml for patients with AF	Prior LVEF $\leq 40\%$	Change from baseline in: 6MWT, KCCQ TS, chronic heart failure questionnaire, self-administered standardized format (CHQ-SAS) dyspnea score, patient global impression of severity (PGI-S) of heart failure symptoms, patient global impression of dyspnea severity, patient global impression of change (PGI-C) in heart failure symptoms, patient global impression of change in dyspnea, N-terminal pro-brain natriuretic peptide (NTproBNP)
NCT02998970 Phase: Phase 4 Sample: 97	Effects of Empagliflozin on cardiac structure in patients with type 2 diabetes EMPA-HEART	Previous myocardial infarction ≥ 6 months ago, or previous coronary revascularization ≥ 2 months ago	LVEF $< 30\%$ NYHA Class IV or recent hospitalization for decompensated heart failure (HF)	Change from baseline in: Left Ventricular (LV) mass LV end-diastolic volume LVEF Regional LV diastolic function
NCT03753087 Phase: Phase 4 Sample: 100	Effects of Empagliflozin on exercise capacity and left ventricular diastolic function in patients with heart failure with preserved ejection fraction and Type-2 diabetes mellitus	Symptoms 4 signs of heart failure (as defined in 2016 European society of cardiology guidelines) LVEF $\geq 50\%$ LV diastolic dysfunction grade II/III	Permanent atrial flutter or atrial fibrillation	Change from baseline in: 6MWD LVMI left atrial volume index (LAVI) average E/e' ratio NT-proBNP minnesota living with heart failure questionnaire (MLHFQ) score
NCT03332212 Sample: NR	EMPA-VISION: A randomised, double-blind, placebo-controlled, mechanistic cardiac magnetic resonance study to investigate the effects of empagliflozin treatment on cardiac physiology and metabolism in patients with heart failure	LVEF $\geq 50\%$ as measured by ECHO structural heart disease NT-proBNP > 125 pg/mL in patient without AF or NT-pro-BNP > 600 pg/mL in patient with AF	Prior LVEF $\leq 40\%$.	Change from baseline in myocardial phosphocreatine-to-ATP ratio
CTRI/2017/09/009734 Phase: NS Sample: NR	Empagliflozin trial in patients with chronic heart failure	Chronic symptomatic heart failure (NYHA class II-IV) LVEF $> 40\%$ Elevated NT-proBNP > 300 pg/mL for patients without AF, OR > 900 pg/ml for patients with AF	NR	The composite endpoint: CV death or hospitalization for heart failure; All-cause mortality; Change from baseline in clinical summary score of the KCCQ; All-cause hospitalizations
JPRN-jRCTs071180091 Phase: NS Sample: NR	Effect of empagliflozin for HFpEF with Type2 DM A clinical study for cardioprotective effect of empagliflozin in T2DM patients with heart failure and exploring associated factors (EMPOWERMENT)	Symptomatic heart failure (NYHA class II-III) LVEF $> 50\%$ BNP ≥ 35 pg/dL	NR	Change from baseline in: uptake efficiency (Peak VO2) BNP LVEF and RVEF(by MRI)

Other SGLT2 inhibitors

Limited evidence from small unpublished Japanese RCTs suggested that luseogliflozin (63 patients), and tofogliflozin (62 patients) improved diastolic dysfunction from baseline in adults with diabetes and HFpEF^[71]. However, luseogliflozin, when compared with voglibose, did not improve diastolic dysfunction or brain natriuretic peptide (BNP) levels in type 2 diabetes patients with HFpEF (defined as LVEF > 45% and BNP = 35 pg/mL²)^[72].

We identified 60 registered studies of ipragliflozin, sotagliflozin, luseogliflozin, or tofogliflozin that did not report enrolling patients with HFpEF.

DISCUSSION

Our review found insufficient evidence that SGLT2 inhibitors can improve cardiovascular mortality, morbidity or hospitalizations in patients with HFpEF. We found no studies that reported adverse effects from SGLT2 inhibitors specifically in adults with HFpEF^[73]. Limited evidence of some improvement in intermediate outcomes of diastolic dysfunction lack clinical significance with valid prediction of better patient-centered outcomes and healthcare utilization required in future studies^[74,75]. The absence of RCTs that met pooling criteria precluded planned meta-analyses. Previously published indirect net-work meta-analyses focused on intermediate outcomes of diastolic dysfunction regardless of baseline HFpEF and did not find consistent superiority of SGLT2 inhibitors when compared with placebo or other anti-diabetic medications^[74,75]. Previously published direct meta-analysis concluded that SGLT2 inhibitors reduced the risk of cardiovascular death or heart-failure hospitalization regardless of baseline heart failure diagnosis^[15]. However, this meta-analysis did not look at patient outcomes depending on baseline LVEF and specifically in patients with HFpEF^[15].

Various definitions of HFpEF preclude valid comparisons of patient outcomes among RCTs of the same SGLT2 inhibitor and across RCTs of different SGLT2 inhibitors [Supplementary Table 2]^[28,33,34,37,76-80]. Ongoing studies use various inclusion and exclusion criterias with a potential threat to external validity of completed in future studies^[81]. Consistent consensus definition of HFpEF in guidelines, RCTs, and real life clinical practice and coding is essential for valid assessment of the best treatment options in adults with HFpEF^[35,82-85]. Patient outcomes can differ depending on HFpEF diagnostic criteria and should be assessed by HFpEF phenotypes^[38,79,80]. Subgroup analyses by HFpEF diagnostic criteria and phenotypes should be conducted with prespecified evidence-based definitions, stratified randomization and adequate sample size^[86,87]. Known interactions between HFpEF phenotypes and treatment effects should guide future studies aimed at efficacious treatments^[11,83]. Registered protocols of ongoing RCTs are inconsistent in addressing recommendations by guidelines hard clinical outcomes including all-cause and cardiovascular mortality, morbidity, hospitalizations, or quality of life in people with HFpEF^[88,89]. Such inconsistency indicates that the most important clinical questions regarding the benefits from SGLT2 inhibitors on patient centered outcomes may not be answered in the upcoming years.

Available heart failure guidelines recommend SGLT2 inhibitors to reduce cardiovascular mortality and hospitalizations in patients with diabetes [Supplementary Table 3]^[5,26-28,89,90]. Some guidelines specify recommendations of SGLT2 inhibitors in heart failure with reduced ejection fraction^[5,29]. Very few guidelines including the Canadian Cardiovascular Society and Canadian Heart Failure Society guidelines and the American Diabetes Association Standard of Care statement acknowledge uncertainty regarding potential benefits of SGLT2 inhibitors for patients with midrange or preserved LVEF^[5,29]. Older guidelines do not make recommendations for or against SGLT2 inhibitors aimed at the prevention of heart failure hospitalizations or mortality^[33,34,88,91].

We found no large observational studies of SGLT2 inhibitors in HFpEF. We can speculate that inconsistencies in diagnostic and treatment recommendations for patients with HFpEF preclude optimal treatment choices

in these patients^[92,93]. Diabetes care should be provided by multidisciplinary teams of endocrinologists, cardiologists and nephrologists and include assessment of HFpEF and consequent decisions of the best treatment choices^[5,6,94,95].

Inconsistency in clinical research and practice policies, market approval, and coverage decisions across countries preclude universal patient access to the optimal treatment options^[96-98]. Harmonization of health technology assessments methodology and data sharing across the countries would improve the quality of care in patients with heart failure and specifically HFpEF^[99,100]. The International Network of Agencies for Health Technology Assessment calls for transparency in evidence collection, data sharing, and consistent evidence appraisal to improve patient outcomes across the globe^[101].

Our work has implications for future research. The emerging epidemic of diabetes, arterial hypertension and HFpEF requires international efforts in improving the quality of evidence and the quality of healthcare^[10,26,40]. Professional associations and health technology assessment groups need to collaborate in the development of consensus definitions of HFpEF, in prospective design of high quality powered RCTs in adults with various phenotypes and underlying causes of HFpEF. Individual patient data meta-analyses of completed RCTs and registries of medical records can shed light on optimal treatment choices in adults with HFpEF^[102-105].

In conclusion, existing evidence is insufficient to support definitive clinical recommendations for use of SGLT2- Inhibitors in adults with HFpEF. Future research should employ consistent definitions of HFpEF and examine the effects from SGLT2- Inhibitors in patients with various HFpEF phenotypes and underlying causes.

DECLARATIONS

Authors' contributions

Designed review protocol, research questions and performed data analysis and interpretation: Shamliyan TA

Conceptualized study objectives and goals and contributed to data analysis and interpretation: Aronow WS

Contributed to study design and execution, data analysis and interpretation: Avanesova AA

Made substantial contributions to the writing of the manuscript: Shamliyan TA, Avanesova AA, Aronow WS

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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Commentary

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Frontiers in endovascular thrombectomy for ischemic stroke

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Abstract

Stroke is a leading cause of morbidity and mortality worldwide. There have been significant advances in the hyperacute treatment of patients with ischemic stroke with the advent and application of reperfusion therapies, including intravenous thrombolysis and endovascular thrombectomy. Endovascular thrombectomy involves the removal of thrombus from an artery using a mechanical retriever or aspiration with angiographic visualization. This review aims to outline the current evidence to support the use of endovascular thrombectomy and highlight areas of ongoing research.

Keywords: Ischemic stroke, endovascular thrombectomy, thrombolysis, tenecteplase, tissue plasminogen activator, alteplase, computed tomography perfusion, magnetic resonance imaging, randomized trial

EVIDENCE FOR ENDOVASCULAR THROMBECTOMY AND THE TIME WINDOW FOR TREATMENT

The benefit of endovascular thrombectomy over standard medical care was proven in 2015, with 5 trials [Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MR CLEAN); Extending the Time for Treatment in Emergency Neurological Deficits - Intra-Arterial (EXTEND-IA); Endovascular Treatment for Small Core and Anterior Circulation Proximal



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Occlusion with Emphasis on Minimizing CT to Recanalization Times (ESCAPE); Solitaire With the Intention For Thrombectomy PRIMARY Endovascular Treatment (SWIFT PRIME); and Randomized Trial of Revascularization with Solitaire FR Device vs. Best Medical Therapy in the Treatment of Acute Stroke Due to Anterior Circulation Large Vessel Occlusion Presenting within Eight Hours of Symptom Onset (REVASCAT)] demonstrating benefit in patients presenting with large vessel occlusion (LVO) of the anterior circulation within 6 h of symptom onset^[1-5]. LVO in the anterior circulation is defined as an occlusion of the intracranial internal carotid artery and/or the first segment of the middle cerebral artery (M1). Patients with intracranial occlusion and tandem extracranial carotid artery occlusion were also included in the trials and clearly benefitted. In an individual patient data meta-analysis of these trials, the rate of functional independence [defined as a modified Rankin scale (mRS) score of 0-2] was significantly higher in the thrombectomy cohort (46%) compared to medical therapy (27%), equaling a number-needed-to-treat (NNT) of 5^[6]. There was a similar rate of serious adverse events, in particular symptomatic intracranial hemorrhage, between thrombectomy and medical therapy groups^[6]. These results were in contrast to 3 previous trials published in 2013 that failed to show clinical benefit of thrombectomy^[7-9]. The difference in outcomes is likely explained by improvements in patient selection (detecting LVO with non-invasive imaging prior to angiography), faster door-to-arterial access times and better devices to achieve faster and more complete reperfusion^[10]. The THRACE (Mechanical Thrombectomy After Intravenous Alteplase vs. Alteplase Alone After Stroke), PISTE (Pragmatic Ischaemic Stroke Thrombectomy Evaluation) and RESILIENT (Randomisation of endovascular treatment with stent-retriever and/or thromboaspiration vs. best medical therapy with acute ischemic stroke due to large vessel occlusion) trials were also subsequently reported with consistent results in favor of thrombectomy^[11-13].

Data from the MR CLEAN trial indicate that the benefit of thrombectomy, when patient selection is simply based on evidence of LVO using non-invasive imaging, is strongly time-dependent and ceases to be statistically significant ~6 h after onset^[14]. The HERMES (Highly Effective Reperfusion Evaluated in Multiple Endovascular Stroke Trials) pooled data found statistically significant benefit out to 7.3 h but included patients selected on the basis of good collateral flow or favorable CT perfusion which has subsequently been shown to extend the time window for treatment^[15].

The DAWN trial (DWI or CTP Assessment with Clinical Mismatch in the Triage of Wake-Up and Late Presenting Strokes Undergoing Neurointervention with Trevo) in patients 6-24 h after the time they were last known to be well used clinical-core mismatch imaging selection. This involved CT perfusion or diffusion MRI to identify a relatively small volume of irreversibly injured brain (ischemic core) compared to the severity of clinical deficit and also factored in patient age^[16]. CT perfusion has been shown to achieve similar perfusion mismatch classification compared to MRI^[17]. Volumetric agreement studies have shown that overestimation of ischemic core using a relative cerebral blood flow threshold < 30% of normal brain^[18] is uncommon and tends to involve white matter in patients with ultra-rapid reperfusion^[19-21]. The DEFUSE 3 trial (Endovascular Therapy Following Imaging Evaluation for Ischemic Stroke) in patients 6-16 h after the time they were last known to be well used CT perfusion or MRI perfusion-diffusion mismatch and required < 70 mL irreversibly injured ischemic core combined with the critically hypoperfused region being both > 15 mL and > 1.8 times the volume of the ischemic core^[22]. In the DAWN and DEFUSE 3 trials, the NNT for functional independence (defined as mRS score 0-2) was 2.8 and 3.6, respectively^[16,22]. There was no significant difference in the rate of symptomatic intracerebral hemorrhage between thrombectomy and medical therapy groups in these trials. The DEFUSE 3 inclusion criteria classified ~60% more patients as eligible for thrombectomy than the DAWN criteria and these additional patients benefitted at least as much from the thrombectomy procedure^[22]. Perfusion imaging selection is therefore recommended in international guidelines for selection of patients for thrombectomy 6-24 h after the time they were last known to be well [Figure 1]^[23,24]. Relatively few patients present with LVO beyond 24 h, and a trial is unlikely to be feasible. However, anecdotal reports have suggested potential benefit in patients > 24 h after onset if imaging is favorable^[25].

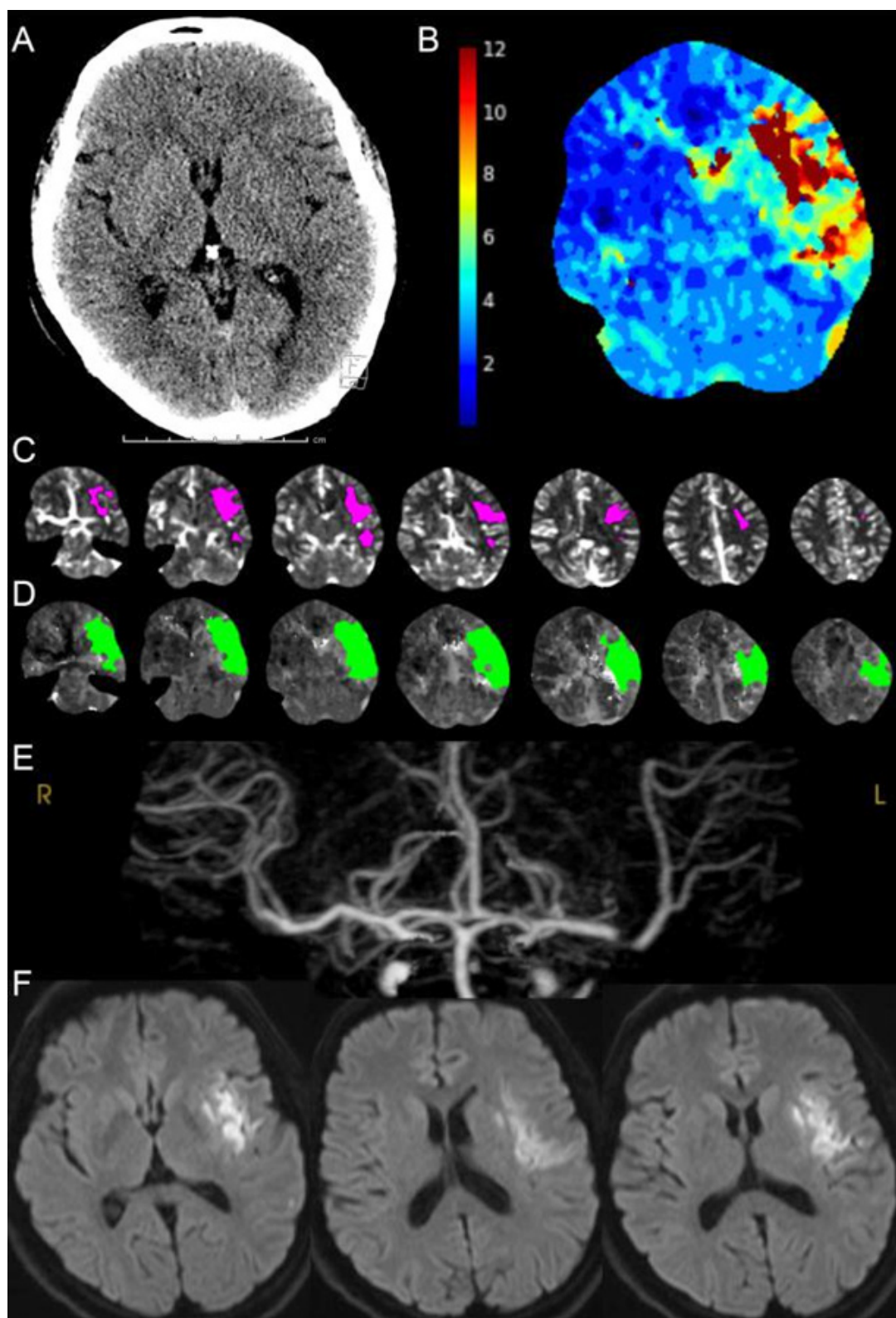


Figure 1. A patient with wake-up onset stroke. Non-contrast CT excluded hemorrhage and indicated subtle loss of grey-white differentiation in the left insular region (A); CT perfusion time to maximum (Tmax) map showing delayed flow via collaterals in the left middle cerebral artery territory confirmed the diagnosis of ischemic stroke (B); Automated segmentation of CT perfusion cerebral blood flow < 30% of normal brain (36 mL magenta region estimated as irreversibly injured ischemic core) (C) and Tmax > 6-s delay (124 mL green region estimated tissue at risk) from RAPID software (D); CT angiography demonstrating distal M1 middle cerebral artery occlusion (E) 24-h diffusion MRI after successful endovascular thrombectomy showing no interval growth in ischemic core (F)

Thrombolysis (without thrombectomy) in perfusion mismatch-selected patients has proven beneficial 4.5-9 h after onset, including patients with wake-up stroke who were within 9 h of the midpoint of sleep^[26,27]. In practical terms this included wake-up stroke patients up to 16 h after the time they were last known to be well, similar to the DEFUSE 3 trial time window. The potential for late-window thrombolysis to improve outcomes in combination with thrombectomy is now being explored in randomized trials [Tenecteplase in Stroke Patients Between 4.5 and 24 h (TIMELESS), NCT03785678 and Extending the time window for Tenecteplase by Effective Reperfusion of penumbral tissue in patients with Large Vessel Occlusion (ETERNAL), NCT04454788].

ENDOVASCULAR THROMBECTOMY FOR PATIENTS PRESENTING WITH MILD STROKE DEFICITS OR DISTAL OCCLUSIONS

The benefit of endovascular thrombectomy in patients presenting with anterior circulation LVO and mild deficits is still unknown, with less than 1% of patients enrolled in recent thrombectomy trials having a NIHSS ≤ 5 ^[28]. Despite this cohort having only mild symptoms on first assessment, evidence suggests that without reperfusion therapy, a substantial proportion subsequently deteriorate and are disabled at 90 days (mRS 2-6; 29%)^[29,30]. Patients with more proximal occlusions, particularly terminal internal carotid artery occlusions, are at the highest risk of neurological deterioration. Deterioration is likely due to leptomeningeal collateral circulation failure over time, in the absence of reperfusion^[31]. Recent observational data indicate that immediate thrombectomy for the mild stroke patient (NIHSS ≤ 5) is safe and may improve clinical outcomes^[28]. This is currently being assessed in two randomized controlled trials [Endovascular Therapy for Low NIHSS Ischemic Strokes (ENDOLOW), NCT04167527 and Exploration of the limits of mechanical thrombectomy indications in a single action - MinOr Stroke Therapy Evaluation - NIHSS 0-5 (IN EXTREMIS-MOSTE), NCT03796468].

In contrast to the unequivocal evidence for the effectiveness of thrombectomy in patients with proximal occlusion, evidence of benefit beyond the M1 segment is less robust. Relatively few patients with M2 occlusions were included in randomized trials and the definition of M2 segments varied, with many representing early bifurcation of the M1 segment. Anterior cerebral artery and posterior cerebral artery occlusions were not included in trials, other than 3 patients in MR CLEAN. Hypothetically, the benefit of thrombectomy should be reduced given the smaller territory supplied by more distal vessels and the increased efficacy of thrombolysis in reperfusion of more distal occlusions. Furthermore, the risk of arterial injury may potentially be increased given the smaller vessel size and increased tortuosity. However, meta-analyses have suggested benefit of thrombectomy in proximal M2 segments in carefully selected patients with significant neurological deficits^[15,32]. Advances in device technology are likely to improve the safety and efficacy of distal thrombectomy. Further research is needed in this area.

ENDOVASCULAR THROMBECTOMY FOR PATIENTS PRESENTING WITH LARGE ISCHEMIC CORE VOLUMES

Increasing ischemic core volume (estimated by diffusion restriction on MRI or critically reduced cerebral blood flow on CT perfusion) is associated with lower likelihood of functional independence^[33]. Despite this association, there is emerging evidence for the benefit of thrombectomy in selected patients with large cores (> 70 mL), particularly within 6h of stroke onset^[33,34]. These data suggest that even with large baseline core volumes, there may be significant volumes of viable but critically hypoperfused tissue that can be salvaged with intervention and translate to clinically meaningful benefit^[34]. Several randomized controlled trials [Thrombectomy for Emergent Salvage of Large Anterior Circulation Ischemic Stroke (TESLA), NCT03805308; Efficacy and Safety of Thrombectomy in Stroke With Extended Lesion and Extended Time Window (TENSION), NCT03094715; Exploration of the limits of mechanical thrombectomy indications in a single action - Large Stroke Therapy Evaluation - ASPECT 0-5 (IN EXTREMIS - LASTE), NCT03811769

and Randomized Controlled Trial to Optimize Patient's Selection for Endovascular Treatment in Acute Ischemic Stroke (SELECT-2), NCT03876457] are currently underway to assess this possible benefit. Undoubtedly, rates of functional independence (defined as mRS 0-2) will be substantially lower compared to the imaging-selected randomized controlled thrombectomy trials, such as EXTEND-IA, DAWN and DEFUSE 3. However, mRS 3 outcomes that allow patients to return home with some supports are clinically and economically meaningful compared to death and requirement for fulltime nursing care. Another potential positive outcome may be a reduction in the requirement for hemicraniectomy.

ENDOVASCULAR THROMBECTOMY FOR BASILAR ARTERY OCCLUSIONS

Basilar artery occlusion is associated with very high levels of morbidity and mortality. Meta-analysis of observational data demonstrated lower rates of death (HR = 0.49, 95%CI: 0.44-0.55) and improved modified Rankin scale 4-6 (HR = 0.67, 95%CI: 0.63-0.72) with thrombectomy as compared to best medical management^[35]. Unfortunately, prospective randomized controlled data are less robust. The BEST randomized trial demonstrated improved outcomes with thrombectomy in an as-treated analysis (mRS 0-2; 39 vs. 19%, OR = 2.81, 95%CI: 1.23-6.41) but was confounded by high crossover rate from medical management to thrombectomy, resulting in a neutral intention-to-treat analysis^[36]. The BASICS (Basilar Artery International Cooperation Study) randomized trial has been reported in abstract form and did not demonstrate a significant benefit in favorable outcome with thrombectomy (mRS \leq 3; 44.2 vs. 37.7%, RR 1.18, 95%CI: 0.92-1.50). However, the subgroup with severe clinical deficit (NIHSS \geq 10) appeared to benefit. Critical details including the rate of successful reperfusion have not yet been released^[37].

THROMBOLYSIS FOLLOWED BY THROMBECTOMY VS. DIRECT THROMBECTOMY

The current standard of care in a patient presenting with a LVO, even in a thrombectomy-capable center, is to give thrombolysis prior to proceeding with thrombectomy. Thrombolysis is more widely available than thrombectomy and should be given at primary stroke centers, if no contraindications exist, prior to transfer to a thrombectomy-capable center. Studies looking at whether thrombolysis can be withheld in patients who present directly to a thrombectomy-capable center are currently underway [Solitaire With the Intention For Thrombectomy Plus Intravenous t-PA Versus DIRECT Solitaire Stent-retriever Thrombectomy in Acute Anterior Circulation Stroke (SWIFT DIRECT), NCT03192332; A Randomized Controlled Trial of DIRECT Endovascular Clot Retrieval Versus Standard Bridging Thrombolysis With Endovascular Clot Retrieval (DIRECT SAFE), NCT03494920 and Multicenter Randomized CLinical trial of Endovascular treatment for Acute ischemic stroke in the Netherlands investigating the added benefit of intravenous alteplase prior to intra-arterial thrombectomy in stroke patients with an intracranial occlusion of the anterior circulation (MR CLEAN-NO IV), ISRCTN80619088]. The first trial published on this topic, Endovascular Thrombectomy with or without Intravenous Alteplase in Acute Stroke [DIRECT-MT], found similar results between direct thrombectomy and combined thrombolysis-thrombectomy arms. Technically, direct thrombectomy narrowly met the specified non-inferiority margin of the lower bound of the 95% confidence interval for the common odds ratio > 0.80 . However, this margin was overly generous and does not provide reassurance that omitting thrombolysis would be appropriate, even in patients who directly present to a thrombectomy center^[38]. The rate of successful reperfusion at end of thrombectomy was numerically higher in the alteplase pre-treated group and there were no significant differences in adverse events, including symptomatic intracerebral hemorrhage, between groups^[38]. Other factors that confound the interpretation of the direct to thrombectomy trials are the use of alteplase rather than the more effective thrombolytic tenecteplase^[39,40] and potentially distorted acute stroke workflow. Usually, thrombolysis can be given in parallel with thrombectomy decision-making, whereas in these trials, all imaging must be completed and the patients accepted by the interventionist for thrombectomy before they can be randomized and eventually receive thrombolysis. The delay in commencing thrombolysis reduces its opportunity to induce reperfusion prior to thrombectomy.

TIME TO TREATMENT IS STILL CRUCIAL: IMPROVING DOOR-TO-REPERFUSION TIMES

The critically time-dependent benefits of reperfusion therapies are well understood, and streamlining workflow to reduce treatment delays remains central to optimizing patient outcomes. Even though individual patients with excellent collateral flow may have slower infarct progression, there is an overall decrease in the proportion of patients eligible for extended-window thrombectomy on the basis of favorable perfusion imaging as time elapses. This corresponds to the time-dependent cerebral blood flow thresholds for infarction and failure of collateral cerebral circulation over time. A meta-analysis looking at time to effective reperfusion demonstrated that for every 4-min delay from emergency department arrival to substantial endovascular reperfusion time, 1 of every 100 treated patients had a worse disability score (higher score by 1 or more on the mRS)^[15]. Minimizing time from onset of symptoms to reperfusion therapy is therefore crucial in maximizing the number of patients eligible for reperfusion therapy.

The largest delay in reperfusion therapy is in the pre-hospital setting^[15]. Several advances have been made in both the pre-hospital and emergency department settings. Paramedic stroke recognition and pre-hospital notification, whereby stroke centers are notified of a potential stroke patient prior to arrival, have been shown to decrease door-to-imaging, door-to-thrombolysis and onset-to-thrombolysis times, while also increasing eligibility for thrombolysis^[41-43]. In addition, screening tools have been developed to identify suspected LVOs in the pre-hospital setting^[44-46]. These tools allow patients with suspected LVOs to be transferred directly to thrombectomy-capable centers, bypassing hospitals not capable of providing this service and subsequently improving time to reperfusion. One study investigating an ambulance pre-hospital clinical triage tool demonstrated high sensitivity (85.7%), specificity (93.5%), and positive predictive value (80%) for the recognition of thrombectomy-eligible LVOs^[46]. Future studies are investigating the application of these tools to accelerate patient delivery to thrombectomy-capable centers by bypassing hospitals not capable of providing this service [Direct Transfer to an Endovascular Center Compared to Transfer to Closest Stroke Center in Acute Stroke with Suspected Large Vessel Occlusion (RACECAT), NCT02795962 and Treatment Strategy In Acute Ischemic large Vessel STROKE: Prioritize Thrombolysis or Endovascular Treatment (TRIAGE), NCT03542188].

There is evidence for the benefit of mobile stroke units (MSUs) in reducing time to reperfusion and subsequent disability. An MSU is an ambulance with on-board CT-scanner and a specialized stroke team capable of assessing and treating patients in the community and directing those eligible to thrombectomy-capable centers. A study from Melbourne, Australia demonstrated that the MSU model resulted in an overall time saving from first ambulance dispatch to thrombolysis of 42.5 min (95%CI: 36.0-49.0) and a median time saving from first ambulance dispatch to the start of thrombectomy (arterial puncture) of 51 min in those with LVO (95%CI: 30.1-71.9)^[47]. The estimated disability reduction, based on time saved, was 20.9 disability-adjusted life years for 100 patients in the thrombolysis group and 24.6 disability-adjusted life years in the thrombectomy group^[47]. Berlin has three MSU and results presented in abstract form indicated improved functional outcomes compared to standard in-hospital care^[48].

Workflow efficiencies in the movement of stroke patients in and between hospitals have decreased time to reperfusion therapy in stroke centers around the world. Universal features of efficient systems include emergency department and stroke team prenotification of suspected stroke patients by ambulance, direct transfer of patients from triage to CT table on the ambulance stretcher and the delivery of thrombolysis, if eligible, on the CT table. These interventions have been shown to reduce door-to-thrombolysis to 20-34 min^[49-51]. Repeated imaging after transfer is a major cause of delays^[52]. A time saving of 59 min from door-to-groin access (at a thrombectomy center) is possible if patients diagnosed with LVOs at an external site are transferred directly to the neuro-angiography suite rather than being admitted via the emergency department^[53]. Another strategy to achieve time savings that is currently under investigation is to transfer patients with suspected LVO (NIHSS score > 10 on arrival) directly from triage to the neuro-angiography

suite, bypassing both the emergency department and conventional CT scanner^[54,55]. A flat panel CT using angiography equipment (or in some cases a separate CT scanner in the angio-suite) can be used to exclude intracerebral hemorrhage, followed by diagnostic angiography and treatment if a LVO is detected. In one observational study, this resulted in significantly shorter median door-to-arterial access times (16 min vs. 70 min; $P < 0.01$) and greater functional independence at 90 days (defined as mRS 0-2; 41 vs. 28%; $P = 0.05$)^[55]. However, whether this is an efficient use of limited angiography suite resources remains to be determined.

CONCLUSIONS AND FUTURE DIRECTIONS

Effective and rapid reperfusion remains the only proven approach to reduce disability in ischemic stroke patients. Thrombectomy has been a major advance, and the indications continue to broaden. Device evolution is likely to continue and the emphasis for technical procedural success is now on rapid “first pass” near-complete reperfusion^[56]. Mild stroke, distal occlusion and patients with large ischemic core are the current frontiers on which randomized trials are focused. Neuroprotection may yet prove beneficial with a recent trial of nerinetide being neutral overall but suggesting benefit in patients who did not receive thrombolysis^[57]. Systems of care innovations to accelerate treatment are highly achievable and of critical importance to continue to reduce the disability associated with LVO ischemic stroke.

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Authors' contributions

Made substantial contributions to conception and design of the commentary and performed data interpretation: Linger M, Campbell BCV

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Original Article

Open Access



Chronic venous disease among nurses in operating room and outside operating room

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Abstract

Aim: Chronic venous disease (CVD) is very common in nurses. Noticeably, operating room (OR) nurses are predicted to have a major prevalence of varicose veins. We investigated whether the prevalence of CVD in OR nurses was more than non-OR nurses.

Methods: Study populations were OR nurses and non-OR nurses at the Faculty of Medicine, Chiang Mai University. Information was compiled by questionnaire. Physical examination was operated by examiners for CVD based on clinical finding using Comprehensive Classification System for Chronic Venous Disorders classification.

Results: 222 nurses were included. The prevalence of C0-C2 was notably different between the two groups ($P < 0.001$). The prevalence of C1 in OR nurses and non-OR nurses was 59.6% and 72.1% while the prevalence of C2 in OR nurses and non-OR nurses was 8.1% and 16.4%, respectively. Nevertheless, the quality of life was not remarkably different between the two groups.

Conclusion: The results demonstrated that CVD in non-OR nurses appear to be higher than OR nurses.

Keywords: Chronic venous disease, operating room nurses, CEAP classification



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INTRODUCTION

Chronic venous disease (CVD) is a condition that effect the veins of the leg. It includes various clinical signs from mild to severe form such as telangiectasia, reticular vein, varicose vein, leg edema, hyperpigmented skin changes, dermal sclerosis, and venous ulcer. Telangiectasia and reticular veins are defined as the modest form of chronic venous disease. They are abnormalities of the vein inside or near the skin layer, which are swollen with blood accumulating to blue or purple lines, usually in the legs or feet. Varicose veins are larger than telangiectasia and are located below the skin layer and in subcutaneous fat. They are common, especially for women, elderly individuals, pregnant women, obese people, family history, and those who walk or stand for long periods of time, which gives fatigue, swelling, and other symptoms^[1-5]. This disease usually does not cause serious complications such as death, but it can affect the quality of life.

This study focuses on nurses. Nurses typically have to walk or stand constantly, which results in an increased risk of developing CVD due to their working conditions^[4-7]. There is often a debate in the nursing field that operating room nurses (OR nurses) walk and stand all day and are more likely to have varicose veins than other nurses (non-OR nurses). However, non-OR nurses have claimed that because they are constantly walking, they are more likely to have varicose veins. Thus far, there have been no studies to settle the debate. Therefore, our study will investigate the prevalence of chronic venous disease in OR nurses and non-OR nurses, as well as a comparative study between the two groups.

METHODS

Patients and data collection

A cross-sectional study was performed between May 2013 and June 2013. The study subjects were nurses including OR nurses and non-OR nurses at Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. This study was approved by our local ethics committee, Faculty of Medicine, Chiang Mai University (SUR-13-1390-EX). All subjects provided written informed consent. This study was conducted per the guidelines of the Declaration of Helsinki. Data was collected by a questionnaire that was divided into two sections. The first section of the questionnaire pertained to individual characteristics, risk factors, and history of CVD. The second section pertained to quality of life by using Chronic Venous Insufficiency Quality of Life Questionnaire-14 (CIVIQ-14) that was validated as a useful measurement in assessing CVD^[8]. The physical examination was operated by the examiners for the varicose vein based on the clinical finding using CEAP classification. The CEAP classification includes clinical, etiologic, anatomic, and pathologic parts^[9]. The clinical classification consists C0 to C6. C0 is no visible sign of CVD. C1 is telangiectasia or reticular veins. C2 is varicose veins. C3 is leg edema. C4 is hyperpigmented skin or dermal sclerosis. C5 is healed venous ulcer and C6 is active venous ulcer.

Statistical analysis

All statistical analyses were performed with STATA 14.0 (StataCorp LP, USA) for Windows. Descriptive statistics of continuous variables were represented using mean \pm standard deviation (SD). Categorized variables were represented with percentages. Differences between the two groups were analyzed with T-test/Mann Whitney *U* test or Chi-square. Statistical significance was set at $P < 0.05$. Sample size calculation for this study were based on the possible proportion of varicose veins from 30% to 35%^[4]. Significance levels are 5% and power levels are 80%.

RESULTS

A total of 222 nurses were included in this study. Two hundred and nine (94.1%) nurses were female, and 99 (44.6%) were OR nurses. The baseline characteristics are shown in Table 1. We found that the height of the OR nurses was greater than that of the non-OR nurses (155.61 ± 5.67 cm vs. 157.77 ± 6.05 cm; $P = 0.013$).

Table 1. Baseline characteristics and comparison between non-OR nurses and OR nurses

	Total <i>n</i> = 222	Non-OR nurses <i>n</i> = 123	OR nurses <i>n</i> = 99	<i>P</i> -value
Gender, <i>n</i> (%)				0.066
Male	13 (5.9)	4 (3.3)	9 (9.1)	
Female	209 (94.1)	119 (96.7)	90 (90.9)	
Age (years), <i>n</i> (%)				0.094
< 35	36 (16.5)	13 (10.8)	23 (23.5)	
35-40	37 (17.0)	22 (18.3)	15 (15.3)	
41-50	82 (37.6)	47 (39.2)	35 (35.7)	
> 50	63 (28.9)	38 (31.7)	25 (25.5)	
Mean ± SD	44.36 ± 9.57	45.80 ± 8.49	42.60 ± 10.51	0.067
Weight (kg)				0.829
Mean ± SD	56.36 ± 9.19	56.09 ± 8.01	56.69 ± 10.49	
Height (cm)				0.013*
Mean ± SD	156.58 ± 5.92	155.61 ± 5.67	157.77 ± 6.05	
BMI (kg/m ²)				0.224
Mean ± SD	22.99 ± 3.51	23.18 ± 3.27	22.76 ± 3.78	
Standing (h/day)				0.459
Mean ± SD	6.03 ± 2.58	6.14 ± 2.64	5.60 ± 2.52	
Sitting (h/day)				0.099
Mean ± SD	4.45 ± 2.61	4.72 ± 2.72	4.11 ± 2.45	
Exercise				0.212
Yes	111 (50.9)	56 (47.1)	55 (55.6)	
No	107 (49.1)	63 (52.9)	44 (44.4)	
Family history of varicose veins				0.167
Yes	49 (22.1)	32 (26.0)	17 (17.2)	
No	173 (77.9)	91 (74.0)	82 (82.8)	

N: number; BMI: body mass index; SD: standard deviation. **P* value < 0.05 was considered statistically significant

However, we found that the body mass index (BMI), weight, standing hours, sitting hours, exercise, and family history of varicose veins to be similar in both nurses.

Concerning the severity of clinical CEAP classification in nurses, the most frequent stages were C1 (66.5%), C0 (20.8%), and C2 (12.7%). The prevalence of C0-C2 was notably different between the two groups (*P* < 0.001) as shown in [Figure 1](#). The prevalence of C1 in OR nurses and non-OR nurses was 59.6% and 72.1%, respectively, while the prevalence of C2 in OR nurses and non-OR nurses was 8.1% and 16.4%, respectively. Nevertheless, the quality of life was not remarkably different (85.75 ± 11.91 vs. 85.60 ± 12.24; *P* = 0.962) between the two groups, as shown in [Table 2](#).

DISCUSSION

The important finding in this study is that the prevalence of C2 and C1 in non-OR nurses was significantly higher than OR nurses. Particularly, C2 in non-OR nurses was two times more frequent than in OR nurses. Telangiectasia and reticular veins are the most common findings of chronic venous disorder in the general population. A study of 5,187 people in Italy found that 64.8% had telangiectasia^[10]. Our study had similar results. C1 was the most common finding of clinical sign; the average prevalence was 66.5%. As we know, nursing is a career that involves standing for a long time at work. Prolonged standing is one of the risk factors of CVD^[1,2]. Some studies showed that prolonged standing was found to be a significant factor for varicose veins among nurses at Dhulikhel Hospital and a university hospital in Busan, South Korea^[6,7]. The standing position results in high venous pressure or venous hypertension in the veins of the leg. Particularly, persons who stand still in the same position for long periods of time are exposed to venous hypertension, which when combined to valvular incompetence or muscle pump dysfunction can aggravate this disease^[11]. Bahk et al.^[1] demonstrated that prolonged standing time was associated with varicose veins and nocturnal leg cramps. However, the standing time per day in our study is not different between the two groups of nurses. The mean standing time per day of OR nurses is 5.6 h and non-OR nurses is 6.14 h.

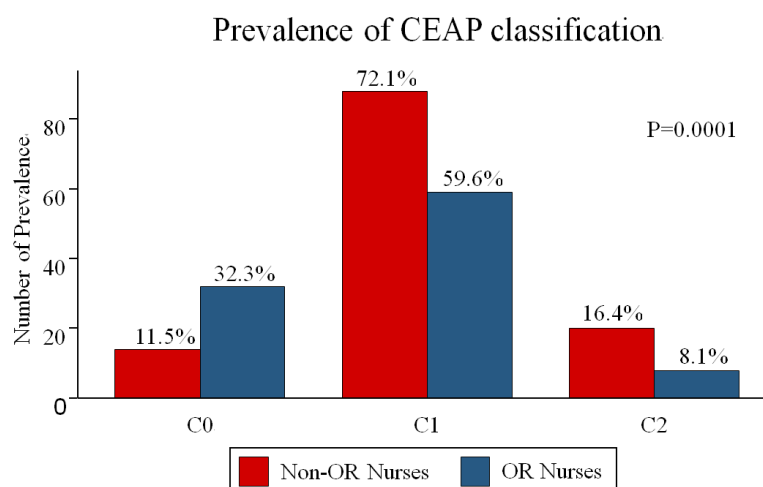


Figure 1. Frequency and percentage of clinical CEAP intensity in nurses. CEAP: Comprehensive Classification System for Chronic Venous Disorders

Table 2. Clinical CEAP classification and CIVIQ comparison between non-OR nurses and OR nurses

	Total n = 222	Non-OR nurses n = 123	OR nurses n = 99	P-value
CEAP, n (%)				< 0.001*
C0	46 (20.8)	14 (11.5)	32 (32.3)	
C1	147 (66.5)	88 (72.1)	59 (59.6)	
C2	28 (12.7)	20 (16.4)	8 (8.1)	
CIVIQ				0.962
Mean ± SD	85.69 ± 12.04	85.75 ± 11.91	85.60 ± 12.24	
Median (min-max)	89 (30-100)	89 (43-100)	88 (30-100)	

N: number; CEAP: Comprehensive Classification System for Chronic Venous Disorders; CIVIQ: Chronic Venous Insufficiency Quality of Life Questionnaire-14. *P value < 0.05 was considered statistically significant

Other risk factors of chronic venous disease such as older age, female gender, obesity, and family history of varicose veins are not different between the groups. The height of the OR nurses is greater than non-OR nurses and have statistical significance, but perhaps the difference around two centimeters might not have any clinical relevance. In short, we found that non-OR nurses had more prevalence of CVD, although our study did not show risk factor differences. The reason behind this difference is not clear. Further cohort study is needed to continue for more specific and further study risk factors. This can help nurses improve both their physical and mental health.

There were limitations in this study. The OR nurse group has a greater frequency of younger nurses (double frequency in the below 35 group) than those in non-OR nurse group. Also, there were more males in OR nurse group than those in non-OR group. As is well known, the prevalence of CVD is age and gender dependent. These might be a bias of this study. There are a large number of risk factors that can determine the progression in CVD including weight, body mass index, number of pregnancy, career, and family history of chronic venous disease. Therefore, our findings need to be confirmed in a large population with adjusting confounders (multivariate analysis). Additionally, this study was a cross sectional study, so it cannot explain cause and effect.

CVD has a substantial effect on the quality of life, especially physical health aspects^[12,13]. The mental health aspect is affected only in a severe stage (C3 or more)^[14]. Our study shows non-OR nurses had more CVD (C1 and C2), but the quality of life was not remarkably different ($P = 0.962$).

In conclusion, CVD in non-OR nurses seems to be more common than in OR nurses; however, the quality of life was not different between the two groups.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Kaima P, Reanpang T, Rerkasem K

Performed data acquisition, as well as provided administrative, technical, and material support: Kaima P, Kulprachakarn K, Pongtam S

Availability of data and materials

Not applicable.

Financial support and sponsorship

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The ethics committee of Faculty of Medicine, Chiang Mai University approved this study (SUR-13-1390-EX). All subjects provided written informed consent. This study was conducted as per guidelines of the Declaration of Helsinki.

Consent for publication

Not applicable.

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Original Article

Open Access



Internal iliac artery sacrifice during endovascular abdominal aortic aneurysm repair

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Abstract

Aim: We aimed to assess the clinical outcomes of the internal iliac artery (IIA) coverage during endovascular abdominal aortic aneurysm repair (EVAR).

Methods: A retrospective observational study was conducted in patients managed with EVAR for the aorto-iliac aneurysmal disease. The IIA was sacrificed by extension of the stent-graft into the external iliac artery in the absence of the distal landing zone, while it was preserved if the landing zone was available.

Results: From 2002 to 2018, 540 patients underwent EVAR for aorto-iliac aneurysmal disease in our center. Sixty-five (12.04%, $n = 65/540$) had iliac aneurysm extension. Among these 65 cases, the IIA was not covered in 32 patients (IIA salvage/spared group), while they were covered in 33 patients (IIA sacrifice group). The IIA sacrifice group consisted of 25 unilateral and 8 bilateral coverages. There was 100% technical success and no 30-day mortality in both groups. The IIA sacrifice group had more postoperative complications in general when compared to the IIA salvage group, but they were not significant ($P < 0.05$). There were one patient with buttock claudication ($P = 1.000$) with bilateral IIA coverage, two cases of lower limb microembolization ($P = 0.492$) and one case of erectile dysfunction ($P = 1.000$) in IIA sacrifice group, while they were not seen in IIA salvage group. There was no ruptured iliac access, device-related malfunction, spinal cord ischemia or bowel ischemia in either group.



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Conclusion: We found coverage of IIA aneurysmal extension during EVAR of AAA to be technically feasible and safe.

Keywords: Abdominal aortic aneurysm, Iliac artery aneurysm, endovascular abdominal aortic aneurysm repair, clinical outcomes

INTRODUCTION

Endovascular aneurysm repair (EVAR) has become progressively common in abdominal aortic aneurysm (AAA) repair. Concomitant aneurysm of the common iliac arteries (CIAs) is seen in around one-third of AAA patients^[1,2]. Internal iliac artery (IIA) embolization and extension of the endograft limb into the external iliac artery (EIA) can widen the indications for EVAR. However, the endovascular repairs of abdominal aortic aneurysms in patients with aneurysmal extensions to the iliac bifurcation could be associated with higher complications and/or secondary procedures^[3].

The optimal endovascular management of the IIA occlusion in aortoiliac aneurysms is controversial. Unilateral occlusion of IIA is relatively safe, but bilateral IIA occlusion could be associated with complications, such as ischemic colitis or neurologic impairment. Therefore, revascularization of at least one of the hypogastric flows might be necessary to maintain pelvic perfusion where the stent-grafts are extended into the bilateral EIAs^[1,4]. Different techniques are utilized to preserve IIA patency; however, most surgical procedures are limited by the increased cost, contrast use and operative time leading to unnecessary radiation exposure^[5]. Therefore, in this study, we aimed to assess the operative outcomes of IIA coverage during EVAR in our tertiary vascular referral center.

METHODS

This is a retrospective observational study of patients who had elective EVAR from 2002 to 2018 in our tertiary vascular referral center. The study was approved by our Institutional Research Ethics Committee. All patients were identified from our medical records and any missing data were obtained from the institutional patient administration system, and archiving of the picture and communication system. We excluded patients with ruptured AAA, aortoiliac occlusive disease and focal abdominal dissection.

Postoperative complications are reported based on the Society for Vascular Surgery reporting guidelines^[6].

Outcomes

Primary outcomes included 30-day mortality and overall survival. Secondary outcomes included freedom from reintervention, aneurysm-related survival and complication rates (new-onset buttock claudication, erectile dysfunction and intestinal ischemia).

Following discharge, follow-up was performed with physical examination and aortic computed tomography angiography (CTA) at six weeks and duplex ultrasound (DUS) imaging at six and twelve months in the first year and annually after that. In selective patients, repeat control CTA was performed if there was evidence of sac expansion and/or endoleak in DUS.

Operative procedures

All patients underwent surgery under general anesthesia. Different stent-grafts were used: AneuRx, Talent, Endurant and Endurant II (Medtronic, Santa Rosa, Calif); Excluder (W.L. Gore & Associates, Flagstaff, Ariz); and Powerlink, AFX and AFX 2 (Endologix, Irvine, Calif). Endograft selection was made based on the preference of the surgeon and vascular anatomy. An iliac limb ≥ 20 mm in distal diameter was considered as a flared limb (FL). The FL extensions were chosen from large-diameter iliac extension limbs with range of 20-28 mm.

Table 1. Baseline characteristics of the patients

	IIA salvage group (n = 32)	IIA sacrifice group (n = 33)	P value
Male, n (%)	19 (59.38%)	25 (75.76%)	0.191
Mean age (years, SD)	78 ± 10	78 ± 13	1
Hypertensive	22	22	1
Hyperlipidemia	15	19	0.46
Diabetes Mellitus	2	6	0.258
Ischemic Heart Disease	20	14	0.138
Peripheral Arterial Disease	8	4	0.215
Carotid Artery Disease	4	2	0.427
Respiratory Disorder	21	22	1
Renal Disorder	0	2	0.492
Smokers	20	27	0.102

IIA: internal iliac artery; SD: standard deviation

Embolization was performed for IIA aneurysm and/or IIA originating from CIA aneurysm. The proximal trunk of IIA was embolized, leaving the branches for collateral formation. We used mainly the contralateral approach for IIA embolization. First, we embolized with coils (MR Eye or Nestor Coils, Cook Medical) at the main IIA orifice before it bifurcates into the anterior and posterior division to achieve IIA occlusion. Then, we inserted the AmplatzerTM vascular plug to close the door. The coils were tightly packed. The overall aim was to maintain the collateral passageway between the anterior and posterior open.

Data analysis

Baseline demographics, preoperative features, surgical details and postoperative complications were noted. Aneurysm morphology and maximum diameter was recorded after analyzing the preoperative and follow-up CTAs. Concomitant common iliac artery aneurysm (CCIAA) was defined as > 20 mm maximum outer wall-to-outer wall CIA diameter.

Statistical Package for the Social Sciences (SPSS) version 23 (IBM, Armonk, NY, USA) was used for statistical analysis. Chi-square tests (or Fisher's exact test based on distribution) for discrete variables and Student *t*-tests (or Mann-Whitney *U* test) for continuous variables were used for comparative analysis. A *P*-value < 0.05 was considered statistically significant. Sustained clinical and hemodynamic improvement, freedom from binary restenosis and re-intervention, amputation-free survival and overall survival were estimated using the Kaplan-Meier survival analysis on a per-patient basis.

RESULTS

From 2002 to 2018, 540 patients underwent EVAR for AAA in our center. Sixty-five (12.04%, *n* = 65/540) had iliac aneurysm extension. Among these 65 cases, the IIA was not covered in 32 patients (IIA salvage/spared group), while they were covered in 33 patients (IIA sacrifice group). The IIA sacrifice group consisted of 25 unilateral and eight bilateral coverages.

The baseline patient characteristics are detailed in Table 1. More male patients were in the IIA sacrifice group than the IIA salvage group (75.76% vs. 59.38%, *P* = 0.191), while the ages of patients were similar (78 ± 13 years vs. 78 ± 10 years, *P* = 1.000). No statistically significant differences in baseline demographics, risk factors and clinical presentations were observed.

The mean AAA diameter was slightly larger in the IIA sacrifice group than the IIA salvage group but not significant (5.70 ± 2.50 cm vs. 5.40 ± 1.65 cm, *P* = 0.569). However, there were significant differences in the right CIA diameter (32.90 ± 20.98 mm vs. 15.40 ± 9.24 mm, *P* = 0.001) and right IIA diameter (16.40 ± 9.40 mm vs. 9.00 ± 2.30 mm, *P* = 0.001) between the IIA sacrifice and IIA salvage groups [Table 2].

Table 2. Procedural and anatomical features

	IIA salvage group (n = 32) (Mean ± SD)	IIA sacrifice group (n = 33) (Mean ± SD)	P value
Operative time (h)	2.20 ± 0.44	3.43 ± 1.54	0.001*
Aneurysm size (cm)	5.40 ± 1.65	5.70 ± 2.50	0.569
Right CIA diameter (mm)	15.40 ± 9.24	32.90 ± 20.98	0.001*
Left CIA diameter (mm)	18.95 ± 4.77	16.82 ± 6.52	0.137
Right IIA diameter (mm)	9.00 ± 2.30	16.40 ± 9.40	0.001*
Left IIA diameter (mm)	9.10 ± 2.80	11.48 ± 6.80	0.071
HDU stay (days)	0.16 ± 0.57	0.75 ± 1.49	0.040*
Total hospital stay (days)	2.91 ± 2.67	5.56 ± 4.88	0.005*

*Statistically significant. IIA: internal iliac artery; SD: standard deviation; CIA: common iliac artery; HDU: high density unit

Table 3. Postoperative complications

Complications	IIA salvage group (n = 32)	IIA sacrifice group (n = 33)	P-value
Thirty-day mortality	0	0	-
Hematoma	1	3	0.613
Infection	1	1	1
Cardiac complications	1	4	0.355
Respiratory complications	0	2	0.492
Renal complications	0	1	1
Type Ib endoleak	0	3	0.238
Lower limb macro-embolization	0	2	0.492
Buttock claudication	0	1	1
Erectile dysfunction	0	1	1
Stroke	0	0	-
Bowel ischemia	0	0	-
Spinal cord ischemia	0	0	-
Deep vein thrombosis	0	0	-
Pulmonary embolism	0	0	-

IIA: internal iliac artery

The IIA sacrifice group compared to the IIA salvage group had significantly higher procedure time (3.43 ± 1.54 h vs. 2.20 ± 0.44 h, $P = 0.001$), mean hospital stay (5.56 ± 4.88 days vs. 2.91 ± 2.67 days, $P = 0.005$) and high dependency unit (HDU) stay (0.75 ± 1.49 days vs. 0.16 ± 0.57 days, $P = 0.040$).

Technical success was 100%, and there was no 30-day mortality in either group. The IIA sacrifice group had more postoperative complications in general when compared to the IIA salvage group, but they were not significant ($P < 0.05$) [Table 3]. As such, there were higher numbers of hematoma (3 vs. 1, $P = 0.613$), cardiac complications (4 vs. 1, $P = 0.355$), respiratory complications (2 vs. 0, $P = 0.492$) and renal complications (1 vs. 0, $P = 1.000$) in the IIA sacrifice group. Similarly, IIA sacrifice group had two cases of lower limb microembolization ($P = 0.492$), one erectile dysfunction ($P = 1.000$), and one buttock claudication ($P = 1.000$), while they were not seen in IIA salvage group. The buttock claudication occurred in a patient with bilateral IIA coverage. There was no ruptured iliac access, device-related malfunction, spinal cord ischemia or bowel ischemia in either group.

The mean follow-up was 3.28 years. At three-years, there were no statistically significant difference in the freedom from reintervention (85% vs. 93.75%, $P = 0.253$) [Figure 1], aneurysm-related survival (97% vs. 97%, $P = 0.982$) [Figure 2] and overall survival (67% vs. 72%, $P = 0.963$) [Figure 3] between the IIA sacrifice and IIA sparing groups.

DISCUSSION

EVAR is progressively being adopted in AAA repair. Many reports focus on the proximal landing zones; however, there could be a need to extend EVAR to EIA due to either short CIA or aneurysmal CIAs. Recent

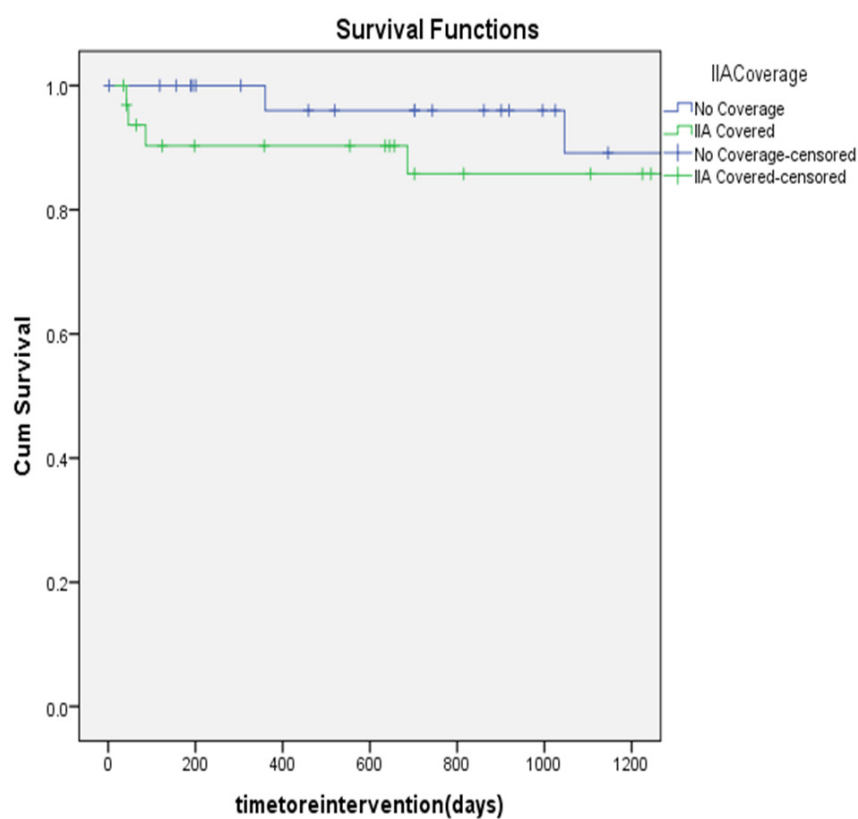


Figure 1. Three-year freedom from re-intervention

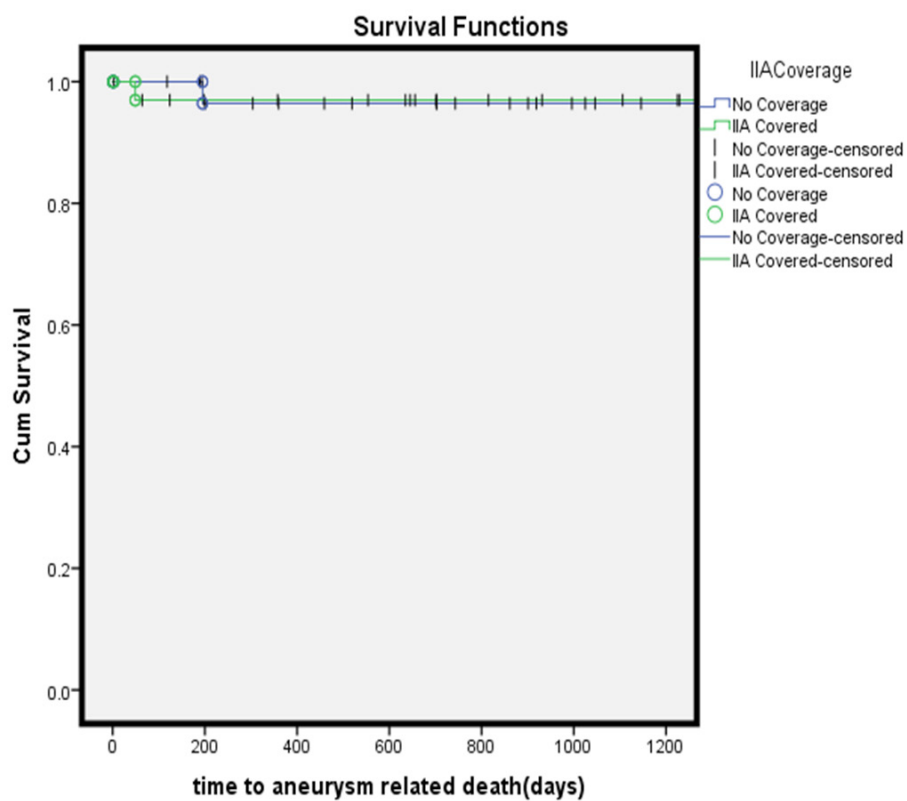


Figure 2. Three-year aneurysm-related survival

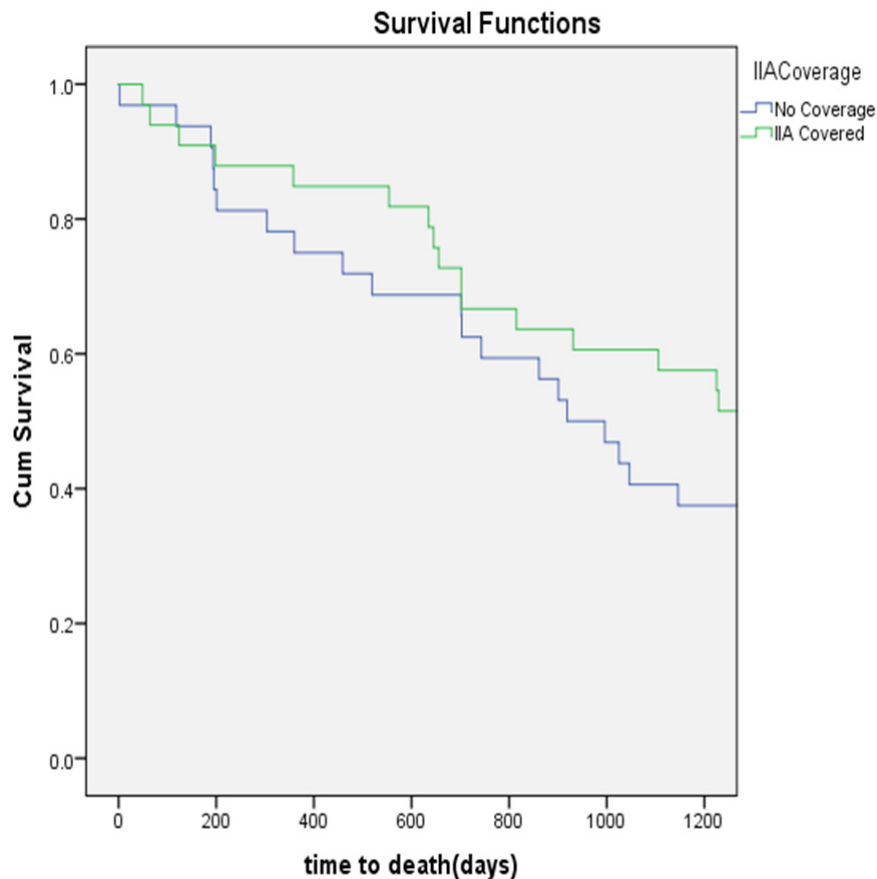


Figure 3. Overall survival at three years

guidelines recommend preserving at least one IIA to maintain the circulation of the pelvis to avoid buttock claudication and bowel ischemia^[7].

The options of preserving IIA include the FL, which can accommodate up to 25 mm CIA; iliac branched devices (IBD); and EIA to IIA bypass either surgically or by endovascular means. Unfortunately, all of these solutions are associated with significant operation time and contrast amount^[8]. In addition, the FL technique could be linked with a high rate of dilatation with Type Ib endoleak, five times more than the corresponding less than 20 mm CIA diameter^[9]. Additionally, the difficulty in later usage of IBD necessitates the usage of trans-axillary access with increased risk of stroke^[9,10].

Anatomical limitations prohibit the usage of IBD in many iliac aneurysms as IBD necessitates certain anatomical features that are only applicable in 40% of CIA aneurysmal anatomy^[8]. Additionally, added financial burden should be taken into account when treating AAA, particularly when compared to the open surgery^[8,11-14]. Similarly, these newer IBD devices have limited evidence on long-term outcomes. Furthermore, the high cost and longer procedure time, absence of the extended follow-up outcomes and poor quality of life may hinder the application of IBD in AAA treatment^[14-17].

The surgical option to revascularize the IIA can also increase the complexity and morbidity of the EVAR. Despite being effective in flow preservation, repositioning of IIA could increase the morbidity and recovery time, increasing the risk of ureteric and venous injuries, primarily in obese patients with IIA aneurysm^[8,18].

Contrary, multiple reports showed that coverage of the IIA could be tolerated without devastating complications. Buttock claudication, the most common complications, is likely reversible after a short period with persistent buttock claudication in less than half of patients. Similarly, reports of the devastating complications, bowel and spinal cord ischemia, seem to be exaggerated and true occurrence seem to be exceedingly rare in the literature^[13,17,19,20].

Mehta *et al.*^[21] reported that the innocence of bilateral IIA coverage and the selective sacrifice of one or both hypogastric arteries could be done safely during EVAR, even in patients with challenging anatomy. Based on them, associated comorbidities, such as shock, distal embolization or inability to salvage collateral branches from the EIA and femoral arteries, may have contributed to the increased morbidity in the previous IIA interruption reports^[21].

Additionally, surgical ligation of one or both IIAs is occasionally needed, for instance in renal transplantation, and life-threatening conditions such as gynecological emergencies have been performed without adverse effects. Iliopoulos *et al.*^[22] studied the circulation of IIA during various open aortoiliac surgeries and found that the ipsilateral EIA branches and common femoral arteries contribute to IIA circulation more than the contralateral IIA. This was proved in the acute setting, and it is likely to be right in the long term^[22].

Furthermore, the preservation of the superior-inferior gluteal system is crucial for the pelvic viscera to maintain the collateral circulation from the ipsilateral deep femoral artery via the inferior gluteal artery and prevent buttock claudication. Fujioka *et al.*^[23] recommended that deep femoral arterioplasty during EVAR may be needed in those with an advanced stenotic lesion at its origin as a valuable means to decrease buttock claudication following the IIA occlusion.

In the current study, we tried hard to preserve the IIA if there was a sufficient landing zone. In those cases where we covered one or two IIAs, there were no significant differences in complications. There was no bowel ischemia and spinal cord ischemia in either group; however, there was one patient who suffered buttock claudication in the IIA coverage group.

Data about buttock claudication should be analyzed cautiously as most of the studies are not comprehensive and do not objectively assess the symptoms^[13,17,24]. Fujioka *et al.*^[23] reported no buttock claudication in their study with 71 patients following the embolization of the IIA proximally and two weeks before the EVAR, allowing the collateral to form properly. Based on the study by Bosanquet *et al.*^[17], catastrophic ischemic events such as gluteal, bowel and spinal ischemia are rare (< 1%), and the actual rate rates could be less than those reported. In their systematic analysis^[17], they showed a clear reduction in reporting of these complications in papers published before 2007 (3.6%) compared to those published after 2007 (0.9%) ($P < 0.001$). The reasons could be multifactorial, such as increased plugs use, a greater understanding of the IIA circulation, and enhanced operator experience^[13,17,23]. Additionally, the etiology and assessment of these reported complications may be complicated as the majority of the patients were of advanced age with common comorbidities, such as diabetes^[8,17,25,26].

In our case, the procedure time, HDU stay and total hospital stay were significantly higher in the IIA sacrifice group compared to the IIA salvage group. However, there was no statistically significant difference in the postoperative complications from sacrificing IIAs.

Study limitations

Our study is a retrospective study, and we were limited to a small number of patients. Similarly, we did not use IBD in our patients. The usage of IBD could be jeopardized by the diameter of IIAs, as there was a

statistically significant higher size of IIA in the IIA sacrifice group. We did not perform quality of life and subgroup analysis with unilateral and bilateral IIA coverage. Furthermore, the lack of standardized way to assess erectile dysfunction and buttock claudication might be responsible for the lower erectile dysfunction and buttock claudication mentioned in the current study.

In conclusion, Although the IIA sacrifice group had higher total hospital and high dependency unit stay, there were no significant differences in postoperative complications, three-year freedom from reintervention and aneurysm-related as well as overall survival. Based on our experience, the coverage of IIA aneurysmal extension during endovascular repair of the aortoiliac aneurysmal disease seems technically feasible and safe.

DECLARATIONS

Authors' contributions

Concept and design: Ghoneim B, Tawfick W, Sultan S

Analysis and interpretation, writing the article, critical revision and final approval of the article, overall responsibility: Ghoneim B, Canning P, Acharya Y, Hynes N, Tawfick W, Sultan S

Data collection: Ghoneim B, Canning P, Acharya Y

Statistical analysis: Ghoneim B, Canning P, Acharya Y, Tawfick W

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The study is approved by the Galway Clinical Research Ethics Committee (C.A. 1210, 13/02/2015).

Consent for publication

Not applicable.

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Original Article

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Usefulness of frozen elephant trunk technique for distal aortic arch aneurysms

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Abstract

Aim: The effectual use of frozen elephant trunk (FET) has been for total aortic arch replacement (TAR) of acute aortic dissection because of positive aortic remodeling. However, the use of FET in the non-dissecting aortic arch aneurysm is still controversial. We aim to investigate the outcomes of TAR using the FET technique for distal aortic arch aneurysms.

Methods: Between August 2014 and February 2020, 40 patients (35 males, mean age 77.0 years) underwent TAR by using the FET technique with the J Graft Open Stent Graft for distal aortic arch aneurysms including 8 patients with shaggy aorta. In 5 of 40 patients, coronary bypass grafting was concomitantly performed. We followed up for 29.0 months.

Results: The mortality were 0%. Stroke occurred in three patients (7.5%) one of whom had shaggy aorta, paraparesis in one patient (2.5%) who recovered fully, and respiratory complication in two patients (5.0%). There was no recurrent nerve palsy. During the follow-up period, death had no relationship with aortic disease.

Conclusion: We conclude the FET has the potential to improve TAR for distal aortic arch aneurysms.

Keywords: Aortic arch aneurysm, frozen elephant trunk, open stent, left recurrent nerve palsy, shaggy, total aortic arch replacement



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INTRODUCTION

The frozen elephant trunk (FET) technique was initially developed in 1994 as one of the first types of hybrid surgery^[1] to combine endovascular treatment and open surgery and has since been disseminated worldwide. This technique has been widely adopted as a single-stage open aortic repair technique for non-dissecting arch aneurysm with a downstream extension or a first-stage procedure^[2], followed by a second open repair or thoracic endovascular aortic repair. In cases of acute or chronic aortic dissection, this technique is a useful additional total aortic arch replacement (TAR) procedure; in such cases, FET is used to reinforce the distal anastomosis of the TAR and close the false lumen of the descending aorta^[3]. In Japan, handmade FET device had been used in some hospitals since 1994. The first commercial product (JG open stent) was introduced in 2014 and modified to Frozenix in 2017. Although this graft has been widely used with concomitant TAR in Japan, the indication for non-dissecting distal aortic arch aneurysm is still controversial^[4]. We studied the outcomes of TAR by the use of the FET technique for distal aortic arch aneurysms.

METHODS

Between August 2014 and February 2020, 40 patients underwent TAR for distal aortic arch aneurysms using the FET technique at Tenri Hospital and Nara Prefecture General Medical Center. Informed consent was obtained from all patients on their information. We have followed up all patients until June 2020. The preoperative patient characteristics are listed in Table 1. The age at surgery was 77.0 ± 6.0 years (16 octogenarians, range 59-87 years), and the number of males was 35 (87.5%). The number of fusiform distal aneurysm was over half. The rate of saccular type aneurysm and the extension of aneurysm was 35% and 10%. Surgical indication of fusiform distal aneurysm is more than 55mm of diameter. But the indication of saccular type aneurysm depends on configuration, diameter and enlargement rate. Preoperative comorbid diseases were hypertension in 32 patients (80.0%), dyslipidemia in 16 (34.7%), chronic obstructive pulmonary disease in 13 (32.5%), coronary artery disease in 10 (25.0%), stroke in 9 (22.5%), diabetes mellitus in 9 (22.5%) and chronic renal dysfunction, (defined serum creatinine ≥ 1.2 mg/dL, in 10 patients (25%). Six patients of them (15%) had aortic surgery previously.

FET selection

We used Frozenix (or JG Open Stent) in all cases. The available sizes of Frozenix as follows, the diameter of Frozenix: 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 mm. The length of stented graft: 6, 9, 12 cm. The optimal FET was selected with use of preoperative computed tomography. The length of FET was decided by measuring the distance between the site of aortic resection and the intended location of the descending aorta. We rarely use the unstented part of FET and therefore removed all but 1 cm of the unstented part to prevent graft kinking. In other word, the distance from the distal end of FET should be no more than the stented part + 1 cm. The diameter of FET was approximately 10% greater than the diameter of the descending aorta at the planned location of attachment.

Operative procedure

The operation was performed through median sternotomy. We used 2 main arterial cannulation sites; the ascending aorta in most of patients and the axillary artery in the shaggy aorta. Venous cannula was inserted into right atrium. Subsequently, the total extracorporeal circulation was initiated. After cooling to achieve a bladder temperature of 25 °C, the ascending aorta was cross-clamped, and cold blood cardioplegia was infused to the aortic root. After circulatory arrest, the proximal left subclavian artery was ligated, and we started cerebral perfusion (200 mL/min) via an 8-mm graft anastomosed to the left axillary artery. After aortic arch incision, selective cerebral perfusion was established from the brachiocephalic artery (400 mL/min) and the left common carotid artery (200 mL/min). Total cerebral perfusion was 800 mL/min and was performed by one roller pump^[5]. We closely monitor near infrared spectroscopy and artery pressure in

Table 1. Preoperative patient characteristics

Number	40
Age	77.0 ± 6.0 years (59-87)
Male	35 (87.5%)
Type of aneurysms	
Fusiform distal aneurysm	21 (52.5%)
Saccular type aneurysm	14 (35%)
The extension of aneurysm	4 (10%)
Penetrating aortic ulcer	1 (2.5%)
Hypertension	32 (80.0%)
DM (insulin)	9 (22.5%)
DL	16 (34.7%)
CVD	19 (47.5%)
Renal dysfunction (Cr ≥ 1.2)	10 (25.0%)
COPD	13 (32.5%)
Precious aortic surgery	6 (15.0%)

DM: diabetes mellitus; DL: dyslipidemia; CVD: cerebrovascular disease; Cr: creatinine; COPD: chronic obstructive pulmonary disease

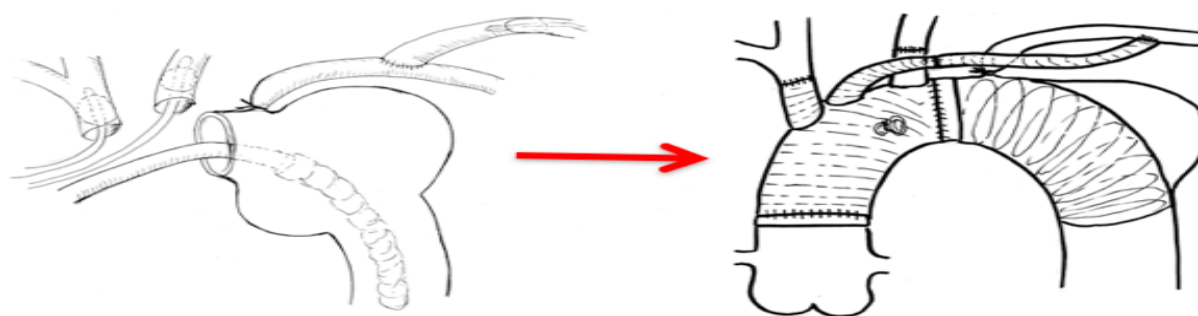


Figure 1. Procedure of total arch replacement with the frozen elephant trunk technique. The frozen elephant trunk was inserted through the transection site into the intended distal landing portion by guiding transesophageal echocardiography. We constructed the distal anastomosis with a separated 4-branched graft and antegrade systemic circulation was restarted through the side branch of the graft. The left common carotid artery and brachiocephalic artery were then anastomosed to their respective graft branches. After completion of the proximal anastomosis, the aortic graft was declamped and the 8 mm graft connected to the left subclavian artery was finally anastomosed with one branch of the graft

bilateral upper extremities under cerebral perfusion in all cases. The aortic arch was dissected transversely between the left common carotid artery and the left subclavian artery. The FET was inserted through the transection site into the intended distal landing portion, which was positioned up to the T8 level to prevent spinal cord injury (SCI). We confirmed the distal end of the FET by transesophageal echocardiography (TEE)^[6]. We constructed the distal anastomosis with a separated 4-branched graft that was reinforced with Teflon felt strips and antegrade systemic circulation was restarted through the side branch of the aortic arch graft and the patient was rewarmed by extracorporeal circulation. The left common carotid artery and brachiocephalic artery were then anastomosed to their respective graft branches. After completion of the proximal anastomosis, the aortic graft was declamped and the 8mm graft connected to the left subclavian artery was finally anastomosed with one branch of the arch graft [Figures 1 and 2].

Statistical analysis

Continuous variables are expressed as the mean ± standard deviation. The Kaplan-Meier method was used to estimate mid-term outcome. Statview for Windows, version 5.0 (SAS Institute Inc., Cary, NC), was used for the statistical analyses. Data were presented as mean ± SD, as appropriate.

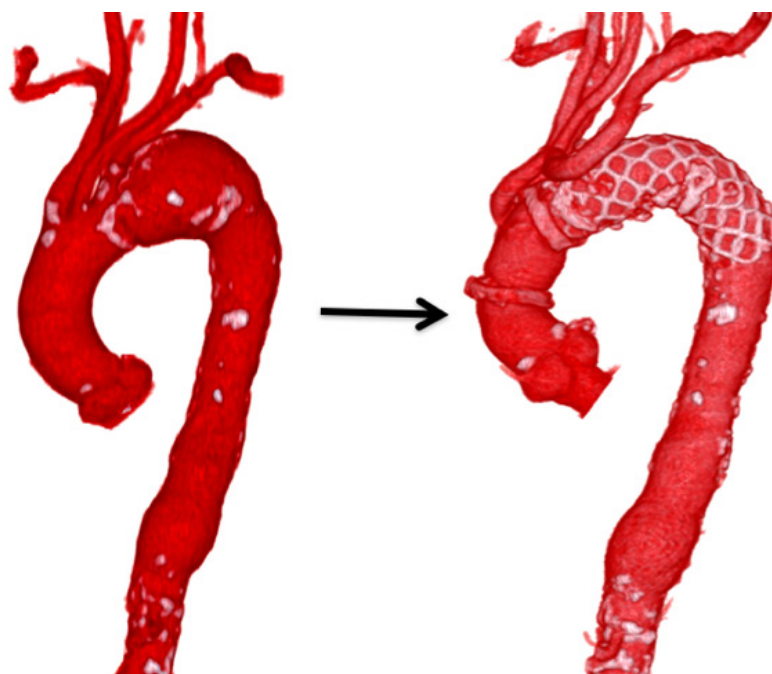


Figure 2. 3D-CT image. Left side: preoperative distal aortic arch aneurysm; right side: total arch replacement with frozen elephant trunk

Table 2. Perioperative data

Operation time	317 ± 66 min
CPB time	164 ± 28 min
SCP time	91 ± 24 min
CA time	45 ± 9 min
CI time	95 ± 29 min
Bladder temperature	25.1 ± 0.5 °C

CPB: cardiopulmonary bypass; SCP: selective cerebral perfusion; CA: circulatory arrest; CI: cardiac ischemia

Table 3. FET data

FET diameter	30.7 ± 3.0 mm
FET length	11.4 ± 1.2 cm
Thoracic vertebra level of FET end	6.4 ± 1.0

FET: frozen elephant trunk

RESULTS

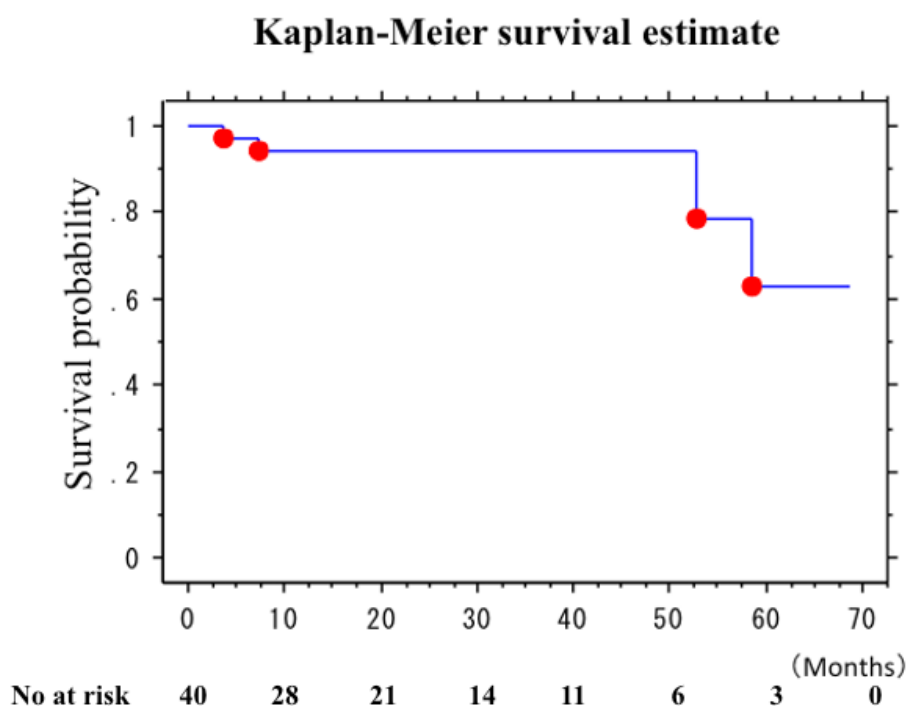
Success rate of aneurysm exclusion by the method was 100%. There was no type II endoleak in all cases and type Ib endoleak in one case, which was not connected to the aneurysm. Perioperative data are listed in [Table 2](#). The implantation of the FET achieved in all cases under moderate hypothermic circulatory arrest. Circulatory arrest was achieved at bladder temperature of 25.1 ± 0.5 °C. The extracorporeal circulation time was 164 ± 28 min, heart ischemic time and arrest time were 95 ± 29 min (range 57-173 min) and 45 ± 9 min (range 34-73 min), respectively. According to postoperative CT, the distal end of the FET was in the T5-T9 range, the mean level of T6.4 [[Table 3](#)].

Postoperative patient characteristics are listed in [Table 4](#). Hospital mortality was 0%. Mean ICU stay was 3.4 ± 5.2 days. Mean hospital stay was 25.3 ± 29.6 days. Stroke occurred in three patients (7.5%) including one with shaggy aorta. One of the three patients developed paralysis on right side of body postoperatively,

Table 4. Mortality and morbidity

Hospital mortality	0 (0.0%)
ICU stay	3.4 ± 5.2 days
Hospital stay	25.3 ± 29.6 days
Cerebral infarction	3 (7.5%)
Paraparesis	1 (2.5%)
Respiratory failure	2 (5.0%)
Recurrent nerve palsy	0 (0.0%)
distal embolism	0 (0.0%)
Re-operation for bleeding	0 (0.0%)

ICU: intensive care unit

**Figure 3.** Kaplan-Meier survival curve for overall survival in patients with total arch replacement with frozen elephant trunk. The cause of death was not related to aortic disease. The survival rate at three years after the frozen elephant trunk was 94.3%

which had disappeared before discharge. The second patient developed paralysis on left side of body while the third patient had small cerebral infarction due to right internal capsule. Paraparesis was reported in one patient (2.5%) who recovered fully and discharged with normal gait. Respiratory complication was reported in two patients (5.0%) who had acute respiratory distress syndrome required tracheotomy. No patients had distal embolism nor new left recurrent nerve palsy.

Postoperative follow-up

All patients were followed up for 29.0 months (range 5-68 months), during which, four patients died. The causes of death were pneumonia in two patients (postoperative 3 and 52 months) and cancer in two patients (postoperative 7 and 58 months). The cause of death had no relationship with aortic disease. The survival rate at three years after the FET was 94.3% [Figure 3].

DISCUSSION

“Open stent” graft implantation in Japan has become established worldwide as the FET technique since the launch of commercially available products for the European and Chinese markets in the 2000s. In Japan,

Frozenix was introduced in 2014, and up to November 2016 the accumulated clinical experience has exceed 4,000 cases. Frozenix, which consists of double-layered oval-shaped nitinol stents, maintains its shape when flexed, thus preventing undue force on the curved aortic wall^[3]. A soft polyester sheath delivery system composes of a slippery material to prevent damage to the descending aorta wall during insertion. Frozenix is deployed by withdrawing the sheath after the device is correctly positioned by TEE guidance. Accordingly, these features of Frozenix enable safe and reliable device insertion. There was no FET failure nor stent fracture of FET in this study. We have had no graft failure of Frozenix (JGOS) in the pilot study since 2008 from 2010^[3]. However, we have the experience of late endoleak (type Ib) in the pilot study, which needed re-intervention by thoracic endovascular aortic repair. The selection of appropriate size of FET (diameter and length) is very important to prevent type Ib endoleak^[7].

The FET technique should be considered suitable for patients with extensive thoracic or thoracoabdominal aortic disease if a second class IIa procedure (open repair or thoracic endovascular aortic repair) is anticipated in downstream aortic segments, according to a European position statement^[8]. This way, the operative indications of the FET for non-dissecting aortic aneurysms are still limited because TAR is gold standard for the aortic arch aneurysm and the FET has the risk of SCI. The exact mechanism of SCI in the FET is unknown. Thromboembolism, long stent graft, and circulatory arrest time are supposed as factors of SCI^[9]. Presumably, the most important risk factor is the distal FET position below the level of T8. We recommend using TEE, fluoroscope, or other modalities to deploy the FET more precisely^[10,11]. We always use TEE for the FET technique. Our three-step method by TEE guidance is easy and safe^[6]. In our institutions, there was no permanent SCI in 113 patients with the FET (48 acute aortic dissections, 19 chronic aortic dissections, and 46 non-dissecting thoracic aneurysms. Although I trust CSF drainage, we don't preoperatively place CSF drain in elective cases because SCI rate was low in our institutions and there is 5%-7% complication related CSF drainage. In the patient with paraparesis, we performed CSF drainage postoperatively, kept more than 80 mmHg mean system pressure and administered Naloxone and steroid. Thereafter, paraparesis disappeared and the patient discharged by foot. The maintenance of high systemic pressure in postoperative period is another important point to prevent SCI. Our target mean systemic pressure is more than 80 mmHg. Therefore, complete hemostasis is essential. In J-ORCHESTRA study (Japanese multicenter study)^[12], the rate of SCI in the FET group was 1.6%. Although the rate is still relatively higher, Japanese surgeons mostly overcame SCI due to the FET.

Another problem is distal embolism. No patient in this study had a distal embolism. Although one of 8 patients with shaggy aorta had small cerebral infarction despite isolation technique, other patients had no cerebral infarction and no distal embolism. It is speculated that insertion of the FET under circulatory arrest never induce disturbed flow or dissipation of plaque and cover the shaggy aortic wall. According to severity or location of shaggy aorta, the use of the FET may prevent distal embolism although the use of the FET in severe shaggy aorta is presumably high risk for embolism. Some surgeons have used distal perfusion from the femoral artery after FET deployment and fixation to remove debris/air and protect distal function and Some surgeons have used thoracic perfusion with balloon-tipped Foley catheter into the end of FET to reduce visceral ischemia time. Although we also tried distal perfusion by these techniques in initial some cases, we gave up distal perfusion because it is difficult to keep bloodless field, and it makes operative procedure more complicated and time-consuming.

As stated above, the complication of the FET technique has been declining in Japan and we suppose the indication of FET can extend some high-risk patients of non-dissecting thoracic aortic aneurysms as the following patients: (1) those with distal aortic arch aneurysms that cannot be treated by thoracic endovascular aortic repair involving the left subclavian artery to the ascending aorta; (2) those in whom a median approach extending to the upper middle descending aorta for distal anastomosis with pulmonary complication is difficult; (3) those requiring redo operation; and (4) and those with an advanced age or frailty who prefer to avoid left thoracotomy.

Additionally, the FET has some attractive advantages as follows: (1) the proximalization of distal anastomosis to zone 2; (2) the simplification of technical complexity; (3) the easy deployment of the FET; (4) the shortening of lower body ischemic time; and (5) fixation of stented graft to avoid graft migration. Furthermore, postoperative hoarseness can be avoided by the use of the FET because the distal anastomosis site lies off the left recurrent nerve at the distal aortic arch. Hoarseness may induce to aspiration pneumonia in elderly patients. The left recurrent nerve palsy was 0% in TAR with the FET (40 patients) and 5% in that without the FET (121 patients) in our other study^[13,14]. Therefore, the FET technique may better preserve postoperative laryngeal function in elderly frail patients.

In conclusion, the FET attained the proximalization of distal anastomosis to zone 2 rather than zone 3^[15]. It caused decreases in incidences of cardiac arrest, selective cerebral perfusion, and visceral ischemia. It further helped to reduce the incidence of recurrent nerve palsy, the ease of anastomosis, and control of hemorrhage. The FET has the potential to improve TAR for distal aortic arch aneurysms.

DECLARATIONS

Authors' contributions

Authors made substantial contributions to the conception and design of the study, and participated in drafting the article: Yamanaka K

Authors provided administrative, technical, and material support: Iwakura A

The author participated in critical revision for important conceptual and intellectual content and gave final approval of the version to be submitted to the Journal: Fujita M or more authors: Nishina T, Sekine Y

Performed data acquisition, as well as provided administrative, technical, and material support: Sato S, Yada M, Tara Y

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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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Current challenges in TAVI: neo-commissural alignment to mimic more physiologic valve implantation

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Abstract

Commissural alignment during transcatheter aortic valve implantation (TAVI) has important clinical implications as TAVI expands to younger patients in whom lifetime treatment of aortic valve disease and coronary artery disease is of particular importance. Numerous studies have shown that lack of commissural alignment may adversely affect coronary reaccess and the feasibility of redo-TAVI in this patient population. To assess the risk of commissural misalignment more accurately, we have pioneered and validated the use of a preprocedural imaging protocol that determines valve orientation using multi-detector computed tomography-fluoroscopy co-registration. Furthermore, we have shown that a modified delivery system insertion technique during initial valve deployment results in improved commissural alignment and reduced coronary artery overlap following TAVI with a self-expanding device. However, numerous unanswered questions remain about the impact of commissural misalignment on balloon-expandable valve-in-valve TAVI, especially in patients with unfavorable aortic root anatomy. It is imperative that clinicians consider these anatomic, device-related, and procedure factors, among others, when evaluating patients for transcatheter therapies.

Keywords: Aortic stenosis, commissural alignment, aortic valve, coronary artery disease



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INTRODUCTION

With the conclusion of the recent low-risk trials, transcatheter aortic valve implantation (TAVI) has now been approved for patients with symptomatic, severe aortic stenosis across all surgical risk categories^[1,2]. Amidst these rapidly evolving clinical indications, several issues related to the long-term durability and feasibility of TAVI in younger patients have been raised by the structural heart community. One such example is transcatheter heart valve (THV) orientation during initial deployment and its impact on commissural alignment. Whereas direct visual inspection and excision of native leaflets during surgical aortic valve replacement (SAVR) readily allows alignment of the surgical valve commissures with the native commissures, commissural alignment with THVs during TAVI is far more inconsistent and random^[3]. Commissural malalignment may lead to varying degrees of overlap between the neo-commissural posts and coronary arteries^[4,5]. Furthermore, experimental models have shown that THV leaflet stress and central aortic regurgitation (AR) may be exacerbated with suboptimal commissural alignment^[3,6]. These findings have significant clinical implications for younger patients who have an increased lifetime risk of complications of aortic valve disease and coronary artery disease. Given that coronary reaccess and redo-TAVI will become more prevalent in the future, achieving commissural alignment during initial TAVI may impact the feasibility of both these procedures. Here, we review some of the salient features of neo-commissural alignment and offer our perspectives on how to achieve a more physiologic valve implantation.

CORONARY REACCESS

Contemporary THV device and delivery system designs do not allow for consistent and precise commissural alignment. Following initial TAVI, an obstructive commissural post may significantly hinder future coronary access by extending above, in front of, or through the coronary ostia^[5]. Coronary reaccess is impeded not just by the obstructive THV stent frame, but also by an *in situ* barrier formed by the native aortic leaflets. Thus, the anatomy relating the aortic root to the valve stent frame must be thoroughly evaluated. The coronary arteries are easily reached when the sinotubular junction (STJ) or coronary ostia are situated distal to the transcatheter valve stent frame. However, when either of these structures is located below the THV frame, the TAVI operator would need to cross the stent frame to access the coronary arteries. This may not be a major issue when using THVs with short stent frame heights provided that the native aortic valve does not obstruct the open cells of the stent frame^[7].

REINTERVENTION AFTER INITIAL TAVI

Commissural misalignment during initial TAVI also jeopardizes the success of future valve-in-procedures for the treatment of prosthetic valve failure. While TAVI-in-SAVR is a relatively simple procedure, redo-TAVI in the current era is associated with a number of anatomic risks, including coronary obstruction, that are exacerbated in the absence of neo-commissural alignment^[8]. Thus, consideration of the THV leaflet height within the anatomic boundaries of a patient's aortic root becomes crucial when evaluating him or her for redo-TAVI. As before, if the leaflets lie proximal to the coronary ostia, valve-in-valve TAVI should be feasible. If not, an adequate margin between the valve stent frame and sinotubular junction is imperative to prevent sinus sequestration and coronary obstruction^[7].

In recent years, the BASILICA (Bioprosthetic or native Aortic Scallop Intentional Laceration to prevent Iatrogenic Coronary Artery obstruction) procedure has been successfully applied in TAVI-in-SAVR cases with a high risk of coronary obstruction^[9]. However, this procedure may not be as easily performed during redo-TAVI, especially with supra-annular THVs since leaflet splitting can be impeded by the valve stent frame. In cases of commissural misalignment that appose the commissural post to a coronary ostium, the BASILICA technique may not completely eliminate the possibility of coronary obstruction in redo-TAVI.

IMAGING-BASED ASSESSMENT OF THV ORIENTATION

Accurate imaging is essential for preprocedural planning. To assess the risk of commissural misalignment and severe coronary overlap, our group pioneered the technique of determining THV orientation using multi-detector computed tomography (MDCT)-fluoroscopy co-registration^[10]. Briefly, we begin by measuring the en-face angle between the left main and right coronary arteries using MDCT. Next, using the 3Mensio Valves software (Pie Medical Imaging version 9.1, Maastricht, Netherlands), we capture the THV orientation in the three-cusp coplanar fluoroscopic view and co-register it onto our coplanar MDCT axial images. We can then superimpose a virtual image of either the SAPIEN 3 THV (Edwards Lifesciences LLC, Irvine, CA, USA) or the Evolut THV (Medtronic Inc., Minneapolis, MN, USA) over the axial MDCT annular and sinus of Valsalva images derived from the 3Mensio Valves software. This allows us to determine the degree of overlap between the neo-commissures and the coronary ostia^[11]. Note that the use of various third-party software systems introduces the risk of operator bias that has to be taken into consideration when performing the aforementioned analyses.

IMPACT OF THV TYPE AND CONTEMPORARY RESULTS

We have previously used the aforementioned co-registration technique to assess the relationship between THV deployment orientation and commissural alignment as part of the ALIGN-TAVR (Alignment of Transcatheter Aortic-Valve Neo-Commissures) study. Here, > 30%-50% of the 828 patients who underwent TAVI from 2016-2019 (483 SAPIEN 3, 245 Evolut, and 100 ACURATE-neo) had overlap with at least one coronary artery. More importantly, commissural alignment was unaffected by initial deployment orientation of the SAPIEN 3 THV, but was significantly improved by specific initial orientations of the Evolut and ACURATE THVs^[12]. The nuances between the two main types of commercially available THVs in the context of the ALIGN-TAVR and other contemporary studies are discussed below.

SAPIEN 3

The balloon-expandable SAPIEN 3 valve can have one commissure crimped at the 3, 6, 9, or 12 o'clock orientation relative to the delivery catheter to track the initial deployment orientation. In the ALIGN-TAVR study, commissural alignment was not improved by crimping the SAPIEN 3 THV at each of the aforementioned orientations^[12]. We speculated that this may be due to the flexibility of the delivery catheter as it courses through the aorta. Fortunately, the unassuming profile of SAPIEN 3 stent frame renders commissural alignment less pertinent for coronary reaccess as wires and catheters can engage the coronary ostia above and through the top row of the stent frame. Coronary access can nevertheless be challenging in certain cases where the SAPIEN 3 stent frame protrudes beyond a narrow STJ^[13,14].

Similar findings were reported by Rogers *et al.*^[15] in their study of 137 low surgical risk patients from the LRT (Low Risk TAVR) trial (NCT02628899) who underwent balloon-expandable TAVI. Using post-TAVI MDCT analysis, the authors found that 9%-13% of patients displayed high-risk alignment due to the valve stent frame extending distal to the coronary ostia and an obstructive commissural post. Similar to the ALIGN-TAVR study, commissural alignment was not significantly influenced by intentional crimping of the transcatheter valve. The THV stent frame protruded beyond the STJ in 21% of patients, and the THV-STJ margin was < 2 mm in 13% of patients. Patients with THV-STJ margin < 2 mm were deemed anatomically unsuitable for redo-TAVI given the excessive perceived risk of coronary obstruction^[15]. Despite limited generalizability, the findings from this anatomic simulation corroborate the results from our pilot angiographic study that show that redo-TAVI may not be possible in > 20% of SAPIEN 3 patients and more than half of those with unfavorable aortic root anatomy (sinus height < THV height)^[16].

Evolut

Commissural alignment is particularly important for facilitating coronary reaccess following Evolut TAVI since this supra-annular valve extends above the STJ and coronary ostia. The Evolut THV has a unique "Hat"

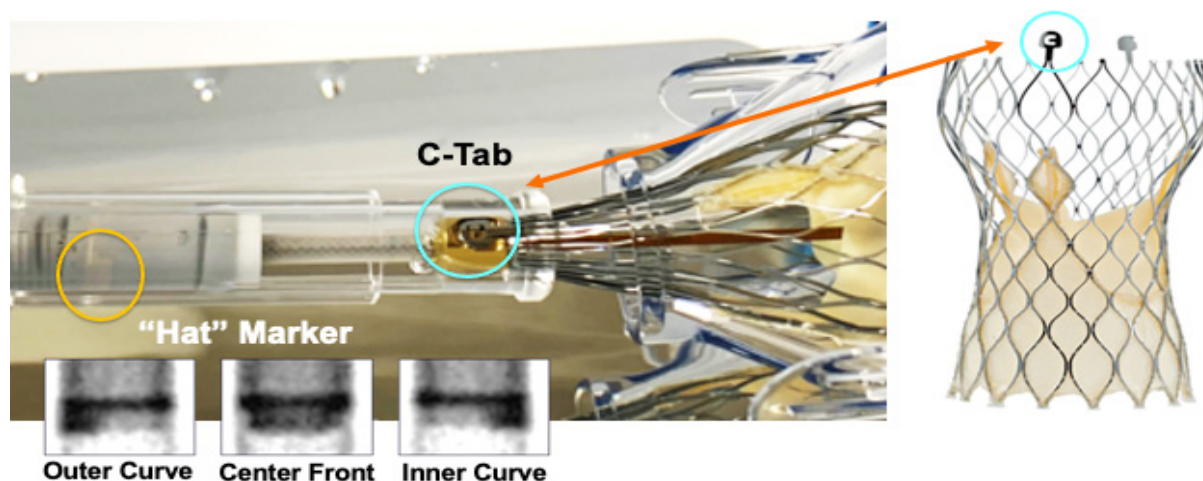


Figure 1. Orientation-specific deployment of the evolut transcatheter heart valve

marker that can be oriented during initial deployment in one of four positions: anterior or posterior in the center of the deployment device [center front (CF) or center back (CB)] or in the inner (IC) or outer curve (OC) of the aortic annulus [Figure 1]. In the ALIGN-TAVR study, positioning the Evolut “Hat” at OC or CF during valve deployment resulted in significantly less severe coronary overlap than IC/CB positioning [Figure 2]. Furthermore, tracking the “Hat” marker at the outer curve of the descending aorta improved deployment at the OC of the aortic annulus from 70.2% to 91.6% ($P = 0.002$) and reduced coronary artery overlap by 36%-60% ($P < 0.05$). Perhaps most importantly, we found that the best method to achieve OC/CF “Hat” orientation involves starting with the flush port at 3 o’clock during insertion of the delivery catheter into the femoral artery^[12].

The above findings were validated in a recent analysis of the impact of delivery system insertion technique on commissural alignment. Here, 154 of 249 patients from the Evolut Low Risk trial (NCT02701283) CT sub-study who underwent transfemoral TAVI using the conventional delivery system insertion technique (flush port at 12 o’clock) were compared to 240 patients from our institution who underwent deployment using a modified technique (flush port at 3 o’clock). Unsurprisingly, the modified technique significantly improved “Hat” marker orientation at OC/CF during initial deployment (93.1% vs. 69.6%, $P < 0.001$), improved commissural alignment, and reduced severe coronary overlap (left main artery: 15.2% vs. 27.7%, $P = 0.004$; right coronary artery: 11.8% vs. 27.7%, $P < 0.001$)^[17]. Note that we insert the delivery catheter at 3 o’clock but let the system self-rotate as it goes inside the body. We do not force the system to maintain the 3 o’clock position as it tracks to the annulus, just like we never force the catheter to maintain the 12 o’clock position when using the conventional technique. We do not recommend force-rotating the delivery catheter inside the patient given the risk of damaging the catheter.

The mechanisms underlying the improved results with the modified insertion technique remain nebulous but may be related to the location of the spine within the Evolut delivery system. Significant columnar rotation may be limited by the two spines inside the delivery system as this is advanced along the aorta, thus maintaining the “Hat” orientation at THV deployment. This technique should only be performed in the descending aorta, and caution other operators that further validation is required to ascertain its safety^[12].

Similar to the Evolut THV, the self-expanding JenaValve system makes use of clipping mechanisms that enhance valve positioning and fixation. It remains to be seen whether this anatomic positioning translates into a reduced risk of coronary artery overlap^[14].



Achieving neo-commissural alignment during initial TAVI has important clinical implications for future coronary reaccess and aortic valve reintervention, especially in younger patients in whom lifetime treatment of aortic valve and coronary artery disease must be taken into consideration. Although we have shown that a modified delivery system insertion technique during initial valve deployment results in better commissural alignment and less coronary overlap following self-expanding TAVI, further studies are needed to affirm the reproducibility of this strategy. Additionally, unanswered questions remain about the impact of commissural misalignment on balloon-expandable valve-in-valve TAVI, especially in patients with unfavorable aortic root anatomy. It is imperative that clinicians consider these anatomic, device-related, and procedure factors, among others, when evaluating patients for TAVI.

Commissural alignment is particularly important for facilitating coronary reaccess following implantation of the Evolut system since this supra-annular valve extends above the sinotubular junction and coronary ostia. The Evolut valve has a unique “Hat” marker that can be oriented during initial deployment in one of four positions: anterior or posterior in the center of the deployment device (center front or center back)

or in the inner/outer curve of the aortic annulus (fluoroscopic insets, bottom left). Note that the “C-tab” is loaded 90° clockwise from the “Hat” marker.

Fluoroscopic images depicting deployment of a 26 mm Evolut Pro valve are shown here. Note that the “Hat” marker is positioned anteriorly in the center of the deployment device (“center front”), with the C-tab seen at the inner curve of the ascending aorta. Multi-detector computed tomography analyses indicate good commissural alignment.

DECLARATIONS

Authors' contributions

Conception and design, analysis and interpretation of data; drafting of the manuscript or revising it critically for important intellectual content; and final approval of the manuscript submitted: Sengupta A, Alexis SL, Kovacic JC, Tang GHL

Availability of data and materials

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Dr. Tang GHL is a consultant for Medtronic, Abbott Structural Heart, and W. L. Gore & Associates. All other 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

Open Access



Routine use of cerebral protection devices during transcatheter aortic valve implantation: what does the evidence say?

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Abstract

Transcatheter aortic valve implantation (TAVI) is a well-established treatment for symptomatic severe aortic stenosis in intermediate and high-risk patients. However, as TAVI indications increase, concerns regarding adverse events and complications rise in the same proportion. Stroke is one of the most feared TAVI complications and a hard endpoint present in all TAVI studies. TAVI-related stroke incidence becomes even more relevant with TAVI indications spreading to younger, low/intermediate-risk patients. Several devices have been developed to prevent this catastrophic event, some of them being broadly used. Nevertheless, the evidence for routine use of cerebral embolic protection devices is still controversial.

Keywords: Transcatheter aortic valve replacement, transcatheter aortic valve implantation, cerebral protection device, cerebral protection system, Sentinel Cerebral Protection System, stroke prevention



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INTRODUCTION

Transcatheter aortic valve implantation (TAVI) is a well-established and widespread option to treat severe aortic stenosis in intermediate and high-risk patients. However, its increased use brings some intrinsic concerns, especially in low-risk and younger patients, a population in which even a low rate of adverse events can be catastrophic^[1-7]. In this setting, to reduce or, ideally, to eliminate neurological complications is especially relevant.

To mitigate neurological events risk, cerebral embolic protection devices (CEPD) were developed aiming to capture embolized debris and/or to prevent them from reaching the cerebral circulation. Even with some evidence supporting CEPD benefits and safety, the lack of a single randomized clinical trial demonstrating reduction in hard outcomes, such as stroke and mortality, has limited the widespread acceptance of CEPD and its routine use.

This article offers an updated state-of-the-art review on CEPD use and which patient profile is most likely to benefit from this therapy.

Issue relevance

The incidence of clinically relevant neurological events after TAVI varies from 1 to 10%, but it can be as high as 94% if silent events detected by brain imaging are also considered^[8]. The majority of post-TAVI strokes have an embolic origin and occur in the early post-TAVI period (64% and 85% at 2 and 7 days, respectively). These are referred to as procedure-related neurological events.

Calcium debris embolization can happen during catheter and wire manipulation, valve implantation, pre-dilatation, and/or post-dilatation^[2,5]. Compared to native valves, valve-in-valve and bicuspid aortic valve are associated with higher stroke rates due to the need for increased valve manipulation or the presence of highly calcified anatomies. Debris embolization can also be secondary to small thrombus formation or embolization from atherosclerotic plaques in the ascending aorta and aortic arch.

Regarding the clinical relevance, patients who suffer a stroke are at high risk for mortality and severe morbidity including physical disability^[9,10]. In a meta-analysis conducted by Eggebrecht *et al.*^[11], patients with cerebrovascular events presented a 3.5-fold higher 30-day mortality than those without events (25.5% vs. 6.9%, respectively). In another study, short- and long-term mortality risks were incremental according to cerebrovascular events severity, with a significantly higher mortality rates in the presence of major stroke [30-day mortality: odds ratio (OR) = 7.43; 95% confidence interval (CI): 2.45-22.53; $P = 0.001$, late mortality: hazard ratio (HR) = 1.75; 95%CI: 1.01-3.04; $P = 0.043$]^[12]. Similarly, a meta-analysis of 29,034 patients showed a 30-day mortality following stroke of 12.27%, with stroke-related mortality of 28.22%, compared with 6.4% mortality in patients without a stroke (OR = 6.45; 95%CI: 3.9-10.66; $P < 0.0001$)^[13]. Furthermore, it is valid to emphasize that 30-day permanent disability is found in around 50% of patients who have suffered a stroke^[14] and that even silent cerebral emboli are associated with worse outcomes, three times higher risk of clinical stroke, two times higher risk of dementia and declined cognitive function.

Notwithstanding, cerebrovascular events present a high impact on patient's quality of life, a consequence even more feared than death. Interesting research showed that, in terms of postoperative perspectives, the majority of patients undergoing TAVI had as their primary objective the maintenance of their independence and being able to practice daily hobbies, but only 7% had staying alive after the procedure as their main goal^[15]. These results highlight the importance of patients' quality of life as endpoint, which should be considered during the TAVI decision-making process.

Table 1. Main cerebral protection devices

	Coverage	Access site	Delivery sheath	Pore size	Mechanism
Sentinel	BCT, LCCA	Radial	6F	140 µm	Capture
TriGUARD	Full arch	Femoral	8F	115 × 145 µm	Deflection
Embella	BCT, LCCA	Radial	6F	100 µm	Deflection
ProtEmbo	Full arch	Radial	6F	60 µm	Deflection
Emblok	Full arch	Femoral	11F	125 µm	Capture
Embol-X	Full body	Transaortic	17F	120 µm	Capture
Emboliner	Full body	Femoral	9F	150 µm	Capture

BCT: brachiocephalic trunk; LCCA: left common carotid artery; µm: micrometers

CEREBRAL PROTECTION SYSTEM

Recent data suggested that CEPD use is associated with less overt strokes, lower total lesion volume, and a smaller number of new ischemic lesions detected by post-procedural magnetic resonance imaging (MRI) studies^[10,16-19].

So far, several CEPD have been developed by many manufactures, including ProtEmbo, Sentinel, TriGUARD, Emblok, Emboline, Embrella, and Embol-X^[20-22]. They vary not only in the mechanism for protection, for instance, capture versus deflection, but also in the access site and delivery sheath size [Table 1]. However, only the Sentinel is already approved by the Food and Drug Administration (FDA), being the most used and studied device. A summary of the current published and ongoing trials regarding cerebral protection during TAVI is presented in Table 2.

Sentinel CPS[®] [Claret Medical (Boston Scientific, Corp, USA)]

The Sentinel CPS is the most studied cerebral protection device. It is made of 2 inter-connected filters deployed into the brachiocephalic trunk and left common carotid artery through a 6 French size sheath^[23]. The most commonly used access is the right radial artery [Figures 1 and 2]^[18].

Three randomized clinical trials (RCT) evaluating the Sentinel's role during TAVI were published in 2016, the MISTRAL-C, the CLEAN-TAVI, and the SENTINEL trial^[22,24,25]. These trials demonstrated device's safety and suggested that Sentinel was associated with fewer and smaller brain lesions on postoperative MRI than unprotected TAVIs.

The MISTRAL-C was the first study to enroll 65 TAVI patients submitted to a protected or unprotected TAVI procedure. New brain lesions on MRI studies were found in 78% of patients, with fewer new lesions number (73% vs. 87%; $P = 0.31$) and total lesion volume [95 mm³ (IQR 10-257) vs. 197 mm³ (95-525); $P = 0.171$] in the protected group. Ten or more new brain lesions were found only in the control cohort (0% vs. 20%; $P = 0.03$), and neurocognitive deterioration was present in 4% of patients with received Sentinel during TAVI vs. 27% in those who did not ($P = 0.017$)^[24]. Similarly, the CLEAN-TAVI study randomized 100 patients in 1:1 fashion to TAVI with or without Sentinel insertion. Post-procedure MRI revealed new cerebral lesions in 98% of patients, with a significant smaller new lesion volume [242 mm³ (95%CI: 159-353) vs. 527 mm³ (95% CI 364-830); $P = 0.001$] and lower number of new lesions two days post-TAVI [4.0 (IQR: 3.00-7.25) vs. 10.0 (IQR 6.75-17.00); $P < 0.001$] in the Sentinel group. These neuro-imaging differences, however, were not translated into a significant reduction in clinical stroke incidence (10% in each group)^[22]. The randomized SENTINEL trial, by its time, included 363 patients with a 2:1 randomization for CEPD vs. no CEPD. Although statistical significance was not achieved, the study demonstrated a strong trend toward stroke reduction within 72 h post-TAVI in the CEPD group compared to the unprotected group (3.0% vs. 8.2%; $P = 0.053$)^[25].

Table 2. Main trials evaluating the use of cerebral embolic protection devices during TAVI

Trial	Year of publication	Device studied	Study design	Endpoints	Population	Main results (device vs. no device)
Published trials						
MISTRAL-C	2016	Sentinel (CE mark and FDA approval)	Randomized clinical trial	Primary endpoint: new cerebral lesions by DW-MRI 5 to 7 days after TAVI	From January 2013 to July 2015, 65 patients randomized 1:1 to transfemoral TAVI with or without Sentinel	Device success: 93% New brain lesions: 78% Absence of new lesions: 13% vs. 27%; $P = 0.31$ Total lesion volume: 95 mm ³ (10-257) vs. 197 mm ³ (95-525) U 10 new brain lesions: 0 vs. 20%; $P = 0.03$ Neurocognitive deterioration: 4% vs. 27%; $P = 0.017$
CLEAN-TAVI	2016	Sentinel	Randomized clinical trial	Primary endpoint: numerical difference in new positive postprocedure DW-MRI brain lesions at 2 days after TAVI in potentially protected territories. Secondary outcome: difference in volume of new lesions after TAVI in potentially protected territories	From April 2013 to June 2014, 100 patients randomized 1:1 to TAVI with or without Sentinel	Device success: 92% New cerebral lesions: 98% Number of new lesions: 4 (3-7.25) vs. 10 (6.75-17); $P < 0.001$ New lesion volume: 242 mm ³ (159-353) vs. 527 mm ³ (364-830); $P = 0.001$ Stroke incidence: 10% vs. 10%
SENTINEL trial	2017	Sentinel	Randomized clinical trial	Primary safety endpoint: MACCE at 30 days Primary efficacy endpoint: reduction in new lesion volume in protected brain territories by MRI at 2 to 7 days after TAVI	19 centers 363 patients randomized 2:1 to TAVI with or without Sentinel	Device success: 94.4% Debris found within filters: 99% MACCE: 7.3% vs. 9.9%; $P = 0.41$ New lesion volume: 102.8 mm ³ vs. 178 mm ³ ; $P = 0.33$ 30 days stroke: 5.6% vs. 9.1%; $P = 0.25$
DEFLECT I	2015	TriGuard (CE mark approval; applied for FDA approval)	Prospective, multicentre trial	Primary safety endpoint: in-hospital device- or procedure-related cardiovascular mortality, major stroke disability, life-threatening bleeding, distal embolisation, major vascular complications, or need for acute cardiac surgery	37 consecutive patients undergoing TAVI with the TriGuard	Successful cerebral coverage: 80% Primary outcome: 8.1% New cerebral ischaemic lesions on post-procedure DW-MRI: 82% Per-patient total lesion volume: 34% lower than historical cohorts (0.2 cm ³ vs. 0.3 cm ³)
DEFLECT III	2015	TriGuard	Randomized clinical trial	Primary endpoint: in-hospital procedural safety (death, stroke, life-threatening or disabling bleeding, stage 2/3 acute kidney injury, or major vascular complications) Secondary device performance endpoint: technical success (successful device deployment, positioning with complete three-vessel coverage)	13 centers in Europe and Israel From February 2014 to March 2015, 85 patients randomized to TAVI with ($n = 46$) or without TriGuard ($n = 39$)	Primary endpoint: 21.7% vs. 30.8%; $P = 0.34$ Technical success: 88.9% Per Treatment population (subjects with complete three-vessel cerebral coverage): new ischaemic brain lesions 26.9 vs. 11.5%; new neurologic deficits 3.1 vs. 15.4%
REFLECT II	2020	TriGuard 3	Randomized clinical trial	Primary safety endpoint (composite of all-cause mortality, stroke, life-threatening or disabling bleeding, stage 2/3 acute kidney injury, coronary artery obstruction requiring intervention, major vascular complication, and valve-related dysfunction requiring intervention at 30 days) Primary efficacy endpoint (composite of all-cause mortality or stroke at 30 days, National Institute of Health Stroke Scale worsening, absence of DWI-MRI lesions post-procedure, and total volume of cerebral lesions by DWI)	25 US centers 295 patients randomized 2:1 to TAVI with or without TriGuard 3	Device successful deployment: 100% Technical success: 71% Primary safety endpoint: 15.9% device vs. 34.4% performance goal; P non-inferiority = 0.0001 Primary efficacy endpoint: 45.7% vs. 54.3%; $P = 0.857$ Median total lesion volume: 215.39 mm ³ vs. 188.09 mm ³ ; $P = 0.405$

PROTAVI-C Pilot	2014	Embrella (CE Mark approval)	Prospective and nonrandomized trial	Periprocedural cerebral lesions assessed by DW-MRI	52 patients who underwent transfemoral TAVI with (n = 41) or without (n = 11) Embrella	Device successfully deployed: 100% 7 days DW-MRI new ischemic lesions: 100% vs. 100% Median number of defects per patient: 8 (3-13) vs. 4 (2-8); P = 0.41 Lesion volume per lesion: 30 mm ³ (20- 50) vs. 50 mm ³ (30-70); P = 0.003 New foci of restricted diffusion: 57% vs. 67%; P = 0.7 Lesion size: 88 ± 60 mm ³ vs. 168 ± 217 mm ³ ; P = 0.27 Lesion volumes in the supply region of the middle cerebral artery: 33 ± 29 mm ³ vs. 76 ± 67 mm ³ ; P = 0.04 Device successfully positioned: 100% Significant debris capture: 90% 30-day MACCE: 0% New ischemic defect post-procedural DW-MRI: 95% Median number of new lesions per patient: 10 (4.75-15.2) Total new lesion volume: 199.9 mm ³ (83.9-447.5) Mean lesion volume per lesion: 42.5 mm ³ (21.5-75.6) Primary safety endpoint: 0% Primary technical performance endpoint: 100% Debris capture: 100%
EMBOL-X trial	2015	EMBOL-X	Prospective, single-center, randomized-controlled trial	Periprocedural cerebral lesions assessed by DW-MRI at baseline and within 7 days post-TAVI	From July 2012 to April 2014, 30 patients randomized 1:1 to TAVI with (n = 14) or without (n = 16) EMBOL-X	
Emblok Embolic study	2020	Emblok	Prospective, nonrandomized, multicenter, first-in-man pilot study	Primary endpoint: technical success and immediate cerebral embolic burden after TAVI (number and volume of new brain lesions detected by DW-MRI at days 2 to 5 post-TAVI compared with baseline)	20 patients submitted to TAVI with Emblok	
SafePass 2	Early clinical results presented at TCT 2019	Emboliner	Prospective, non-randomised, multicentre, open-label study	Primary safety endpoint: 30-day MACCE incidence Technical performance: technical success (ability to successfully access the aortic arch, position the device and retrieve and remove it)	3 centers in New Zealand 24 patients submitted to TAVI with Emboliner	
Ongoing trials						
PROTECTED TAVR	Recruiting NCT04149535	Sentinel	Prospective randomized trial	Primary endpoints : rate of stroke through 72 hours post-TAVR or discharge (whichever comes first)	Estimated enrollment: 3000 patients randomized to TAVR with or without Sentinel	
PROTECT TAVI	Recruiting NCT02895737	Sentinel	Prospective randomized trial	Primary endpoint: total new lesion volume in protected brain regions detected by MRI Secondary endpoint: number of new cerebral lesions detected by MRI; occurrence of clinical stroke and/or neurocognitive dysfunction; postoperative outcome according to VARC 2 criteria	Estimated enrollment: 328 patients submitted to TAVI with or without Sentinel	
PROTEMBO SF Trial	Recruitment completed, not published NCT03325283	ProtEmbo	Prospective, observational, multi-center, intention-to-treat study	Primary endpoint: procedural success (successful access, delivery to, deployment within, and retrieval of the ProtEmbo System from the aortic arch, adequate coverage of side branch vessels and maintenance of position for duration of the TAVR procedure) In-hospital procedural safety up to 7 days (MACCEs, including device-related safety outcomes) Stroke severity at 3 and 30 days Occurrence of Serious Adverse Events at 3 and 30 days	Original estimated enrollment: 10 patients submitted to TAVI with ProtEmbo	

MACCE: major adverse cardiac and cerebrovascular events; DWI-MRI: diffusion-weighted magnetic resonance imaging

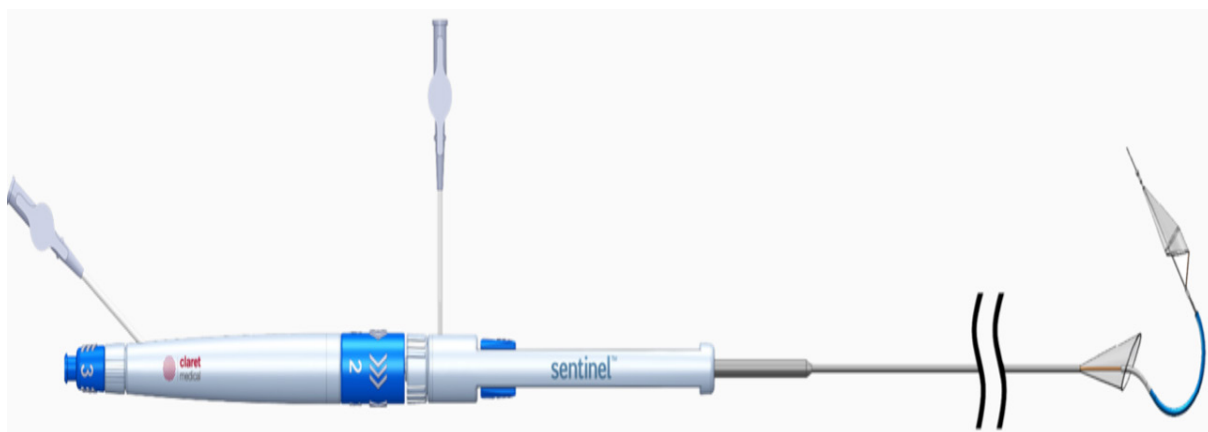


Figure 1. Sentinel cerebral protection system. Image provided courtesy of Boston Scientific. © 2020 Boston Scientific Corporation or its affiliates. All rights reserved

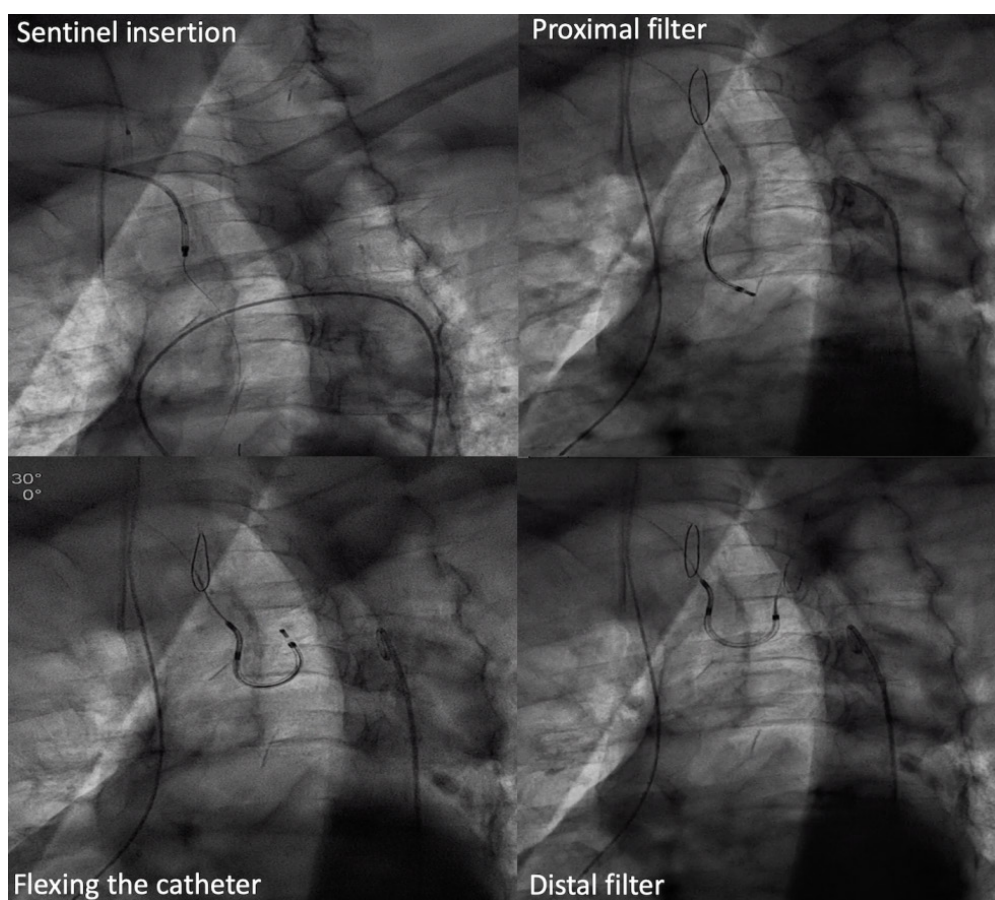


Figure 2. Sentinel implantation

Regardless of the fact that none of these trials have, individually, demonstrated superiority in terms of hard outcomes, such as stroke and mortality, recent meta-analyses showed that CEPD use was associated with lower rates of stroke and 30-day mortality^[18,23]. A propensity-matched patient cohort including 533 patients also showed lower rates of procedural all-stroke (1.88% vs. 5.44%, OR = 0.35, 95%CI: 0.17-0.72, relative risk reduction 65%; $P = 0.0028$), and the combined endpoint of all-cause mortality and all-stroke (2.06% vs.

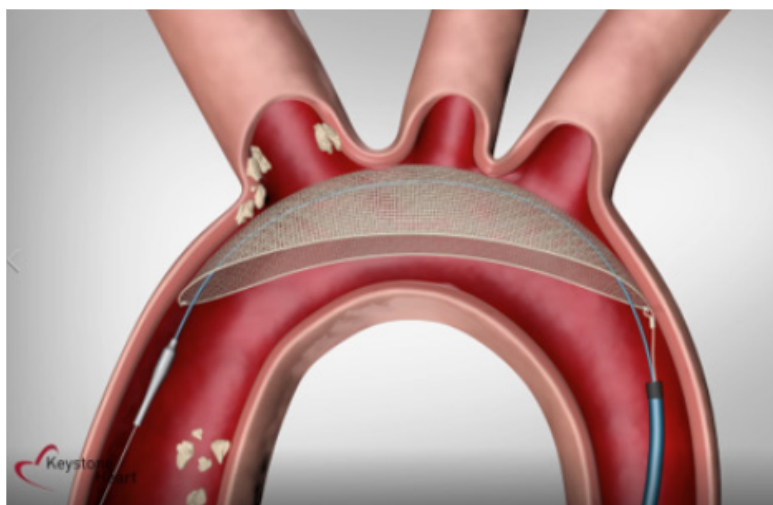


Figure 3. TriGUARD device

6.00%, OR = 0.34, 95%CI: 0.17-0.68, relative risk reduction 66%; $P = 0.0013$) in the protected TAVI group. The rate of disabling stroke was also substantially lower in the Sentinel group (0.38% vs. 2.44%; $P = 0.0045$)^[26].

Furthermore, in the last months, evidence from two large US databases has suggested that Sentinel use during TAVI was associated with statistically significant reduction in stroke risk. In the Society of Thoracic Surgeons/American College of Cardiology (STS/ACC) TVT Registry, the rate of in-hospital stroke was not significantly lower when the Sentinel device was used according to an instrumental-variable analysis (1.39% vs. 1.54%; RR = 0.90; 95%CI: 0.68-1.13). A secondary propensity-weighted analysis of the data, however, indicated that cerebral protection was associated with a reduction in the rates of in-hospital stroke (1.30% vs. 1.58%; RR = 0.82; 95%CI: 0.69-0.97), in-hospital death or stroke (2.1% vs. 2.5%; RR = 0.84; 95%CI: 0.73-0.98), 30-day stroke (1.9% vs. 2.2%; RR = 0.85; 95%CI: 0.73-0.99), and 30-day death (1.7% vs. 2.2%; RR = 0.78; 95%CI: 0.64-0.95)^[27]. Corroborating these findings, a propensity-weighted analysis of the National Inpatient Sample showed that Sentinel use was associated with a lower risk of in-hospital ischemic stroke (1.0% vs. 3.8%; OR = 0.24; 95%CI: 0.09-0.62) and in-hospital death (0% vs. 1%; $P = 0.036$)^[28].

Despite the aforementioned, it is essential to remember that the Sentinel does not protect the left vertebral artery since it is a branch of the left subclavian artery. There are still concerns about leaving the left vertebral artery unprotected, thus some companies are developing devices to eliminate this blind spot.

TriGUARD™ (Keystone Heart, Herzliya, Israel)

The TriGUARD is the only CE mark approved system designed to cover and protect all three major cerebral aortic arch vessels [Figure 3]. Currently, the device is only in investigational use in the US, planning to apply for FDA approval.

The TriGUARD is inserted through a transfemoral 8F sheath, via the femoral artery access already in use during TAVI, usually at the pigtail insertion side, thus eliminating the need for a third arterial puncture. The device is made of nitinol and consists of a self-positioning, self-stabilizing polymetric mesh with pore sizes 115×145 micrometers (μm) opened in the aortic arch, covering the three aortic arch vessels^[29].

There are clinical trials already published showing the efficacy and safety of the device. The DEFLECT I and DEFLECT III trial demonstrated that the technical success, which included complete 3-vessel cerebral coverage, was achieved in 80%-90% of the patients. The DEFLECT III demonstrated that this device use is

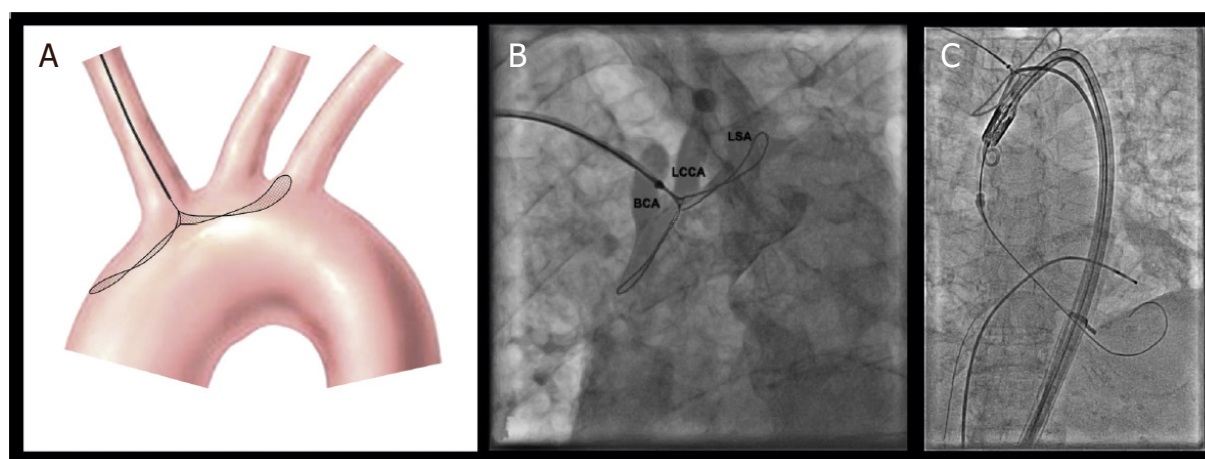


Figure 4. Embrella device. Reproduced from Samim et al.^[35]. A: the Embrella Embolic Deflector System; B: device positioned in the aortic arch; C: TAVI deployment

associated with greater freedom from new ischemic brain lesions, fewer new neurologic deficits, and better performance on a delayed memory task at hospital discharge^[30,31]. From DEFLECT I to DEFLECT III study, the device has changed from a 250 μm to 130 μm pore size. The REFLECT trial is another randomized clinical trial with larger population, designed to study the TriGUARD 3 device. The TriGUARD 3 is the new generation device, designed to bring some improvements such as a simplified frame design, which eliminates the need for a dedicated stabiliser. It is fully visible via fluoroscopy, contains a reduced filter mesh pore size for deflection of smaller particles (145 $\mu\text{m} \times 115 \mu\text{m}$ vs. 250 $\mu\text{m} \times 250 \mu\text{m}$), and has a refined delivery system that reduced the delivery profile (8F instead of 9F)^[32]. Recently, Jeffrey W. Moses presented the results from the Reflect II trial during a late-breaking trial session at TCT Connect 2020. The results showed the safety of the TriGUARD 3, but did not demonstrate superiority for the primary hierarchical efficacy endpoint^[33].

Embella Embolic Deflector System (Edwards Lifesciences, Irvine, CA, USA)

The Embrella Embolic Deflector (EED) system consists of an oval-shaped nitinol frame (length 59 mm, width 5-25 mm), covered with a porous polyurethane membrane (100 μm pore size). Its porous membrane allows blood flow to the brain while simultaneously deflecting embolic material [Figure 4]. The device is composed of 2 petals and a delivery cable in a 6F sheath system inserted via the right radial or brachial arteries. The two opposing petals are positioned along the aorta greater curvature, protecting the brachiocephalic and common carotid arteries from embolism^[34,35].

The EED System has been assessed in a limited clinical study in Europe and received CE Mark approval in May 2010. Two main RCT have studied the EED system. In these studies, Rodés-Cabau et al.^[34] (PROTAVI-C Pilot) and Samim et al.^[35] demonstrated the feasibility and safety of using the EED. However, the device failed to prevent cerebral microemboli or new transient ischemic lesions, as evaluated by Diffusion Weighted Imaging Magnetic Resonance Imaging (DW-MRI). In fact, the studies showed a higher number of brain lesions in the EED group compared to the control group, even though the device was associated with lower lesion volume^[34,35]. The PROTAVI-C editorial comment also raises doubts about the real utility of the device^[36].

ProtEmbo^R (Prtembis, GmbH, Germany)

The ProtEmbo is an intra-aortic embolic protection filter device comprising a filter connected to a delivery unit enabling delivery of the unexpanded device with a 6F sheath via the left radial artery [Figure 5]. The device is delivered in the aortic arch, protecting its three major vessels (i.e., brachiocephalic trunk, left

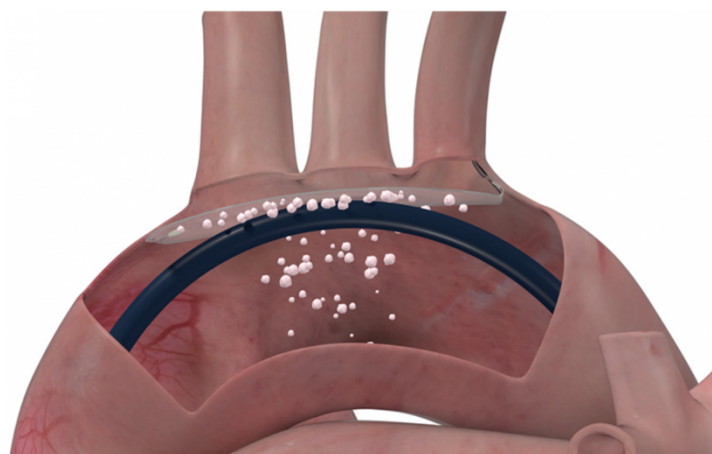


Figure 5. Illustration of ProtEmbo device

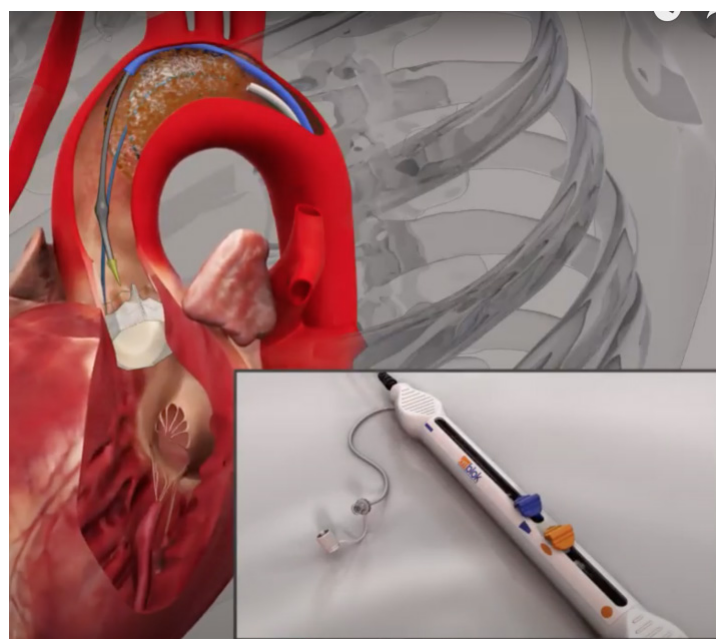


Figure 6. Illustration of Emblok device

common carotid artery, and left subclavian artery). The filter consists of a porous polymeric material with 60 μm pores. The PROTEMBO SF Trial is the first randomized clinical trial to test the ProtEmbo device clinically. It has already completed the recruitment phase, but the results have not been published yet.

Emblok (Innovative Cardiovascular Solutions, Grand Rapids, MI, USA)

The Emblok embolic protection system is an 11F sheath device containing a 4F pigtail, delivered via femoral artery access in the aortic arch [Figure 6]. The device covers all three aortic arch vessels, and the filter consists of 125 μm of polyurethane.

The first trial testing the device was published on JACC, in 2020, by Latib *et al.*^[37]. This prospective, nonrandomized, multicenter study had no control group. Nevertheless, it demonstrated that the use of this device appears to be feasible and safe. It was successfully placed and retrieved in all twenty cases, and no neurological events were observed.

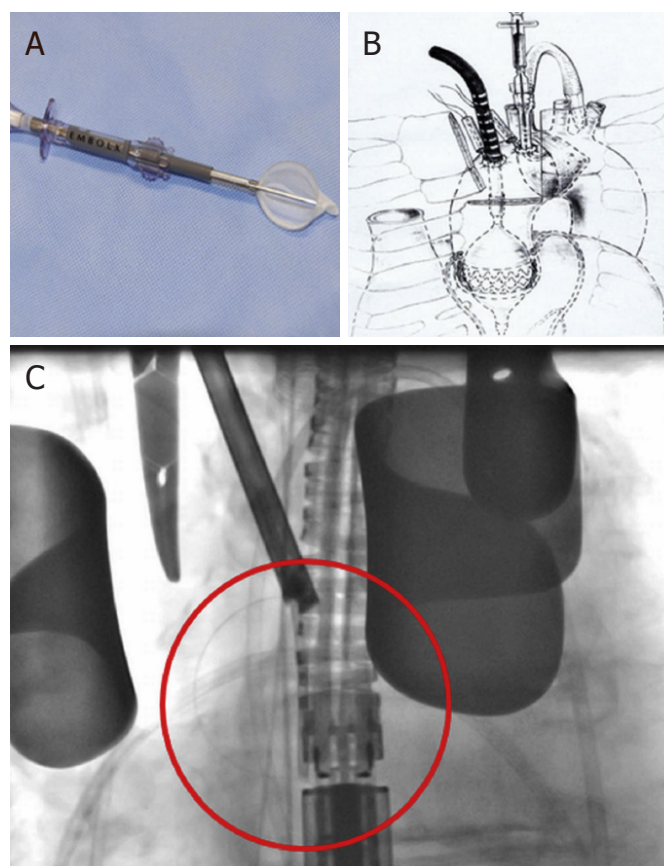


Figure 7. Transaortic Embol-X device. Reproduced from Wendt et al.^[38]. A: EMBOL-X system; B: transaortic TAVI; C: EMBOL-X intraprocedural control

Embol-X (Edwards Lifesciences, Irvine, CA, USA) - Transaortic

The Embol-X device was first developed to be used during open-heart surgery at the aortic cannulation site [Figure 7]. A randomized clinical trial tested its effectiveness in TAVI by a transaortic approach. In this trial, the device was shown to be safe and effective in reducing the incidence and the volume of new cerebral lesions. The device is placed inside the aorta and is available in 5 sizes covering an aortic diameter of 22 to 40 mm. It is delivered by a 17F sheath^[38].

Emboliner Embolic Protection Catheter (Emboliner)TM

The Emboliner Cerebral Protection Catheter is the first device designed to prevent both cerebral and body embolism [Figure 8]. It is delivered through a transfemoral 9F sheath, the same sheath used for the 6F pigtail. Therefore, no additional access is required. Its pore size is 150 μm . The SafePass 2 trial is the first trial with the Emboliner device; it has completed enrollment but has not been published yet. However, the device seems to be safe and effective with little adverse events related to it, capturing up to five times more debris than Sentinel, according to informal data.

Cost-effectiveness analysis

There is no published cost-effectiveness analysis defining the real role of routine cerebral embolic protection device use during TAVI procedures. Therefore, the benefit of preventing a stroke should be balanced against the device costs, taking into consideration that strokes have an unpredictable, but often devastating impact, not only in terms of mortality but also in terms of sequelae (50% of patients develop permanent disability, more than 50% are unable to return to work, and more than 30% end up with serious financial problems). In this setting, Shiyovich et al.^[39] estimated that the cost added by a moderate disability

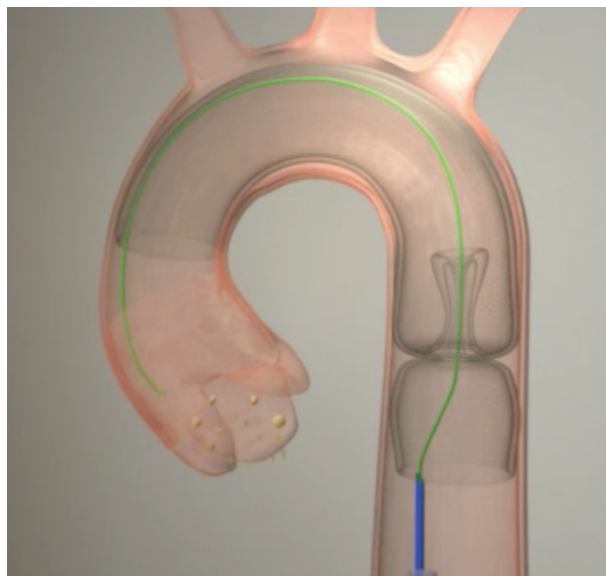


Figure 8. Illustration of Emboliner full body protection device

due to a neurologic event is around \$25,000, followed by a subsequent annual cost increase of up to \$60,000. Hence, as the device cost (Sentinel CPS) is approximately \$2,800, and the CEPD number needed to treat is around 20, CEPD cost-effectiveness is suggested.

DISCUSSION

To critically evaluate the CEPD trials presented above, some critical points should be taken into account. First, the studies showed important discrepancy between imaging and clinical outcomes since the observed reduction in new cerebral lesions number and volume did not reflect the expected benefit in hard outcomes. Trying to explain this discrepancy, it has been hypothesized that the lack of validated models to assess neurocognitive function in TAVI patients, the certain degree of pre-procedural cognitive dysfunction in some patients, and the high prevalence of inter and intra-observer variability for neurological tests, could blunt the real CEPD benefit^[15]. Second, stroke incidence varies according to the study type, being significantly higher when the results are adjudicated based on formal neurologist clinical assessment (up to 10%) than when they are adjudicated by non-neurologists (2%-6%)^[24-26]. Third, CEPD randomized trials have not been designed or powered to demonstrate an unequivocal impact on hard clinical endpoints. These observations make the search for preventive strategies even more relevant, especially in younger patients with longer life expectancy.

Regarding the best procedure strategy, we believe that it is still too early to affirm that CEPD should be universally used or that there is a specific patient population in which protected TAVI is more cost-effective. During the TAVI decision-making process, several factors should be balanced, such as age, the amount of leaflet and/or left ventricular outflow tract calcification, and the presence of aortic plaques or atrial fibrillation^[26]. Therefore, from our perspective and considering the available evidence discussed above, two strategies could be possible:

1. Tailored preventive strategy: If TAVI is performed in a center with limited CEPD availability, one possible strategy could be to limit its use to high-risk scenarios based on preoperative risk factors (e.g., age, previous atrial fibrillation, history of cerebrovascular events, renal failure, concomitant coronary artery disease), transoperative risk factors (e.g., increased catheter and guidewire manipulation, extremely severe aortic stenosis, complex valve-in-valve procedures, multiple valve repositioning maneuvers, need

for pre- and post-dilatation), and highly calcified anatomies (e.g., extensive atherosclerosis, complex aortic atheroma, bicuspid aortic valve, severe left ventricular outflow tract calcification).

2. Routine preventive strategy: If TAVI is performed in a center without CEPD use restrictions, one possible approach could be to offer it routinely as long as there is adequate anatomy, heart team indication, and patient concordance. This approach is based on the fact that captured debris are presented in almost all patients^[40], regardless of preoperative risk factors or type of device used.

CONCLUSION

This review article discusses the pros and cons of cerebral embolic protection use during TAVI procedures. Despite CEPD's high cost, recent evidence, especially with the Sentinel system, has suggested that cerebral protection employment may lower stroke and even mortality rates. Ongoing and upcoming trials will help to fill some of the current evidence gaps related to CEPD use during TAVI.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design and review of this manuscript: Saadi EK, Saadi RP, Tagliari AP, Taramasso M

Availability of data and materials

Not applicable.

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None.

Conflicts of interest

Dr. Saadi EK is a consultant and Proctor for Medtronic, Abbott and Edwards and received speaker honoraria from Edwards and Medtronic. Dr. Tagliari AP has received a Research Grant from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (Capes) - Finance Code 001. Dr. Taramasso M is a consultant for Abbott Vascular, Boston Scientific, 4TECH, and CoreMedic; he has received speaker honoraria or Consultant fees from Edwards Lifesciences, CoreMedic, SwissVortex and Mitraltech.

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Review

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PCSK9 inhibitors and their use in advanced heart failure and heart transplant recipients

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Abstract

The use of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors has garnered widespread attention in the medical community over the past ten years. A number of landmark trials have demonstrated the efficacy of PCSK9 inhibitors in lowering low-density lipoprotein (LDL) levels dramatically when added to background statin therapy. Importantly, their use has led to a significant reduction in adverse events in patients at risk and with established cardiovascular diseases. Published evidence is sparse in the heart failure (HF) population, especially in those with Stage D disease. While the use of PCSK9 inhibitors has not been reported in patients with durable mechanical circulatory support devices, limited data exist in heart transplant recipients. Management of dyslipidemia is critically important in post-heart transplant population as it contributes to the development of cardiac allograft vasculopathy (CAV). However, most 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) interfere with the metabolism of commonly used immunosuppressant agents, such as tacrolimus. Case studies in post-heart transplant patients demonstrated significant LDL reduction with PCSK9 inhibitor use, without significant drug-drug interactions or adverse events. Two trials are currently underway examining their efficacy in reducing CAV progression. This paper aims to review the available clinical evidence for PCSK9 inhibitor use in HF patients, with specific focus on the advanced heart failure group.

Keywords: PCSK9 inhibitors, left ventricular assist device, heart transplant, heart failure



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INTRODUCTION

The association between dyslipidemia and the risk of atherosclerotic cardiovascular diseases (ASCVD) is well established. Numerous clinical studies have documented the strong correlation of elevated serum low-density lipoprotein cholesterol (LDL-C) levels with plaque development and progression over the past decades^[1-3]. This has led to a surge in research aimed at identifying new molecules with more profound cholesterol lowering effect. The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors were developed in the mid-1980's, and lovastatin was the first statin approved by the Food and Drug Administration (FDA) in 1987. Several more potent drugs in this class have been more recently developed and introduced into clinical practice, as their routine use has been widely endorsed by both the American College of Cardiology/American Heart Association (ACC/AHA) and European guidelines for many years^[4,5]. For the past few decades, statins have served as the backbone for LDL-C reduction and have revolutionized cardiovascular prevention^[1,4]. However, it became evident that medications in this class may not be well tolerated by many, may be contraindicated, or may provide suboptimal lipid control in some patients. Therefore, research in the field of cardiovascular prevention continued and several novel agents in multiple drug classes have been developed with profound lipid lowering effect. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are one of those new classes.

THE BIOLOGY OF PCSK9

LDL-C receptors (LDL-R) are transmembrane proteins that function primarily to attract, bind to, and remove circulating LDL-C particles from the blood. While a limited number of LDL-R are present on the surface of most cells of the human body, their expression is abundant on hepatocytes^[6]. After binding to a target LDL-C particle, the receptor complex enters the hepatocyte through endocytic clathrin-coated pit formation. Subsequently, the ligand separates, the LDL-R is recycled and is transported back to the cell surface to bind additional LDL-C particles^[6]. This cycle may repeat several times per hour, up to 150 times total, which is thought to be the lifecycle of the LDL-R^[7]. PCSK9 is a proteolytic enzyme secreted by the liver that regulates the number of LDL-R expressed on the cellular surface. It interferes with the release of the LDL-C particle from its receptor following endocytosis, ultimately prompting proteolysis of the entire complex^[1,6]. Premature degradation leads to the reduced expression of LDL-R on the hepatocyte surface and therefore decreased LDL-C clearance. On the contrary, inhibiting the PCSK9 enzyme leads to increased LDL-R recovery and a significant reduction in circulating LDL-C^[1].

In addition to hepatocytes, PCSK9 is expressed in cardiac myocytes and vascular smooth muscle cells in response to pro-inflammatory mediators such as lipopolysaccharide, TNF alpha, ox-LDL, reactive oxygen species (ROS) and damaged mitochondrial DNA^[8-12]. In turn, PCSK9 increases the expression of various scavenger receptors, particularly LOX-1, through a positive feedback loop. This facilitates ox-LDL uptake by macrophages promoting the development and progression of atherosclerosis^[13]. In addition, it mediates the further release of pro-inflammatory cytokines from macrophages, hepatocytes and other tissues^[9,13].

Myocardial ischemia and elevated ROS levels during reperfusion promote mitochondrial stress, cardiomyocyte apoptosis, autophagy, and increase PCSK9 expression in the zone bordering the infarction^[14-16]. Animal models demonstrate that pre-treatment with PCSK9 inhibitors reduces the infarct size by attenuating mitochondrial dysfunction, mitochondrial fission and the apoptotic process. However, administration following the ischemic injury was not protective^[17]. Further studies are needed to determine the possible protective role of PCSK9 inhibitors in reducing infarct size following coronary occlusion.

ESTABLISHED AND EMERGING PCSK9 INHIBITORS

Soon after discovery of the PCSK9 enzyme, inhibiting its function became a target of intense research. This led to the development of a novel class of agents with prominent cholesterol lowering effect, the

PCSK9 inhibitors. Two subcutaneous products, evolucumab (Amgen Inc, Thousand Oaks, CA, USA) and alirocumab (Sanofi SA, Paris, France; and Regeneron Pharmaceutical Inc, Eastview, New York, USA), are currently approved by the FDA, both of which are human monoclonal antibodies with identical mechanism of action. Three landmark clinical trials have evaluated these medications with their results transforming the landscape of lipid management. OSLER, ODYSSEY, and FOURIER have been recently published. These were randomized, controlled, outcome trials that explored the impact of evolucumab or alirocumab on serum LDL-C reduction, risk of cardiovascular events and safety outcomes^[18-20]. All enrolled patients were at high risk or had documented ASCVD when entering the clinical studies. PCSK9 inhibitors were used on top of moderate or high-intensity background statin therapy in the treatment groups. The three trials confirmed a sustained, approximately 60% reduction in serum LDL-C level when compared to placebo. This was accompanied by a significant reduction in adverse clinical outcomes, including death, unstable angina, myocardial infarction and ischemic stroke. Importantly, progressively lower serum LDL-C levels, even below previously reported target values, were not associated with worse outcomes^[21]. The drugs are well-tolerated in general with injection-site reactions, mild influenza-like illness and self-limiting myalgias as the most frequently reported side effects in real-world experience^[22]. However, they are quite expensive. Their role may be of greater importance in patients with familial hypercholesterolemia and in post-heart transplant population where long-term treatment is necessary.

In addition to the monoclonal antibodies already in clinical practice, small interfering RNAs (siRNAs) are also under investigation with the aim to reduce circulating PCSK9 levels. SiRNAs interfere with the expression of specific genes by promoting mRNA degradation prior to translation. Inclisiran is a long-acting siRNA that targets hepatic PCSK9 synthesis and has been shown to significantly reduce circulating LDL-C levels. It has the distinct advantage of twice per year dosing and acting at the intracellular level of hepatocytes^[23]. It is currently awaiting FDA approval that is expected by the end of 2020. Vaccination aiming to develop PCSK9-specific antibodies are also under investigation. DSPE-PEG-maleimide lipid (L-IFPT) adsorbed to Alhydrogel® (L-IFPTA+) administration has shown to induce a high IgG antibody response, specific against the PCSK9 peptide in hypercholesterolemic mice^[24]. This was paralleled by a 42% reduction in circulating LDL-C levels. This approach could provide safe and long lasting PCSK9 inhibition, as well as decrease the cost and frequency of administration^[24,25].

CURRENT GUIDELINES ON THE USE OF PCSK9 INHIBITORS

The 2018 ACC/AHA/NLA (American College of Cardiology/American Heart Association/National Lipid Association) cholesterol guidelines for clinical practice utilize the ASCVD risk calculator to determine if a patient would benefit from interventions reducing LDL-C levels. Two large meta-analyses have confirmed that ASCVD risk declines progressively as serum LDL-C is lowered using statin therapy^[26,27]. Guidelines now define cholesterol-lowering goals in terms of absolute LDL-C level and percentage LDL-C reduction. The calculated 10-year ASCVD risk is classified into “low risk” < 5%, “borderline risk” 5%-7.5%, “intermediate risk” 7.5%-20% and “high risk” > 20% groups^[4], which determines the recommended intensity of statin therapy (low, moderate, or high). The guidelines also identify a group of patients who are thought to benefit from additional lower levels of LDL-C, with target levels below 70 mg/dL. At times, this goal may only be achieved when prescribing a high-intensity statin combined with a medication from a different drug class. Ezetimibe is currently the first line adjuvant agent, and PCSK9 inhibitors are considered second line adjunctive agents^[4]. Current guidelines also identify additional groups of patients who should be initiated on a PCSK9 inhibitor given their high ASCVD risk. These include individuals with heterozygous familial hypercholesterolemia with an LDL-C of 100 mg/dL or higher, patients with LDL-C level exceeding 220 mg/dL, and persistently elevated serum LDL-C above 130 mg/dL despite a combination of high-intensity statin and ezetimibe^[4].

PCSK9 INHIBITORS IN PATIENTS WITH HEART FAILURE

The medical management of patients with heart failure has become increasingly more complex over the past decades with several new drug classes added to the treatment pool. Diuretics are the most commonly used agents aiming to achieve euvolemia. Medications that reduce sympathetic nervous system activity or block the renin-angiotensin-aldosterone axis have been shown to improve outcomes significantly and are recommended by all guidelines^[28,29]. In contrast, the routine use of lipid lowering agents remains controversial in this population. Statins may be indicated for patients with ischemic cardiomyopathy or with high 10-year ASCVD risk score. However, their use is not established in individuals with non-ischemic heart failure etiology. In two randomized controlled trials (CORONA and GISSI-HF), moderate dose rosuvastatin administration was not associated with improved mortality or a decrease in adverse cardiovascular events in patients with heart failure of any cause, despite significant reduction in LDL-C^[30,31]. The possible benefit of targeting the PCSK9-LDL-R pathway in this population also remains uncertain. In a recent sub-study of BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure), frozen serum samples obtained from patients with worsening heart failure were analyzed for circulating levels of PCSK9 and LDL-R^[32]. Authors described an independent and significant association between the activity of this axis and adverse clinical outcomes. However, it remains unclear if elevated circulating PCSK9 level is merely a marker or possibly a contributor to increased mortality. New evidence suggests a possible additional benefit of PCSK9 inhibitors stemming from their anti-inflammatory properties^[11,33]. However, as of today, no randomized controlled trials have been published assessing the efficacy of PCSK9 inhibitors in patients with heart failure. Further studies are warranted to establish their benefit in this population.

PCSK9 INHIBITOR USE IN PATIENTS WITH DURABLE MECHANICAL CIRCULATORY SUPPORT DEVICES

The use of durable mechanical circulatory support (MCS) devices has been steadily rising over the past decade in patients with advanced heart failure. Despite the technological advancements and dramatic improvement in clinical outcomes with newer generation devices, neurological complications remain relatively high in these patients^[34,35]. With the newest generation Heart Mate 3 LVAD (Abbott Laboratories, Minneapolis, MN), the cumulative rate of stroke remains at around 10% at two years^[36]. Many of these ischemic and hemorrhagic cerebrovascular events are related to hypertension, micro embolization, infection and changes in cerebral autoregulation, owing to the continuous flow profile. Although many patients supported with MCS have underlying ischemic cardiomyopathy and diffuse atherosclerotic vascular disease, there is limited evidence for statin therapy to improve survival in this population. Vieira and colleagues found that statin use after LVAD implantation is associated with lower rates of ischemic, but not hemorrhagic strokes^[37]. On further evaluation, it was hypothesized that the pleiotropic effects of statins, including their anti-inflammatory, immunologic and anti-thrombotic effects, are primarily responsible for these clinical benefits^[37]. No specific guidelines currently address statin use in patients receiving LVAD, and providers often rely on risk calculators and consider other indications when prescribing statins. Similarly, there are no published data or guidelines on PCSK9 inhibitor use in this population. More studies are needed to evaluate how these novel lipid lowering agents are tolerated in patients supported with an LVAD and how these may affect long-term clinical outcomes, including stroke.

PCSK9 INHIBITORS IN HEART TRANSPLANT RECIPIENTS

Heart transplantation is the most definitive treatment option for patients with end stage heart failure. Advances in post-transplant care has led to significant reduction in rejection rates, infections, and the incidence of malignancies. The improvement in graft and patient survival unmasked cardiac allograft vasculopathy (CAV) as the leading cause of morbidity and mortality a few years following transplantation. With the prevalence of CAV rising to 47% at 10 years, effective and early prevention are critically important^[38,39]. The predominant histological feature of CAV is the progressive, diffuse thickening of the

coronary intima affecting all large epicardial vessels, intramuscular arteries as well as the microvascular bed^[40,41]. The initial, immune-mediated arteritis is followed by the diffuse deposition of cholesterol particles within the intima. Risk factors include the host-mediated immunological response towards the graft as well as non-immune factors, such as dyslipidemia, hypertension, smoking, CMV infection and ischemia-reperfusion injury^[42,43]. Dyslipidemia is extremely common in heart transplant recipients. Many patients have long-standing hyperlipidemia prior to transplantation, and it is also a well-established side effect of immunosuppressive agents, including corticosteroids, rapamycin and calcineurin inhibitors (e.g., tacrolimus, cyclosporine)^[44]. As such, statin therapy is a Class I recommendation in current guidelines for heart transplant recipients, irrespective of serum cholesterol levels^[38]. Despite being the standard of care, many statins have significant interactions with immunosuppressants, may cause myositis, rhabdomyolysis or myalgias, and may provide suboptimal lipid control^[45].

Due to the above limitations and the unfavorable side effect profile of statin therapy in the post-transplant population, there are ongoing investigations to test therapeutic alternatives for lipid management, such as PCSK9 inhibitors. Agents in this class bind specifically to an extracellular target and do not interact with the cytochrome P450 system. Therefore, they have low risk for significant drug-drug interactions, including with immunosuppressants, and their properties render them well tolerated overall^[46,47]. Interestingly, Simha and colleagues reported that mammalian target of rapamycin (mTOR) inhibition with sirolimus increases PCSK9 expression in both humans and *in-vitro* cell culture studies; however, the increased PCSK9 levels did not correlate with sirolimus-induced hypercholesterolemia. It is postulated that sirolimus may cause hyperlipidemia via multiple pathways and further studies are under-way^[48]. Although no large, randomized clinical trials have yet been completed, several case series reported single center experiences on the use of PCSK9 inhibitors in statin-intolerant heart transplant recipients [Table 1]. The reduction in serum LDL-C in response to PCSK9 initiation averaged between 40% and 70%^[44,49]. Both drugs in the class demonstrated a favorable safety profile with adverse reactions limited to injection site erythema, rhinorrhea, nausea and clinically insignificant transaminitis^[49,50]. These case series reported no increase in the risk of graft rejection, infections or fluctuation in serum immunosuppressant levels^[44,49,51,52]. In addition to their direct benefit on circulating LDL-C levels, the anti-inflammatory properties of PCSK9 inhibitors may also reduce the activation of the innate immune system encountered after solid organ transplantation^[33,53]. While the results of current observational data on the use of PCSK9 inhibitors in heart transplant recipients are promising, most reports are limited by the number of patients and short-term follow-up, highlighting the need for additional studies in the field.

A recent paper by Bjerre and colleagues reported elevated levels of PCSK9 in patients with macrovascular CAV, as detected by coronary angiography and optic coherence tomography^[54]. In addition, the authors found a trend towards higher circulating PCSK9 levels in the subgroup taking mTOR inhibitors. Note, however, that it is routine practice to initiate patients on an mTOR inhibitor once CAV is established, and therefore this observation might be unrelated to the immunosuppressant used. Nevertheless, further larger scale randomized studies are needed to establish the possible benefit of PCSK9 inhibition in de novo heart transplant recipients to prevent CAV development and progression.

FUTURE DIRECTION

Given their clinical efficacy in reducing serum LDL-C, limited side effect profile, and the lack of interactions with immunosuppressants, it is imperative to further explore the idea of PCSK9 inhibitor use in heart transplant recipients for CAV prevention. Two clinical trials are currently open and are actively enrolling patients. EVOLVD is a multicenter, randomized, controlled, double-blind study aiming to determine whether the addition of evolocumab on top of background statin therapy, can ameliorate CAV in de novo heart transplant patients at 12 months, as assessed by coronary intravascular ultrasound^[55]. First results are expected to be published in 2022. The PCSK9 Inhibition After Heart Transplantation trial lead

Table 1. Published reports on PCSK9 inhibitor use in heart transplant recipients

Author	Title/PMID	Drug used and number of patients	Outcome	Adverse effects
Warden <i>et al.</i> ^[49]	Use of PCSK9 inhibitors in solid organ transplantation recipients	alirocumab <i>n</i> = 2; evolocumab <i>n</i> = 9; sequential <i>n</i> = 1	60% median LDL-C decrease	25% mild and self-limiting injection site reactions, nausea and rhinorrhea
Di Nora <i>et al.</i> ^[57]	Safety and efficacy of pcsk9 inhibitor treatment in heart transplant patients PMID: 30399127	evolocumab <i>n</i> =1	87% decrease in LDL	None
Jennings <i>et al.</i> ^[52]	PCSK9 inhibitor use in heart transplant recipients: a case series and review of the literature PMID: 31478991	evolocumab <i>n</i> = 7	45% decrease in LDL	None
Kühl <i>et al.</i> ^[44]	Treatment of hypercholesterolemia with PCSK9 inhibitors in patients after cardiac transplantation PMID: 30650126	evolocumab <i>n</i> = 8; alirocumab <i>n</i> = 2	40% decrease in LDL; 30% decrease in cholesterol	alirocumab patient developed HCC and Hep E
Moayed <i>et al.</i> ^[45]	Safety and efficacy of PCSK9 inhibitors after heart transplantation PMID 30595172	evolocumab <i>n</i> = 6	> 70% reduction in LDL	None
Groba-Marco <i>et al.</i> ^[51]	Treatment of hypercholesterolemia with PCSK9 inhibitors in heart transplant recipients. first experience in Spain. PMID 31474577	alirocumab <i>n</i> = 5	45%-76% reduction in LDL	None
Sandesara <i>et al.</i> ^[50]	PCSK9 inhibition in patients with heart transplantation: a case series PMID 31353230	alirocumab <i>n</i> = 1; evolocumab <i>n</i> = 2	64% reduction in LDL; 49% reduction in total cholesterol	None

PCSK9: proprotein convertase subtilisin/kexin type 9; LDL: lowering low-density lipoprotein

by Fearon^[56] from Stanford University (NCT03537742) aims to determine alirocumab's safety, efficacy, and impact on CAV when administered early after heart transplantation. The expected study completion date is in September 2023. Further, long-term outcome trials will be needed to establish the safety and efficacy of PCSK9 inhibitors in heart transplant recipients.

CONCLUSION

With the discovery of PCSK9 inhibitors, cardiologists are given a novel but expensive tool to manage hyperlipidemia in patients at high risk for ASCVD, and those intolerant to more conventional therapies. Several large, randomized, outcome trials have established their safety, efficacy and favorable side effect profile. Trials are ongoing for heart transplant recipients, yet current evidence is lacking for advanced heart failure patients and those with durable mechanical circulatory support. New PCSK9 inhibitors are on the horizon with a more patient-friendly administration schedule. When evaluated and introduced into clinical practice, these will hopefully reduce the overall cost burden, thereby enabling a more widespread utilization.

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Authors' contributions

Literature review, manuscript preparation, review, editing: Agdamag ACC, Maharaj VR, Fraser M, Edmiston JB, Charpentier V, Francis GS, Alexy T

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AUTHOR INSTRUCTIONS

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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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Case Report	A Case Report details symptoms, signs, diagnosis, treatment, and follows up an individual patient. The goal of a Case Report is to make other researchers aware of the possibility that a specific phenomenon might occur.	Unstructured abstract. No more than 150 words.	3-8 keywords	The main text consists of three sections with fixed section titles: Introduction, Case Report, and Discussion.
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2.3 Manuscript Structure

2.3.1 Front Matter

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The title of the manuscript should be concise, specific and relevant, with no more than 16 words if possible. When gene or protein names are included, the abbreviated name rather than full name should be used.

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Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email addresses for all authors should be listed. At least one author should be designated as corresponding author. In addition, corresponding authors are suggested to provide their Open Researcher and Contributor ID upon submission. Please note that any change to authorship is not allowed after manuscript acceptance.

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Methods should contain sufficient details to allow others to fully replicate the study. New methods and protocols should be described in detail while well-established methods can be briefly described or appropriately cited. Experimental participants selected, the drugs and chemicals used, the statistical methods taken, and the computer software used should be identified precisely. Statistical terms, abbreviations, and all symbols used should be defined clearly. Protocol documents for clinical trials, observational studies, and other non-laboratory investigations may be uploaded as supplementary materials.

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Organization as author	Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. <i>Hypertension</i> 2002;40:679-86. [PMID: 12411462]
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Journal articles ahead of print	Odibo AO. Falling stillbirth and neonatal mortality rates in twin gestation: not a reason for complacency. <i>BJOG</i> 2018; Epub ahead of print [PMID: 30461178 DOI: 10.1111/1471-0528.15541]
Books	Sherlock S, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub; 1993. pp. 258-96.
Book chapters	Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. <i>The genetic basis of human cancer</i> . New York: McGraw-Hill; 2002. pp. 93-113.
Online resource	FDA News Release. FDA approval brings first gene therapy to the United States. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm . [Last accessed on 30 Oct 2017]
Conference proceedings	Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.
Conference paper	Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. <i>Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming</i> ; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. pp. 182-91.
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Diagrams with describing words (including, flow chart, coordinate diagram, bar chart, line chart, and scatter diagram, *etc.*) should be editable in word, excel or powerpoint format. Non-English information should be avoided;

Labels, numbers, letters, arrows, and symbols in figure should be clear, of uniform size, and contrast with the background; Symbols, arrows, numbers, or letters used to identify parts of the illustrations must be identified and explained in the legend;

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2.4.6 Tables

Tables should be cited in numeric order and placed after the paragraph where it is first cited;

The table caption should be placed above the table and labeled sequentially (e.g., Table 1, Table 2);

Tables should be provided in editable form like DOC or DOCX format (picture is not allowed);

Abbreviations and symbols used in table should be explained in footnote;

Explanatory matter should also be placed in footnotes;

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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.

2.4.10 Numbers

Numbers appearing at the beginning of sentences should be expressed in English. When there are two or more numbers in a paragraph, they should be expressed as Arabic numerals; when there is only one number in a paragraph, number < 10 should be expressed in English and number > 10 should be expressed as Arabic numerals. 12345678 should be written as 12,345,678.

2.4.11 Equations

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2.5 Submission Link

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