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REVIEW

Translational research of adult stem cell therapy

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Abstract

Congestive heart failure (CHF) secondary to chronic coronary artery disease is a major cause of morbidity and mortality world-wide. Its prevalence is increasing despite advances in medical and device therapies. Cell based therapies generating new cardiomyocytes and vessels have emerged as a promising treatment to reverse functional deterioration and prevent the progression to CHF. Functional efficacy of progenitor cells isolated from the bone marrow and the heart have been evaluated in preclinical large animal models. Furthermore, several clinical trials using autologous and allogeneic stem cells and progenitor cells have demonstrated their safety in humans yet their clinical relevance is inconclusive. This review will discuss the clinical therapeutic applications of three specific adult stem cells that have shown particularly promising regenerative effects in preclinical studies, bone marrow derived mesenchymal stem cell, heart derived cardiosphere-derived cell and cardiac stem cell. We will also discuss future therapeutic approaches.

Key words: Congestive heart failure; Adult stem cells; Mesenchymal stem cell; Cardiosphere-derived cell; Cardiac stem cell

Core tip: Cell-based therapy emerged as a new approach to restore damaged heart function. Although cell therapy in experimental animal models is promising, beneficial effects in clinical trials are variable. This review summarizes recent preclinical and clinical applications on three specific adult stem cells (bone marrow derived mesenchymal stem cell, heart derived cardiosphere-derived cells and cardiac stem cell) and discuss about future approaches.

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INTRODUCTION

The prevalence of congestive heart failure secondary to chronic coronary artery disease is increasing in spite of

Table 1 Clinical Trials of mesenchymal stem cells, cardiosphere-derived cells and cardiac stem cells in heart disease

Trial name	Study design	No. of patients	Delivery method	Cell dose	End point evaluation	Follow-up period	Outcome
MSCs							
Chen *et al*[10]	Randomized, controlled study	MSC n = 34 Control n = 35	Intracoronary	48-60 × 10^9 cells	Echocardiography	3 and 6 mo	LVEF↑
POSEIDON[28]	Randomized, Pilot study	MSC n = 30 Auto *vs* Allo	Intramyocardial (transendocardial)	20, 100, 200 × 10^6 cells	Cardiac CT	12 mo	LVEF↔ LVEDV↓
PROMETHEUS[29]	Randomized, Pilot study	MSC n = 6 No control	Intramyocardial (transepicardial)	20, 200 × 10^6 cells	MRI	18 mo	LVEF↑ Scar size↓
C-CURE[30]	Randomized, controlled study	MSC n = 21 Control n = 15	Intramyocardial (transendocardial)	7 × 10^6 cells	Echocardiography	6 and 24 mo	LVEF↑ LVESV↓
CDCs							
CADUCEUS[36,37]	Randomized, controlled study	CDC n = 17 Control n = 8	Intracoronary	12.5-25 × 10^6 cells	MRI	6 and 12 mo	LVEF↔ Scar size↓
ALCADIA	Pilot study	CDC n = 6 No control	Intracoronary	25-30 × 10^6 cells	MRI	12 mo	LVEF↑ Scar size↓
TICAP[38]	Randomized, controlled study	CDC n = 7 Control n = 7	Intracoronary	2-3 × 10^6 cells	MRI	18 mo	LVEF↑
CSCs							
SCIPIO[50]	Randomized, controlled study	CSC n = 20 Control n = 13	Intracoronary	1 × 10^6 cells	Echocardiography MRI	12 mo	LVEF↑ Scar size↓

Auto: Autologous; Allo: Allogeneic; MSCs: Mesenchymal stem cells; CSCs: Cardiac stem cells; CDCs: Cardiosphere-derived cells; CT: Computed tomography; MRI: Magnetic resonance imaging.

recent advances in medical and device therapies that delay the progression of disease[1]. Currently available medical interventions attenuate neurohormonal activation (*e.g.*, renin-angiotensin-aldosterone system, sympathetic nervous system, and arginine vasopressin), reducing myocyte apoptotic cell death, reducing interstitial connective tissue proliferation and attenuating the progression of myocyte cellular hypertrophy. However, none of the current therapies are effective in reversing myocyte loss and cellular abnormalities associated with myocyte contractile performance which are impaired in the failing heart. Recent investigations have demonstrated that there is an endogenous cardiac repair system that arises from resident cardiac stem cells regulating cardiac engraftment by maintaining a low level of myocyte proliferation, regeneration and cell death[2]. Nevertheless, the regenerative capacity of this endogenous stem cell pool is limited.

Expansion of adult stem cells *ex vivo* can stimulate the heart to induce endogenous or exogenous cell based repair. Cell-based therapy has emerged as a promising therapy to regenerate the failing heart through its potential to repair dead myocardium and improve left ventricle (LV) function[3-5]. Although clinical trials have demonstrated the safety and feasibility of using bone marrow-derived stem cells [Bone marrow mononuclear cells (MNCs) or mesenchymal stem cells (MSCs)] or heart-derived stem cells [cardiac stem cells (CSCs) or cardiosphere-derived cells (CDCs)] in humans with MI who do not have severe heart failure, the long term clinical efficacy of this approach is variable with a small improvement in LV function[6-8]. Although the biological action of adult stem cells *in vivo* is still controversial, for now, the beneficial effects of adult stem cells are considered to be associated with the secretion of paracrine factors rather than direct differentiation of *de novo* cardiac cells[9]. Accordingly, stem cells secrete multiple growth factors and cytokines which reduce scar volume and myocyte apoptosis, increase myocyte proliferation and activate endogenous CSCs to produce new myocytes. Therefore, current research using adult stem cells has focused on optimizing cell based therapy that effectively improves LV function and decreases disease progression. This would have a major impact on the survival and quality of life in patients with ischemic heart disease as well as reduce healthcare expenditures related to recurrent hospitalizations from advanced disease. In this review we will discuss three types of adult stem cells, MSCs, CDCs and CSCs, which are involved in the early phase of clinical trials (Table 1) and address current problems and future directions (Table 2).

MSCS IN ISCHEMIC CARDIOMYOPATHY

MSCs arise from a small proportion of bone marrow mononuclear cells (0.001%-0.01% of nucleated cells in the bone marrow). Although it has been reported that MSCs can be differentiated into cardiomyocytes and vascular-like structures[10-14], actual *in vivo* differentiation is infrequent. Moreover, current approaches using direct myocardial injection or intracoronary infusion of cells in the infarcted region result in a low myocardial retention of stem cells[15]. Thus, most of the beneficial effects derived from MSCs are considered to be related to a paracrine mechanism. MSCs produce a wide variety of cytokines, chemokines and growth factors, and many are involved in restoring cardiac function or regenerating myocardial tissue. Factors such as basic fibroblast

Table 2 Alternative strategies of stem cell therapy

Enhancement of cell survival, mobilization and paracrine secretion
Pharmacology (Statins, *etc.*)
Genetic modification (Akt and Ang1, VEGF and SDF-1, HO-1, bFGF/IGF-1/BMP2)
Preconditioning (Hypoxia, TLR3 stimulation)
Combination of different cell types or delivery approaches
MSCs and CSCs
Stop-flow (infarct area) and global intracoronary infusion (viable area)
Others
Cell infusion immediate after revascularization (allogeneic MSCs, CDCs, *etc.*)
Repeated cell infusion
Stimulation of exosome release
Direct exosome (or microRNAs) injection
Cell therapy in hypertrophied myocardium or dysfunction due to congenital heart disease

MSCs: Mesenchymal stem cells; CSCs: Cardiac stem cells; CDCs: Cardiosphere-derived cells; VEGF: Vascular endothelial growth factor; SDF: Stromal cell-derived factor; bFGF: Basic fibroblast growth factor; IGF: Insulin-like growth factor; BMP2: Bone morphogenetic protein 2; HO-1: Heme-oxygenase 1; Ang1: Angiopoietin 1.

growth factor (bFGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, and stromal cell-derived factor (SDF)-1 inhibit LV remodeling[16] and apoptosis, stimulate proliferation of endogenous myocytes and angiogenesis, activate endogenous CSCs[4] and mobilize bone marrow progenitor cells to the heart[17]. Importantly, MSC are immunoprivileged because they do not express MHC class II molecules therefore they escape immune-rejection, release immunomodulatory factors and inhibit T-cell proliferation. Allogeneic cells can be expanded *ex vivo* and stored to use in patients[18,19]. This would allow for "off-the-shelf" treatment of patients with severe LV dysfunction, without the need to wait for cell processing and expansion[19].

A large number of preclinical investigations have been performed using MSCs, and demonstrate a significant beneficial effect on cardiac structure and function[13,20-23]. In a large animal model, Quevedo *et al*[18] demonstrated that administration of allogeneic MSCs to a swine model of chronically infarcted myocardium resulted in improvements in regional contractility and myocardial blood flow, as well as engraftment, differentiation and enhanced survival. Williams *et al*[24] assessed serial cardiac MRI in animals with post-MI LV remodeling and showed progressive scar size reductions, improvements in ejection fraction (EF) and reverse LV chamber remodeling in animals receiving allogeneic MSCs as compared to controls[24]. Mesenchymal precursor cells (MPCs) are subpopulation of MSCs expressing the STRO-3 cell surface marker. MPCs are highly proliferative and secrete abundant paracrine factors. Houtgraaf *et al*[25] demonstrated that slow infusion of allogeneic MPCs (12.5 to 37.5 million cells) to an bovine model with acute MI improved regional and global function and reduced scar volume and LV remodeling. Interestingly, MPC infusion in the infarct-related coronary artery caused myocyte cell size reduction in the infarcted and remote regions. Based on these data a clinical trial is currently ongoing (NCT01781390, phase Ⅱ) that investigates the safety of MPCs in patients with *de novo* anterior MI.

We have demonstrated that slow infusion of allogeneic MSCs into the three major coronary arteries in swine with hibernating myocardium increased regional cardiac function in both the ischemic left anterior descending (LAD) artery and remote regions (wall thickening: LAD: 24% to 43%, Remote: 60% to 85%, $P < 0.05$)[17]. Intracoronary MSCs (icMSCs) significantly increased cKit+/CD133 positive cells (or bone marrow-derived progenitor cells) in the bone marrow and circulation corresponding to the increase in myocardial localization of cardiac progenitor cells (cKit+/GATA4 or Nkx2.5+). icMSCs also induced myocytes to enter the cell cycle and increased the production of small cardiac myocytes indicating the presence of cardiac regeneration. Although some laboratories have identified rare myocytes arising from MSCs in swine[18], our own studies using multiple reporter genes could not identify cardiac myocytes differentiating from labeled MSCs[26]. Thus, cardiac regeneration after icMSCs is related to a bone marrow-derived progenitor cell mediated endogenous cardiac repair mechanism.

Chen *et al*[10] administered 48-60 billion bone marrow derived MSCs by intracoronary injection into 34 patients and reported a 13% increase in EF compared to placebo groups at 3-6 mo follow-up. The Percutaneous Stem Cell Injection Delivery Effects on neo-myogenesis (POSEIDON) trial, by Hare's group, tested the ability of autologous and allogeneic MSCs (20, 100 and 200 million cells) in patients with ischemic cardiomyopathy to promote cardiac recovery following transendocardial stem cell injection[27,28]. Using multidetector computed tomography and biplane left ventriculography, this study reported a 32% reduction in scar size in allogeneic MSCs group *vs* a 35% reduction in autologous MSCs groups without improvement of LV EF. Subgroup analysis demonstrated that 20 million MSCs improvement in LV EF and LVEDV. Furthermore, autologous MSCs showed improvement in the 6 min walk test and allogeneic MSCs reduced LVEDV. Additionally, allogeneic MSCs did not stimulate a donor specific alloimmuno reaction. Thus, this study clearly demonstrates the importance of cell injection site and the safety of using allogeneic MSCs in patients. The Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) trial investigated injection of autologous MSCs (20-200 million cells) into akinetic or hypokinetic areas in hearts that were unsuitable for surgical revascularization during coronary artery bypass graft surgery (CABG)[29]. Cardiac MRI analysis demonstrated that MSC injection increased EF by 9.4% as well as increased scar reduction by 48%

and contractile improvement in dysfunctional areas where surgical reperfusion was not performed[29]. Although this study lacked a placebo control group and had a limited patient number (6 patients), it demonstrates the potential benefits of injection of MSCs directly into non-revascularized myocardium. The Cardiopoietic stem Cell therapy in heart failure (C-CURE) trial tested the ability of a "cardiogenic cocktail" to enhance the therapeutic benefits of autologous MSCs[30]. Bartunek *et al*[30] pretreated MSCs with growth factors to enhance their cardioprotective functions. Twenty-one patients with class 2 or 3 heart failure received over 700 million cardiogenic cocktail treated MSCs by electromechanically guided endomyocardial injections. No adverse events or systemic toxicity was observed. Moreover, in LV EF, end-systolic volume and the 6-min walking test were significantly improved. Subsequently, the Safety and Efficacy of Autologous Cardiopoietic Cells for Treatment of Ischemic Heart Failure (CHART-1) trial is currently ongoing. This study is investigating the efficacy and safety of Bone Marrow-derived Mesenchymal Cardiopoietic Cells for the Treatment of Chronic Advanced Ischemic Heart Failure. The safety and efficacy of MSCs and modified MSCs in patients have been confirmed. In the future, randomized controlled trials involving a large population of patients are anticipated.

CDCS IN ISCHEMIC CARDIOMYOPATHY

Smith *et al*[31] expanded in culture tissue from percutaneous myocardial biopsies to form cardiospheres as the basis for cardiac stem cell expansion. They selected floating cardiospheres (out-growing cells) for culture and expanded them in a monolayer to isolate what is termed CDCs. Cardiospheres and CDCs express antigens specific for stem cells (cKit, CD90, CD105 and the absence of CD34 and CD45) as well as proteins vital for cardiac contractile (Nkx2.5, GATA4) and electrical function (Cx43)[32]. This defines cardiospheres and CDCs as a population of cardiac progenitor cells. Cardiospheres are heterogeneous groups of cells that contain not only adult CSCs, which are capable of long-term self-renewal and cardiomyocyte differentiation, but also vascular cells and differentiated progenitor cells[33]. Preclinical investigations were exclusively reported from Marban's group, they demonstrated that administration of CDCs in an experimental acute MI model reduced LV remodeling, improved contractility and reduced the infarct size without improvement in cardiac function[5]. Specifically they show that injection of 10 million of autologous CDCs to a swine model of infarcted myocardium resulted in a significant reduction in infarction size (approximately 5%) compared to a 2.4% reduction in placebo with no change in global function[5]. Malliaras *et al*[34] showed that injection of 12.5 million of allogeneic CDCs significant reduced scar size (3.6%) and preserved EF in a swine model of MI compared to no reduction in scar size (0.4%) and deterioration of EF (approximately 9.9%) in placebo. Lee *et al*[33] compared the effects of CDCs and their precursor cells, cardiospheres, in a swine MI model. They found that the effects on infarct reduction and preservation of EF were similar in both CDCs and cardiospheres whereas there was improved hemodynamics and regional function and preservation of LV chamber remodeling (all quantified by serial cardiac MRI) in animals receiving cardiospheres.

We previously demonstrated that slow infusion of CDCs into the three major coronary arteries (total dose 30 million CDCs) in swine with hibernating myocardium improved regional function in ischemic LAD (wall thickening: 23% to 51%, $P < 0.05$) as well as in the normal right coronary artery (RCA) regions (68% to 107%, $P < 0.05$) and global function (EF: 54% to 71%, $P < 0.05$)[35]. Quantitative histochemical analysis demonstrated that CDCs increased myocyte nuclear density and significantly reduced myocyte cellular hypertrophy in hibernating LAD and normal RCA regions indicating viable myocardium is a main therapeutic target.

The cardiosphere-derived autologous stem cells to reverse ventricular dysfunction (CADUCEUS) involved 25 patients who were given 12.5-25 million autologous CDCs[36] after successful percutaneous coronary intervention. The CDCs were expanded for approximately 36 d in culture from right ventricular endomyocardial biopsies taken 2-4 wk after acute MI. After expansion CDCs were injected into the previously stented coronary artery between 6-12 wk after heart attack. Despite the lack of improvement in left ventricular EF or patient reported outcomes, the scar reduction was 28% and 46% at 6 and 12 mo respectively and regional wall thickening was significantly improved in treated patients by 7.7%[37]. Serious adverse events were also reported to be three times higher in the treated group, however due to the relatively small number of patients enrolled, this trial cannot ascertain to the safety of CDCs. The autologous human cardiac-derived stem cell to treat ischemic cardiomyopathy (ALCADIA) trial investigated CDCs expanded from cardiac (endomyocardial) tissue isolated during CABG. This trial combined the use of stem cells, bioengineered scaffolds and biologics to create a hybrid therapy. CDCs were cultured for 1 mo before intracoronary injection followed by placement of a gelatin sheet containing bFGF over the injection site. Six months after therapy, cardiac MRI indicated a 12.1% increase in EF, a 3.3% reduction in infarct size and a significant improvement in wall motion as well as maximum aerobic exercise capacity. Since this study enrolled only 6 subjects, study is anticipated to enroll larger patients.

The transcoronary Infusion of Cardiac Progenitor Cells in Patients with Single Ventricle Physiology trial involved in 14 patients who had hypoplastic left heart syndrome. Tissue was isolated from the right atrium of patients receiving stage 2 (Glenn) or stage 3 (Fontan) surgeries[38]. Cardiospheres were expanded from this right atrium tissue for 2-3 wk in culture. CDCs (2-3

million autologous cells, n = 7) were injected into the 3 major coronary arteries 1 mo after surgery[38]. At 18 mo post injection, cardiac echo and MRI indicated an increase in right ventricular EF from 46.9% to 54.0% (P = 0.0004) compared to no change in EF (46.7% to 48.7%, P-ns) in control. This was a small study (only 7 patients received CDCs) but indicates that viable and dysfunctional myocardium can be treated with autologous CDCs. Although CDCs are beneficial in patients with heart disease, CDCs have many characteristics that overlap with MSCs[39]. Therefore, it is necessary to identify the similarities and differences in biological responses of both MSCs and CDCs prior to further proceeding with clinical applications.

CSCS IN ISCHEMIC CARDIOMYOPATHY

Several investigators have demonstrated the presence of small clusters of Sca-1+, cKit+ or a side population cells (multipotent stem cells identified by the ability to efflux Hoechst dye) in the cardiac atria and apex[40-42]. These cells were named CSCs and are most abundant during postnatal cardiac development after birth. Progeny of CSCs acquire a cardiomyocyte phenotype therefore resident CSCs are optimal candidates for cardiac regeneration studies. CSCs are self-renewing, can replace senescent and apoptotic CSCs *via* mobilization of BM-derived stem cells, and participate in maintaining the CSC pool in the heart[43-45]. In adulthood, the cells are quiescent and reside within the heart. Following ischemic injury, activation by paracrine signals induces CSCs to divide. Nevertheless, their proliferative potential is limited and the extent of the myocardial injury (*e.g.*, necrosis and fibrosis following MI) is frequently too large to be compensated by new cardiomyocytes formed from dividing resident CSCs[40]. In the normal organism the heart retains a pool of CSCs that regulate cardiac homeostasis by maintaining a low level of myocyte proliferation, regeneration and cell death[2]. It is well known that CSCs are a rare population in the myocardium making their isolation and cultivation difficult and time-consuming. Since these cells are located in the heart and are primed for cardiac repair, protocols to enhance their endogenous activity or expand these cells *in vitro* before re-implanting them in the heart are currently being tested. A limited number of animal studies indicate that the administration of CSCs can slow left ventricular remodeling and cardiac improve function after ischemic injury[40,46,47]. Welt *et al*[48] demonstrated that intramyocardial injection of autologous CSCs in a canine infarct model with permanent LAD occlusion resulted in the preservation of global function (31% to 33%) and reduced LV remodeling compared to functional deterioration (35% to 26%, P < 0.05) and LV remodeling in vehicle animals[48]. Bolli *et al*[49] demonstrated that administration of autologous CSCs to a swine model of chronically infarcted myocardium resulted in improvements in regional and global contractility (45.4% to 51.7%, P < 0.05) as well as engraftment and differentiation of injected CSCs[49].

The stem cell infusion in patients with ischemic cardiomyopathy (SCIPIO) trial isolated autologous CSCs during CABG[50]. SCIPIO involved 23 patients who had experienced MI in the past and exhibited an EF of under 40%. One million of cKit positive and lineage negative CSCs were isolated with magnetic beads from cultures of right atrial appendage tissue and administered *via* intracoronary infusion 1 mo after CABG. Twelve months after the treatment, infarct size was decreased by 30.2%, regional wall thickening was increased by 18% and left ventricular EF was increased by 8.2%. The benefits of treatment continued to increase and left ventricular EF was increased by 12% after 2 years[51]. Although studies have shown the beneficial effects of CSCs on the infarcted myocardium, their biological actions in the heart are still controversial[52]. Further studies are necessary to clarify the significance of CSCs in clinical applications.

FUTURE DIRECTIONS

Based on current achievements in experimental large animal studies and clinical trials of cell-based therapies, it is evident that cell therapies still require significant progress to be registered in the daily practice of modern medical therapies. The following strategies are solutions to overcome current limitation of cell-based therapies.

PRECONDITIONED MSCS

Since the safety and efficacy of MSCs has been demonstrated by clinical work, there has been an increasing interest on enhancing the benefits of MSC therapy. For example, combining MSC and pharmacotherapy[53], genetically modifying MSCs[54-56] and pre-conditioning MSCs[57] are approaches that are being explored to augment MSC-mediated cardiac repair. MSCs transfected to overexpress Akt or cell survival protein promoted myocardial protective function[16,55]. Furthermore, MSCs engineered to express combinations of gene products such as Akt and angiopoietin-1 (Ang1) have also shown functional benefits in experimental animal models[58]. MSCs overexpressing VEGF and SDF-1 improved cardiac function by activating Akt pathway[54]. MSCs transfected to express heme-oxygenase 1 (HO-1), an enzyme that improves MSC tolerance to hypoxia, infused into a cardiac ischemia-reperfusion model improve EF and lower end systolic volume compared to controls[59]. MSCs pretreated with growth factors, bFGF, IGF-1 and bone morphogenetic protein 2 (BMP2), improved myocardial repair in a rat model of MI[60]. Those preconditioned MSCs improved engraftment and survival of transplanted cells. Although data are promising, the safety of these cells must be carefully evaluated before use in humans.

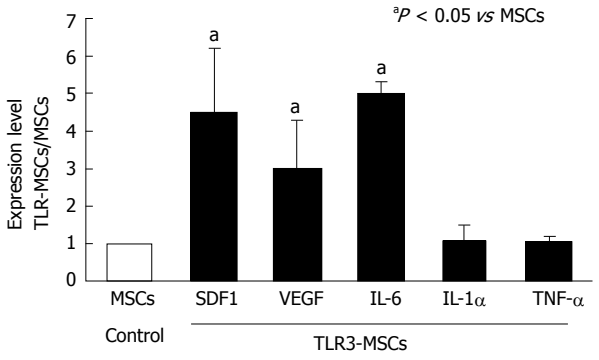

Figure 1 Toll-like receptor 3-mesenchymal stem cells enhance to secrete paracrine factors. RNA mimetic polyinosinic-polycytidylic acid [poly(I:C)] stimulated TLR3 system on MSCs. TLR3-MSCs secreted a variety of paracrine factors. RT-PCR detected significant upregulation of SDF1, VEGF and IL6 while inflammation related cytokines (IL-1α, TNFα) were downregulated. Injection of TLR3-MSCs in cardiomyopathy model improved cardiac function more than standard MSCs in association with increasing myocyte proliferation, reducing fibrosis and myocyte apoptosis. TLR3: Toll-like receptor 3; MSCs: Mesenchymal stem cells; SDF1: Stromal cell-derived factor-1; VEGF: Vascular endothelial growth factor; IL: Interleukin; TNF: Tumor necrosis factor.

PRECONDITIONED MSCS WITHOUT GENETIC MODIFICATION

As mentioned above, the currently used approaches to enhance stem cells are mostly through genetic modification. Thus, modified cells are not considered as a clinically relevant approach because genetically engineered stem cells may have increased unwanted long-term side-effects. We demonstrated that stimulation of toll-like receptor 3 (TLR3) produced many trophic factors without induction of inflammatory-related cytokines[26]. Poly (I:C) is structurally similar to double-stranded RNA and is known to interact with TLR3, which is expressed on the membrane of B-cells, macrophages, dendritic cells, MSCs and CDCs. Poly (I:C) directly reacts with the TLR3 receptor on the surface of MSCs/CDCs. Thus, after washing and collecting MSCs/CDCs after stimulation, poly (I:C) does not reside within the cells and does not affect the heart environment after injection of cells. Interaction of Poly (I:C) with TLR3 on MSCs causes secretion of the growth factors VEGF and the cytokine IL-6 without up-regulation of the inflammatory cytokines IL-1 and TNFα (Figure 1). Injection of TLR3 activated MSCs (TLR3-MSCs) in a non-ischemic cardiomyopathy model improved cardiac function more than standard MSCs in association with increasing myocyte proliferation, reducing fibrosis and myocyte apoptosis[61]. Activation of TLR3 on CDCs (TLR3-CDCs) stimulated the secretion of HGF, IGF1 and IL-6 without up-regulation of inflammatory cytokines. TLR3-MSCs or TLR3-CDCs are safe and feasible to use in the human heart. Further investigation is necessary to confirm the safety and feasibility to use in the heart.

MSCS AND CSCS

Combining MSC and CSC in post-MI treatment may further enhance the therapeutic effects of each cell type. Recent work by Williams *et al*[62] demonstrated that the combined use of 1 million human CSCs with 200 million human MSCs provided greater recovery, almost to baseline, in a swine model of anterior wall MI[62]. While all stem cell treated animals demonstrated improved LV EF compared to placebo controls, notably, animals receiving dual cell therapy had a 2-fold greater reduction in scar size (21.1% for CSC/MSC *vs* 10.4% for CSC alone or 9.9% for MSC alone) and had improved rates of pressure change during diastole. Overall left ventricular chamber dynamics were improved in both the dual therapy and CSC or MSC alone treated groups. Interestingly, CSC alone treated animals demonstrated better isovolumic relaxation as compared to controls, while MSC alone treated animals exhibited improved diastolic compliance, indicating that the enhanced effect of dual therapy on both systolic and diastolic function may be due to a synergistic effect between CSC and MSC targeted mechanisms.

REGIONAL INFUSION WITH STOP-FLOW *VS* GLOBAL INFUSION WITH SLOW INFUSION

Clinically applied techniques for cell delivery include endomyocardial injection using an injection needle or infusion of cells into a coronary artery supplying the infarcted region using a stop coronary flow technique. Although both approaches elicit significant improvements in cardiac function, they increase the risk of endomyocardial hemorrhage and MIs caused by stem cells plugs in the capillaries which could potentially limit the beneficial effects of cell-therapy. We previously demonstrated that slow infusion of MSCs into the three major coronary arteries without stop flow technique (global infusion) did not cause microembolization and stimulated prominent cardiac regeneration in ischemic as well as normally perfused RCA regions in swine with hibernating myocardium[17]. Likewise, intracoronary injection of autologous CDCs[35] without a stop-flow technique in swine with hibernating myocardium stimulated myocyte proliferation and regeneration in an ischemic LAD region as well as the normally perfused RCA regions. Subsequently, we applied the global infusion approach in an acute MI model, CDC infusion significantly improved cardiac function despite no changes in the size of infarction area. These results indicates that scar reduction and functional improvement are independent phenomenon[63]. Accordingly, the approach of stem cell injection in the entire heart is safe and feasible to improve LV dysfunction and our results indicate that normally perfused and viable myocardium could be the target for regenerative therapy. Alternatively, combining stop-flow infusion in the infarcted area with slow flow infusion into the viable myocardium may be a method to enhance therapeutic efficacy.

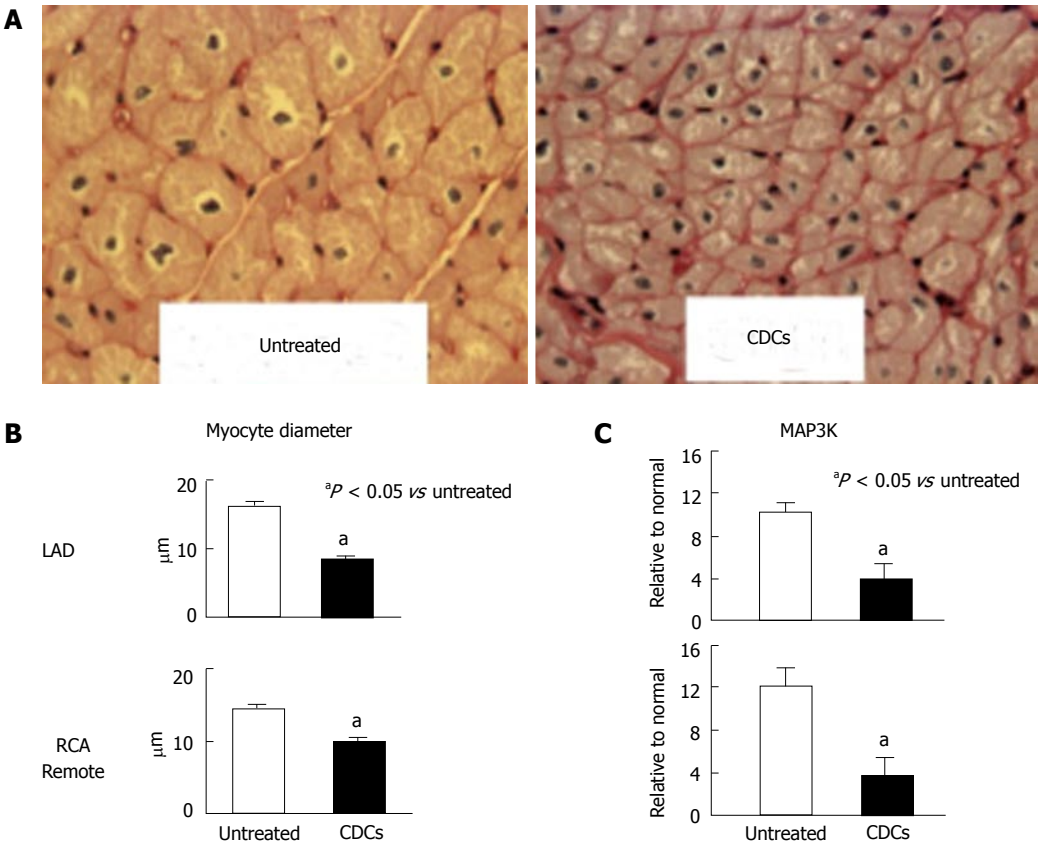

Figure 2 The effect of cardiosphere-derived cells on myocyte cell size and MAP kinase in the dysfunctional left anterior descending *vs* remote regions. A: Images (PAS staining) demonstrate that hypertrophied myocytes in untreated hibernating LAD became smaller after CDCs. Myofibrils were condensed indicating the production of healthy myocytes; B: Myocyte diameter was significantly reduced in hibernating LAD and remote regions; C: Corresponding to the morphological change, protein level of MAP3K was downregulated in LAD and remote regions. Data indicates CDCs induced myocyte regeneration and hypertrophy regression. CDCs: Cardiosphere-derived cells; LAD: Left anterior descending; RCA: Right coronary artery.

ALLOGENEIC CDCS INFUSION IMMEDIATE AFTER REPERFUSION

Allogeneic CDCs can escape direct recognition of helper T cells due to the lack of expression of MHC class Ⅱ antigen (SLA class Ⅱ on pig)[34,64] and therefore are immunoprivileged. Based on these observations, a recent clinical trial was initiated using allogeneic human CDC treatment in patients with chronic myocardial infarction (ALLSTAR trial). These allogeneic cells can be expanded *ex vivo* and stored for use at a future time[18]. This "off-the-shelf" treatment for patients with AMI immediately after revascularization is unique in that *ex vivo* expanded cells are available immediately for treatment and the patient does not need to wait for cell processing and expansion[19]. Recently, administration of CDCs immediately after reperfusion demonstrated the protective effects in swine with acute myocardial infarction[65]. Thirty minutes after ischemia-reperfusion, CDCs were injected into the infarct-related coronary artery and reduced the size of the infarct area and myocyte apoptosis in the border region. Although data were collected 48 h post CDCs injection, we recently demonstrated that functional improvement and myocyte regeneration were maintained up to 1-mo follow-up. These data indicate that the cardioprotective effects at early times were maintained. Previously bone marrow cell and endothelial progenitor cells injection in patients were performed within 7 d after AMI and demonstrated superiority to cell injection within 24 h[66,67]. Since stem cell homing factors (mobilization, migration and adhesion) are maximized between day 3 and day 7[68], these therapies are effective for stem cell homing. However, the inflammation caused by MI is already developed and the potential cardioprotective effects (*i.e.*, *via* anti-apoptotic effects or modulation of the inflammatory response) are limited when cells are delivered. Since CDCs secrete multiple cytokines (SDF-1, Akt)[69], growth factors (HGF, IGF-1, VEGF)[69,70] and exosomes[71,72], CDCs early after reperfusion might reduce the inflammatory response and protect the heart from functional deterioration due to reperfusion injury.

REPEATED INJECTION OF STEM CELLS

Since single injection of CDCs improved regional function and reduced scar volume[36,37], repeated injection of stem cells has been considered a more effective approach to regenerate myocardial tissue[73,74]. However, the initial infusion of cells activates and enhances the immune response[34,64] and the subsequent injected cells are quickly eliminated and ineffective. This quick

reaction is mainly associated with acquired/adaptive rather than innate immunity. Repeated infusion of autologous/allogeneic CDCs may overcome the limited functional recovery from a single injection[73,74]. However, the extent of immune activation caused by repeated injections is unclear and optimal immunosuppressive therapy is still undetermined. Development of efficacious CDC platforms administered with optimal immune suppression would circumvent barriers related to multiple injections of stem cells and allow the widespread application of "off-the-shelf" cell therapy to treat the large number of patients in need[64,75,76].

EXOSOME ACTIVATION AND MICRORNAS

The beneficial effects of adult stem cells are mainly associated with the secretion of paracrine factors rather than direct differentiation of *de novo* cardiac cells[9]. They secrete multiple growth factors and cytokines which reduce scar volume and myocyte apoptosis, increase myocyte proliferation and activate endogenous CSCs to produce new myocytes. Recently, it was reported that CDCs secrete exosomes and they play important roles for cardiac regeneration[71]. Exosomes transfer microRNAs from cell to cell and they inhibit inflammation and apoptosis and increase angiogenesis and myocyte proliferation. Therefore, a new method of treatment may focus on how to effectively stimulate secretion of exosomes from stem cells or may be directly injecting exosomes in the infarcted myocardium.

ANTI-HYPERTROPHIC EFFECT

Besides their regeneration potential, adult stem cells have other beneficial effects such as anti-apoptosis, anti-inflammation, extracellular matrix reduction, contractile alternation and anti-hypertrophy. Pathological cardiac hypertrophy in post MI remodeling is a major cause of mortality and morbidity including the risk of sudden cardiac death and heart failure in patients[77-81]. It is associated with increased interstitial fibrosis, cell death and cardiac dysfunction. LV assist devices used in heart failure patients as a bridge to heart transplantation not only improved peripheral circulation but also reversed the geometric remodeling of the heart and restored the function of the heart[82-85].

We demonstrated that global infusion of CDCs into hearts with chronically ischemic myocardium's improved myocardial function in the ischemic and remote regions[86]. CDCs significantly increased newly formed small myocytes. Interestingly, CDCs also reduced the cell size of pre-existing myocytes and hypertrophic signaling (mitogen activated kinases) in the ischemic and remote regions. Data indicate that CDCs have the potential to reverse cardiac hypertrophy (Figure 2). Future studies are necessary to determine whether hypertrophy regression is primary or secondary to myocardial regeneration and functional improvement.

CONCLUSION

Promising data derived from experimental models indicate the potential success of using cell based therapy in clinical applications. To overcome the current limitations in the field, development of new methods to enhance cardiac repair is necessary. In light of their proven safety profiles, MSC, CDC and CSC are prime candidates for cell based therapies. Recently, it was reported that a combination of CSCs and MSCs may be more effective than either one alone, and this approach is under investigation. Similarly, pre-conditioning MSC and CDCs are also promising approaches, and further investigation is anticipated. Optimizing the dose and method of delivery, as well as the timing for delivery are important variables that should be studied. It is anticipated that cell based therapies will become a mainstream treatment for heart diseases due to their potential ability to improve functional outcomes and decrease mortality.

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REVIEW

Vascular endothelial dysfunction and pharmacological treatment

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Abstract

The endothelium exerts multiple actions involving regulation of vascular permeability and tone, coagulation and fibrinolysis, inflammatory and immunological reactions and cell growth. Alterations of one or more such actions may cause vascular endothelial dysfunction. Different risk factors such as hypercholesterolemia, homocystinemia, hyperglycemia, hypertension, smoking, inflammation, and aging contribute to the development of endothelial dysfunction. Mechanisms underlying endothelial dysfunction are multiple, including impaired endothelium-derived vasodilators, enhanced endothelium-derived vasoconstrictors, over production of reactive oxygen species and reactive nitrogen species, activation of inflammatory and immune reactions, and imbalance of coagulation and fibrinolysis. Endothelial dysfunction occurs in many cardiovascular diseases, which involves different mechanisms, depending on specific risk factors affecting the disease. Among these mechanisms, a reduction in nitric oxide (NO) bioavailability plays a central role in the development of endothelial dysfunction because NO exerts diverse physiological actions, including vasodilation, anti-inflammation, antiplatelet, antiproliferation and antimigration. Experimental and clinical studies have demonstrated that a variety of currently used or investigational drugs, such as angiotensin-converting enzyme inhibitors, angiotensin AT1 receptors blockers, angiotensin-(1-7), antioxidants, beta-blockers, calcium channel blockers, endothelial NO synthase enhancers, phosphodiesterase 5 inhibitors, sphingosine-1-phosphate and statins, exert endothelial protective effects. Due to the difference in mechanisms of action, these drugs need to be used according to specific mechanisms underlying endothelial dysfunction of the disease.

Key words: Endothelial dysfunction; Endothelium-dependent vasodilation; Endothelial nitric oxide synthase; Inflammation; Nitric oxide; Pharmacological treatment; Reactive nitrogen species; Reactive oxygen species; Risk factors

Core tip: The endothelium is involved in the regulation of vascular tone and permeability, coagulation and fibrinolysis, inflammatory and immunological reactions

and cell growth. Cardiovascular risk factors cause vascular endothelial dysfunction through impairing endothelium-derived vasodilators, enhancing endothelium-derived vasoconstrictors, producing reactive oxygen species and reactive nitrogen species, activating inflammatory and immune reactions and promoting thrombosis. Among these mechanisms, a reduction in nitric oxide bioavailability plays a central role in the development and progression of endothelial dysfunction. A variety of currently used or investigational drugs exert endothelial protective effects according to specific mechanisms underlying endothelial dysfunction of the disease.

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INTRODUCTION

The endothelium is formed by a monolayer of endothelial cells. It constitutes a physical barrier between blood and tissues and regulates the exchange of molecules between blood and tissues. In addition, endothelial cells metabolize, synthesize and release a variety of substances, including vasoactive substances regulating vascular tone, blood pressure and local blood flow, the substances participating in coagulation, fibrinolysis and inflammatory and immunological reactions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) involved in oxidation and nitrosylation of proteins and lipids, and growth factors promoting cell growth (Table 1). Any perturbation affecting the capacity and equilibrium of the endothelium as a physical barrier and to metabolize, synthesize and release these substances will cause endothelial dysfunction, which contributes to the development and progression of cardiovascular diseases. After summarizing the role of a number of endothelium-derived vasoactive substances and risk factors of endothelial dysfunction, this review focus on several categories of pharmacological substances that may be used for improving endothelial function.

Table 1 Some of endothelium-derived substances

Vasoactive substances
Endothelium-derived vasodilators
Adrenomedullin
Endothelium-derived hyperpolarizing factors
Kinins
Nitric oxide
Prostacyclin
Endothelium-derived vasoconstrictors
Angiotensin II
Endothelin-1
Vasoconstrictor prostanoids
Coagulation and fibrinolysis
Coagulation
Factor V
Heparan sulfate
Protein C
Protein S
Thrombomodulin
Tissue factor
von Willebrand factor
Fibrinolysis
Plasminogen activator inhibitor
Tissue plasminogen activator
Urokinase
Growth factors
Basic fibroblast growth factor
Insulin-like growth factor
Platelet-derived growth factor
Transforming growth factor
Inflammatory and immunological mediators
Cytokines
Interleukins
Monocyte chemoattractant protein 1
Transforming growth factor
Tumor necrosis factor-α
Adhesion molecules
Intercellular adhesion molecules
Platelet-endothelial cell adhesion molecules
Selectins
Vascular cell adhesion molecules
Reactive oxygen species and reactive nitrogen species
Reactive oxygen species
Hydrogen peroxide (H_2O_2)
Hydroperoxyl (HO_2)
Superoxide (O_2^-)
Reactive nitrogen species
Nitrite (NO_2^-)
Nitrogen dioxide (NO_2)
Peroxynitrite ($ONOO^-$)
Nitryl chloride (NO_2Cl)

ENDOTHELIUM-DERIVED VASOACTIVE SUBSTANCES

The endothelium releases a variety of vasoactive substances, including different vasodilators such as nitric oxide (NO), prostacyclin, kinins, and endothelium-derived hyperpolarizing factors (EDHF), vasoconstrictors such as endothelin-1 and PGH_2, and ROS. Among endothelium-derived vasodilators, NO occupies a central position because changes in the release of endothelial NO play a crucial role in the perturbation of vascular homeostasis and in the development of endothelial dysfunction associated with various cardiovascular disorders.

NO

NO is synthesized from the amino acid L-arginine by a family of enzymes, the NO synthase (NOS), through the L-arginine-NO pathway. Three isoforms of NOS have been identified. Neuronal NOS (nNOS), initially found in the nervous system, is also constitutively expressed in skeletal and cardiac muscles, vessels and many other tissues. Endothelial NOS (eNOS) is constitutively expressed mainly in endothelial cells, whereas the expression of inducible NOS (iNOS) can

be stimulated by diverse factors such as cytokines and endotoxin in different circumstances. The endothelium-derived NO release is primarily ensured by eNOS and complemented by nNOS expressed in vascular endothelial cells. Therefore, eNOS is primordial in the regulation of NO production by endothelial cells. The activity of eNOS depends on several factors, including eNOS mRNA and protein expression, the abundance of asymmetric dimethylarginine (ADMA, an endogenous eNOS inhibitor that competes with L-arginine for binding to eNOS)[1,2], the quantity and quality of cofactors such as tetrahydrobiopterin (BH4) and NADPH that are necessary for eNOS catalyzing NO production from L-arginine[3,4], its interaction with caveolin and heat shock protein 90 (hsp90)[5,6], and its translational modifications such as phosphorylation at different sites by multiple kinases or phosphatases [for example, phosphorylation at ser-1179 by phosphotidylinositol-3-kinase (PI3K)/protein kinase B (Akt) activates eNOS to initiate NO synthesis][7,8] and S-nitrosylation at cysteine residues[9]. In addition, excessive superoxide (O_2^-) and hydrogen peroxide (H_2O_2) production due to increased NAD(P)H oxidase[10,11] and eNOS uncoupling induced by changes in oxidized low density lipoprotein (OxLDL), caveolin-1, BH4, a switch from S-nitrosylation to S-glutathionylation and oxidation of the zinc-thiolate complex by peroxynitrite ($ONOO^-$) also affects effective eNOS activity[12-15].

NO released by eNOS participates in the regulation of vascular tone. NO activates soluble guanylate cyclase by binding to its ferrous heme, leading to the conversion of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) that causes vascular smooth muscle relaxation. Moreover, NO exerts antiinflammatory, antiplatelet, antiproliferative and antimigration actions that contribute to the maintenance of an adequate environment for the endothelium. Lacking eNOS gene in mice induces insulin resistance, hyperlipidemia and hypertension[16]. NO released by nNOS also participates in the regulation of vascular tone, especially for the regulation of vascular tone in skeletal muscles. Altered nNOS activity and protein levels contribute to muscular damage due to sustained vasoconstriction in patients with Duchenne muscular dystrophy[17,18] and endothelial dysfunction in dogs with Duchenne muscular dystrophy[19].

Prostacyclin

Prostacyclin (also called PGI2) is generated from arachidonic acid by cyclooxygenase (COX) in endothelial cells. Activation of IP receptors by PGI2 activates adenylate cyclase to synthesize cyclic adenosine monophosphate (cAMP) from adenosine triphosphate, causing vascular smooth muscle relaxation. However, PGI2 synthesis can be inactivated by increased cytokines[20] and under certain conditions, PGI2 exerts vasoconstrictor action and contributes to endothelial dysfunction[21].

EDHF

The term "EDHF" describes a set of substances causing vascular myocyte hyperpolarization and spreading endothelial hyperpolarization to vascular myocytes, resulting in vascular myocyte relaxation, which is not affected by blocking NO or prostacyclin production[22]. Interestingly, in eNOS/COX-1 double-knockout mice, EDHF-mediated vasodilation plays a compensatory role for the absence of endothelial NO[23]. EDHF hyperpolarize myocyte membranes by opening calcium-activated potassium channels named as BKca, IKca, and SKca according to their conductance (big, intermediate, and small conductance), leading to K^+ efflux[24-26]. EDHF-mediated vasodilation also involves epoxyeicosatrienoic acids (EETs), gap junction, reactive oxygen species and hydrogen peroxide, depending on the vascular beds and vessel types[24,25,27]. Cytochrome P450 epoxygenase catalyzes the production of EETs from arachidonic acid in different vessels and EETs participate in, at least partly, the hyperpolarization and relaxation of myocytes in these vessels[28,29]. It is worth noting that EDHF-mediated vasodilation is a complex phenomenon, which involves multiple signaling pathways that may be not exclusive in response to different stimuli[24-26]. Altered EDHF signalings may account for endothelial dysfunction in some cardiovascular disorders as suggested by studies in animals. For example, a defect in connexins that compose gap junctions is partly responsible for impaired vasodilator responses in hypertensive rats[30] and in diabetic rats[31]. However, the role of EDHF in endothelial dysfunction in human cardiovascular diseases remains elusive. This may be related to the difficulty that the function of EDHF can only be deciphered after impairment of NO and prostacyclin-mediated responses.

Kinins

Kinins such as bradykinin can be generated in vessel walls, especially in endothelial cells that contain the components such as kinin precursors and kinin-generating enzymes, necessary for the production of kinins[32]. The biological effects of kinins are mediated by stimulation of constitutively expressed B2 receptors and inducible B1 receptors. In endothelial cells, activation of B2 receptors by bradykinin releases NO, prostacyclin, EDHF and tissue plasminogen activator, which exert diverse physiological and pathological actions on cardiovascular system, including regulation of coronary vascular tone and local blood flow of organs, coagulation, fibrinolysis, and water-electrolyte while[33,34]. Stimulation of B1 receptors by its agonists also induce NO-mediated vasodilation[35]. Due to very short half-life in the blood, bradykinin essentially plays an autocrine/paracrine role. Experimental studies have demonstrated a protective role of bradykinin B2 receptors on cardiovascular function. It is, at least in part, due to opposing effects of bradykinin B2 receptor activation on angiotensin Ⅱ AT1 receptor activation because of multiple cross-talks between the kallikrein-kinin system and the renin-

angiotensin system[36]. This explains the contribution of kinins to the cardiovascular protective effects of angiotensin-converting (ACE) inhibitors and angiotensin AT1 receptor blockers. Deletion of both B1 and B2 receptors in diabetic mice exacerbates nephropathy as indicated by increased oxidative stress, mitochondrial DNA deletions and renal expression of fibrogenic genes, suggesting a protective role of the kallikrein-kinin system on diabetic nephropathy[37]. However, these mice exhibit neither accelerated cardiac dysfunction nor ROS production, challenging the protective role of kinins in this setting[38]. Otherwise, kinins can increase endothelial permeability[39] and are involved in inflammatory responses by activating phospholipase A2 to release arachidonic acid that is used for the production of vasoconstrictor prostanoids, which may be harmful for endothelial function.

Adrenomedullin

Adrenomedullin (AM), a vasodilator peptide initially identified from human pheochromocytoma, can be secreted by vascular cells, especially by endothelial cells[40-42]. AM exerts its action in the cardiovascular system through receptor complexes composed of the calcitonin receptor-like receptor and receptor activity-modifying proteins. In vessels, the receptors for AM are expressed in both endothelial and smooth muscle cells[43,44]. AM-induces endothelium-dependent and -independent vasodilation, depending upon species and vascular beds[42]. AM-induced endothelium-dependent vasodilation is mediated by PI3K/Art/NO/cGMP pathway, the activation of cGMP-stimulated protein kinase G and/or the production of a vasodilator prostanoid (likely prostacyclin)[42,45,46], whereas AM-induced endothelium-independent vasodilation involves the opening of K^+ channels (calcium-activated K^+ channels or ATP sensitive K^+ channels, probably depending upon vascular beds) and the activation of cAMP-dependent protein kinase A[42,47]. In addition to vasodilator effect, AM was shown to inhibit angiotensin II-induced ROS generation by NAPDH[48], and AM -deficient mice developed insulin resistance due to increased ROS[49]. AM protects bone marrow-derived mononuclear cell and endothelial progenitor cells from apoptosis and exerts a vascular protective role[50,51]. AM also exerts an protective effect on endothelial barrier function and reduces endothelial permeability in response to inflammation and endotoxin[52-56]. Otherwise, AM posses angiogenesis property and participates in vascular remodeling [50,57,58]. Plasma AM levels were shown to be higher in many pathological situations such as arteriosclerosis, sepsis, essential or pulmonary hypertension and heart failure[52,59-63], whereas intracoronary AM levels were lower in patients with stable coronary disease[64] and in infants with brain damage after surgery under cardiopulmonary bypass[65]. Increased AM levels were interpreted as a compensatory mechanism to protect cardiac and vascular function[61,66]. Expression of receptors for AM was shown to be increased in rats with heart failure though its signification remains elusive[67,68]. Despite its protective role in the cardiovascular system, AM was shown to be involved in the growth of different tumors such as prostate, colorectal and bladder tumors, and AM and its receptors are potential targets for the treatment of these tumors[69-71].

Angiotensin Ⅱ

Endothelial cells express ACE and angiotensin AT1 and AT2 receptors. Once released, angiotensin Ⅱ immediately binds to these receptors and those expressed on smooth muscle cells. Although angiotensin Ⅱ causes both vasoconstriction *via* AT1 receptors and vasorelaxation by stimulating AT2 receptors, angiotensin Ⅱ-induced vasoconstriction is predominant in many circumstances. Moreover, angiotensin Ⅱ exerts multiple actions affecting endothelial function. Angiotensin Ⅱ upregulates endothelial receptors for OxLDL, stimulates OxLDL uptake, and enhances OxLDL-mediated ROS generation and endothelial cell apoptosis[72]. Angiotensin II increases receptors for vascular endothelial growth factors and matrix metalloproteinases (MMPs), which may account for increases in endothelial permeability and vascular remodeling[73-75]. Angiotensin Ⅱ increases the expression of plasminogen activator inhibitor type 1 (a natural inhibitor of tissue-type plasminogen activator and urokinase-type plasminogen activator) in endothelial cells[76], thereby favoring thrombosis. Angiotensin II favors inflammation by inducing COX-2 expression[74] and increasing cytokine tumor necrosis factor-alpha (TNF-α)[75]. Although angiotensin Ⅱ can upregulate eNOS and inducible NO synthase (iNOS) expression, it reduces eNOS-derived NO by promoting eNOS uncoupling through monocyte-dependent S-glutathionylation[77]. In addition, the activation of AT2 receptors also contributes to the angiotensin Ⅱ-induced vascular remodeling[78]. These actions of angiotensin Ⅱ may contribute to endothelial dysfunction as the renin-angiotensin system is activated, as is the case in atherosclerosis[79] and heart failure.

Endothelin-1

Although endothelin-1 can upregulate eNOS expression by enhancing eNOS mRNA stability *via* protein tyrosine kinases and protein kinase C-dependent pathways[80,81], endothelin-1 *via* type A endothelin receptors induces expression of adhesion molecules and neutrophil adhesion to endothelial cells, and promotes cytokine and ROS generation[82-84]. Elevated endothelin-1 blood levels can be seen in atherosclerosis[85], pulmonary hypertension[86] and heart failure[87,88], which may account for the development of endothelial dysfunction under these circumstances. Although the activation of Type B endothelin receptors generally induces vasodilation, these receptors appear to mediate endothelin-1-induced ROS production and contribute to endothelial dysfunction in obese rats[82].

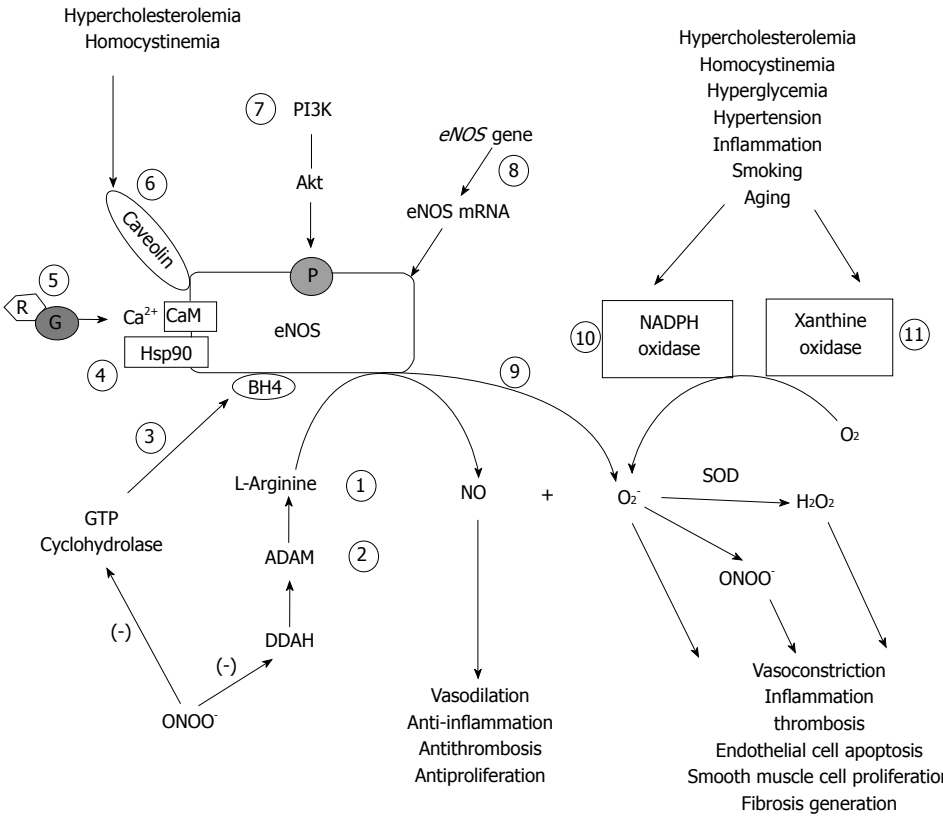

Figure 1 Mechanisms underlying the reduction in nitric oxide bioavailability involve both reduced nitric oxide production and increased nitric oxide scavenging. A reduction in NO production can be resulted from: (1) decreased L-arginine availability due to L-arginine deficiency and/or changes in L-arginine transporter; (2) accumulation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial NO synthase (eNOS); (3) deficiency or modification of cofactor tetrahydrobiopterin (BH4); (4) altered interactions between eNOS and caveolin due to increased caveolin-1; (5) changes in receptor-coupled G proteins; (6) altered eNOS-heat shock protein 90 (Hsp90) interaction due to changes in Hsp90 abundance; (7) changes in calcium-independent phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)-mediated eNOS activation by tyrosine or serine phosphorylation; and (8) decreased eNOS expression due to reduced *eNOS* gene transcription and/or decreased eNOS mRNA stability. Increased NO scavenging by reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be due to: (9) eNOS uncoupling related to changes in BH4, cavelin-1 and oxidized low density lipoproteins; (10) increased NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) expression and activity; and (11) increased xanthine oxidase expression and activity. CaM: Calmodulin; DDAH: Dimethylarginine dimethylaminohydrolase; SOD: Superoxide dismutase; NO: Nitric oxide.

Vasoconstrictor prostanoids

Endothelial cells can produce vasoconstrictor prostaglandin H2 (PGH2), thromboxane A2 (TXA2) and PGF2α. The production of these prostanoids is enhanced in hypertension, hypercholesterolemia, diabetes and vitamin E deficiency[89], which in turn upregulates NAPDH oxidase and type 4 and type 5 phosphodiesterases (PDE4 and PDE5)[90,91], resulting in increases in ROS production, cAMP and cGMP degradation, and vasoconstriction. TXA2 is also a potent activator of platelets, while activated platelets in turn release a large amount of TXA2 to promote thrombosis. Furthermore, TXA2 interacts with EDHF by inhibiting potassium channels, EETs and gap junction-mediated signaling pathways[27], which could account for the development of endothelial dysfunction.

RISK FACTORS CAUSING ENDOTHELIAL DYSFUNCTION

Clinically, endothelial dysfunction is characterized by impaired endothelium-dependent vasorelaxation in response to endothelium-dependent agonists such as acetylcholine and bradykinin, or to maneuvers that increase shear stress such as flow-mediated dilatation. Although mechanisms leading to endothelial dysfunction are multiple, a reduction in NO bioavailability is largely observed in many cardiovascular disorders. As shown in Figure 1, reduced NO bioavailability can be the consequence of decreased L-arginine availability[92], increased ADMA[1,2], altered interaction with hsp90[93] and phosphorylation of eNOS[94], as well as increased NO scavenging by excessive ROS generated by NADPH and xanthine oxidases[10,11,95] and eNOS uncoupling. It is worth noting that changes in caveolin-1[96], BH4[97], S-glutathionylation of eNOS[15,98] and OxLDL[12] are all involved in eNOS uncoupling. Importantly, a reduction in eNOS protein expression also leads to impaired eNOS activity and NO production, which can be observed in different cardiovascular diseases such as atherosclerosis, acute myocardial infarction and heart failure in animals and in humans[99-103]. However, mechanisms underlying endothelial dysfunction in different cardiovascular

disorders may be different, depending on risk factors contributing to the development of specific disease.

Hypercholesterolemia/atherosclerosis

Atherosclerosis is a chronic arterial disease involving the formation of multiple atheromatous plaques within arteries by accumulation of lipids due to the inability to remove LDL from macrophages. In this process, endothelial dysfunction related to hypercholesterolemia plays a pivotal role in the development of atherosclerosis. Hypercholesterolemia induces endothelial cell activation, leukocyte recruitment and adherence, platelet activation and adhesion within the vasculature, reflecting an inflammatory response and high thrombotic state that may cause endothelial dysfunction. Hypercholesterolemia increases superoxide and hydrogen peroxide production by increasing NAD(P)H oxidases[79], xanthine oxidase[95], and myeloperoxidase[104]. Increased superoxide reacts with NO, resulting in the formation of RNS and reduced eNOS-derived NO bioavailability. ROS induce oxidation of lipids, proteins and DNA, which cause cell damage, necrosis and cell apoptosis. Increased RNS induce nitrosylation reactions that modify the structure and function of proteins. Hypercholesterolemia increases caveolin-1 levels[105], also contributing to impaired eNOS activity. In addition, hypercholesterolemia disturbs reactions between oxygen radicals or enzymatic oxidation and lipoproteins, particularly LDL phospholipids and results in the production of oxidized phospholipids. These phospholipids contain arachidonic acid and bind to their membrane receptors, resulting in their accumulation within the cellular membrane, immune and inflammatory responses and ROS generation, which in turn induces eNOS uncoupling that impairs endothelium-dependent vasodilation induced by endogenous vasodilator such as kinins but enhances the role of endogenous vasoconstrictors such as angiotensin Ⅱ and endothelin-1, and promote endothelial dysfunction[106,107]. Therefore, endogenous vasoactive substances such as NO, prostanoids, ROS, RNS, AM, angiotensin Ⅱ, endothelin-1 and other substances interact and reduced NO bioavailability due to eNOS uncoupling is a key event contributing to the development of endothelial dysfunction and ultimately atherosclerosis.

Hyperhomocysteinemia

Homocysteine is a non-protein α-amino acid synthesized from methionine. Hyperhomocysteinemia is observed in patients with coronary disease and is correlated with endothelial dysfunction[108]. Homocysteine causes endothelial dysfunction through NO inhibition, vasoconstrictor prostanoid production, EDHF inhibition[109,110], angiotensin AT1 receptor activation, and ROS generation[111]. Homocysteine reduces eNOS activity by increasing asymmetric dimethylarginine production[112] and eNOS uncoupling *via* decreasing intracellular do novel synthesis of BH4[97], leading to decreased NO bioavailability and increased ROS generation. Furthermore, homocysteine downregulates eNOS expression in human endothelial cells[113], and induces endothelial loss, vascular deendothelialization and increases platelet adherence and consumption in baboons[114]. Homocysteine also increases ROS generation by phosphorylating NAPDH oxidase[115], and/or by increasing ACE activity *via* ACE homocysteinylation to generate angiotensin Ⅱ that activates NAPDH oxidase[116].

Hyperglycemia/diabetes mellitus

Endothelial dysfunction is associated with both insulin-dependent and independent diabetes mellitus. In this setting, hyperglycemia increases ROS generation through activating protein kinase C-mediated NAD(P)H oxidases[117] and peroxynitrite-mediated eNOS uncoupling[118], which also leads to reduced NO bioavailability. In addition, hyperglycemia increases iNOS expression and iNOS-derived NO and peroxynitrite production, leading to increased ROS and RNS levels and pancreatic islet endothelial cell apoptosis[119]. Moreover, hyperglycemia promotes platelet aggregation by increasing expression and circulating levels of endothelial adhesion molecules through protein kinase C-NFκB signaling pathway[120-122], and increases endothelial apoptosis[123]. All of these effects of hyperglycemia contribute to endothelial dysfunction observed in diabetes mellitus. Otherwise, increased release of vasoconstrictors such as prostanoids and endothelin-1 through protein kinase C-mediated pathway in response to hyperinsulinemia and hyperglycemia appears to precede changes in vascular complication or NO production[124,125]. Changes in EDHF also contribute to endothelial dysfunction, especially in type 2 diabetes as suggested in rat models in which an impaired EDHF-mediated vasorelaxation was observed before marked alteration in NO-mediated responses[126-129]. Therefore, altered NO bioavailability in type 2 diabetes appears to be a relatively late event worsening endothelial dysfunction.

Hypertension

An impaired endothelium-dependent vasorelaxation has been observed in patients with essential hypertension[130] and in several animal models of hypertension. This is related to a lower production of endothelium-derived vasodilators and/or over production of vasoconstrictors. An increased endothelin-1 production also plays a role in endothelial function, especially in pulmonary hypertension as lung is an important metabolic organ of circulating peptides such as adrenomodullin and endothelin-1. In this regard, pulmonary endothelin-1 extraction affects the incremental resistance of pulmonary vascular bed in response to increased cardiac work[131] and plasma endothelin-1 levels are closely related to clinical worsening of patients with pulmonary hypertension[132]. An impaired NO and EDHF-mediated vasorelaxation linked to an increased ADMA that inhibit eNOS and downregulates SKca in

endothelial cells has been reported in hypertensive patients and in spontaneous hypertensive rats[133]. Interestingly, alterations in EDHF appears to occur before alterations in NO pathways in different rat models of hypertension[134]. An reduced vasodilator response to AM has been observed in hypertensive patients[45]. However, an increased eNOS expression is generally observed in animal models of hypertension associated with angiotensin Ⅱ. In this case, angiotensin Ⅱ-induced oxidative stress and increases in the production of vasoconstrictor prostanoids and cytokines may account for the development of endothelial dysfunction. Furthermore, a reduced NO bioavailability has been reported in some models of hypertension. This appears to be linked to reduced substrate availability due to L-arginine deficiency and changed L-arginine transport[92] and to eNOS uncoupling due to oxidation of BH4 and/or S-glutathionylation, leading to increased ROS production[4,15]. Thus, although altered NO bioavailability may not be an initial event to induce endothelial dysfunction, it participates in its progression in hypertensive subjects.

Smoking

Endothelial dysfunction is one of the primary damages induced by cigarette smoke. Circulating cigarette toxins such as free radicals and reactive glycation products can react with endothelial cells and cause vascular impairment[135]. Cigarette smoking induces inflammatory state as indicated by elevation of white blood cells, adhesion molecules and cytokines, and increases ROS production and lipid peroxidation[136-141]. These mechanisms may contribute to impaired endothelium-dependent vasodilation observed in active smokers, even at young healthy adult, and in passive smokers[142,143]. However, despite a reduced NO bioavailability, eNOS expression has been shown to be increased in different endothelial cells or decreased in platelets in response to cigarette smoke[144,145]. Cigarette smoke extracts inhibits eNOS activity of pulmonary arterial endothelial cells through modifying eNOS phosphorylation pattern, which cannot be protected by antioxidants such as vitamin E and C[146,147]. In this setting, decreased NO bioavailability is probably the consequence of decreased eNOS activity due to modified eNOS phosphorylation and uncoupling as well as NO scavenging by increased ROS.

Inflammation

Endothelial cells produce inflammatory and immune mediators (Table 1) and undergo morphological modifications in response to inflammatory stimuli. The inflammatory and immune mediators increase endothelial permeability and promote adhesion of leukocyte to endothelial cells and interactions between chemokine receptors on leukocyte and proteoglycans on endothelial cells, leading to leukocyte transendothelial migration to inflammation sites. Inflammation induced endothelial dysfunction is often associated with impaired NO bioavailability. For example, typhoid vaccination induced an inflammatory response as indicated by increased cytokines and oxidative stress as well as a decreased endothelium-dependent vasodilation that was partially restored by antioxidant vitamin C[148]. In patients with viral myocarditis, acetylcholine induced a coronary vasoconstriction rather vasodilation[149]. Similar responses were also observed in mice with virus-induced myocarditis, which was attributed to reduced eNOS activity and expression[150]. In some autoimmune diseases, anti-endothelial antibodies cause abnormal immune activation that activates endothelial cells to release adhesion molecules and cytokines, leading to inflammation, increased permeability of the endothelium, thrombosis and cell apoptosis[151-153], which are, at least in part, responsible for endothelial dysfunction in this setting. Patients with rheumatoid arthritis have increased levels of cytokines and ADMA and impaired flow-mediated dilation[154]. Similarly, increased arterial stiffness is closely correlated with ADMA blood levels in systemic lupus erythematosus patients[155]. The increase in ADMA levels may account for reduced NO bioavailability in these autoimmune diseases.

In some cases, an over production of NO occurs in response to inflammation. Septic shock associated with a severe infection and sepsis is characterized by a profound hypotension, widespread endothelial injury and activation, multiple organ failure and death. In this setting, toxic microbe products, including endotoxins (bacterial membrane lipopolysaccharides, LPS) of gram-negative bacteria and analogous molecules in the walls of gram-positive bacteria and fungi, dramatically activate mononuclear cells to release cytokines[156] that upregulate bradykinin B1 receptors[157,158], inducible NO synthase[159] and COX-2[160], which increase NO and prostaglandin E2. In this regard, blocking or deleting bradykinin B1 receptors might yield benefits for the treatment of septic shock. However, experimental studies showed conflicting results regarding the role of kinins in septic shock in animals. Mice with overexpression of B1 receptors exhibited an increased susceptibility to develop septic shock and mice lacking B1 receptors or both B1 and B2 receptors had an enhanced resistance to LPS-induced sepsis[161-163], whereas mice lacking B1 receptors had an higher mortality in response to LPS[164] and additional B1 receptor blockade suppressed the beneficial effect of B2 receptor blockade[165]. Similarly, B2 receptor blockade showed no effect or amelioration in porcine sepsis[165,166]. Results regarding the role of NO, particularly iNOS in septic shock are also elusive. Experiments in rats and in human blood cells showed that iNOS expression is correlated with cell apoptosis in septic shock[167,168]. Selective iNOS inhibition improved hemodynamics and mortality in nondiabetic rats with LPS-induced sepsis but not in diabetic rats[169], whereas depletion of iNOS resulted in increased dysfunctional mitochondria, IL-

1β production and caspase-1 activation in response to LPS in myeloid cells from both mice and humans and increased NLRP3 inflammasome-mediated cytokine production and mortality in mice with LPS-induced sepsis, which was prevented by NLRP3 deficiency[170]. Although treatment with methylene blue that has the ability to scavenge NO and to inhibit NO synthase showed a transient and reproducible beneficial effect on systemic vascular resistance, arterial pressure and organ function in patients with septic shock, but its effect on mortality remains unknown[171,172].

Aging

Aging is accompanied by complex structural and functional modifications of the vasculature, leading to dysfunction of both the endothelium and smooth muscle cells. Changes in aged smooth muscle cells are characterized by changed migration, proliferative and apoptotic behavior, increased response to vasoconstrictors and decreased expression of Ca^{2+}-activated K^+ channels in coronary arteries[173,174]. Aged endothelial cells are associated with decreased NO synthesis and sensitivity to agonist and mechanic stimuli that promote eNOS expression but increased sensitivity to be apoptotic[175,176]. Loss of PI3K/Akt-dependent eNOS phosphorylation seems to be a main mechanism explaining the reduction in NO production in old rats[94]. In addition, aging of endothelial cells is associated with increased production of vasoconstrictor prostanoids, endothelin-1 and ROS[176-178]. ROS are mainly produced by mitochondrial respiratory chain and NADPH oxidases, although eNOS uncoupling my also contribute to increased ROS during aging[179].

METHODS FOR MEASURING ENDOTHELIAL DYSFUNCTION

In animals, endothelial dysfunction can be measured by examining vasodilator responses to endothelium-dependent substances such as acetylcholine, bradykinin and serotonin in comparison with responses to endothelium-independent molecules such as NO donor in the absence and presence of NOS inhibitor and COX inhibitor *in vivo*[180,181] and in isolated vessels[19,182].

The methods used in clinical practice to measure endothelial dysfunction are detailed elsewhere[183]. This includes invasive methods by using quantitative angiography and intracoronary Doppler wire within coronary circulation and non-invasive methods, including venous occlusion plethysmography to measure forearm blood flow, flow-mediated dilatation in brachial artery, and peripheral arterial tonometry measuring pulsatile volume changes in the distal digit[183].

In addition, some circulating biomarkers such as endothelin-1, E-selectin, von Willebrand factor, thrombomodulin, intercellular adhesion molecules and vascular cell adhesion molecules can also be analyzed to detect endothelial dysfunction, although none of them are specific[183].

CARDIOVASCULAR DRUGS IMPROVING ENDOTHELIAL FUNCTION

Experimental and clinical studies have shown that numerous currently used or investigational drugs can improve endothelial function, although they have different structure and mechanisms of actions.

ACE inhibitors and AT1 blockers

Since the success of ACE inhibitors in the treatment of heart failure and discovery of their multiple actions, ACE inhibitors and AT1 blockers are widely used to the treatment of hypertension, arthrosclerosis, diabetes and some autoimmune diseases. It is well established that ACE inhibitors can improve endothelial function in animals with heart failure[184] and in patients with coronary artery disease[185,186]. This effect is related to both reduction in angiotensin Ⅱ and increase in bradykinin accumulation. In addition, ACE inhibitors upregulate eNOS expression in animals[102,187]. The effect of ACE inhibitors on eNOS expression is mediated by bradykinin B2 receptors, which can be blocked by B2 receptors blockers[102,187]. ACE inhibitors and AT1 blockers also inhibit ROS production and COX-2-derived vasoconstrictors, which contribute to endothelial protective effects of these drugs[188]. It appears that the combination of both ACE inhibitor and AT1 blocker does not produce more beneficial effects on endothelial dysfunction than monotherapy in a murine model of atheroclerosis[189], whereas the combination of a stain with an ACE inhibitor or an AT1 blocker produces additive effects on systemic inflammation biomarkers[190]. Also, the combination of ramilapril with felodipine, an calcium channel blocker does not induce more effect on endothelium-dependent vasodilation than each drug alone but increases endothelium-independent vasodilation in spontaneous hypertensive rats[191].

Antioxidant agents

Several substances having very different molecular structure and proprieties, such as vitamin C and E, N-acetylcysteine and genistein exert antioxidant effects through different mechanisms.

Vitamin C can improve endothelium-dependent response in circumstances such as chronic smoking, diabetes mellitus, hypercholesterolemia and hypertension[136,192-195]. Vitamin C protects the endothelium by scavenging superoxide, which in turn prevents NO scavenging, lipid peroxidation, platelet and neutrophil activation, and adhesion molecule upregulation[136,196]. Vitamin C scavenges peroxidase-generated reactive nitrogen species and inhibits myeloperoxidase/H_2O_2/nitrite-mediated LDL oxidation[197]. Vitamin E also exerts endothelial-protective effects in smoking and hypercholesterolemia[194,198] but its effects in diabetes remains controversial[199,200]. Vitamin E acts as a lipid

soluble antioxidant, scavenging hydroperoxyl radicals in lipid milieu[201].

N-acetylcysteine is a non-essential amino acid, essentially used in the treatment of cough. However, experimental studies have demonstrated that N-acetylcysteine is a potent antioxidant. It acts on the production of glutathione, which protects the cardiovascular system from harmful effects of TNF-α that induces glutathione depletion and ROS production *via* NADPH oxidase and ceramide[202-206]. For example, N-acetylcysteine improves coronary and peripheral vascular endothelium-dependent responses in patients with or without atherosclerosis[203]. The effect of N-acetylcysteine on endothelial dysfunction is associated with inhibition of NADPH oxidase expression, leukocyte adhesion and inflammatory cytokine secretion[204]. In addition, N-acetylcysteine inhibits von Willebrand factor dependent platelet aggregation and collagen binding in human plasma and in mice[207], attenuates MMPs expression in microvascular endothelial cells and in rats[202,208], and inhibits caveolin-1 upregulation and improves endothelial barrier function in mice[209], which may also contribute to the endothelial protective effect of N-acetylcysteine. N-acetylcysteine interacts with endogenous and exogeneous vasodilators. For example, in patients with systemic sclerosis, N-acetylcysteine induces vasodilation in association with a reduction in plasma AM concentrations[210] and potentiates hypotensive effects of ACE inhibitors in hypertensive patients[211].

Genistein is a soya-derived phytoestrogen and exerts an antioxidant effect. Genistein attenuates endothelial dysfunction in hypertensive rats and hyperhomocysteinemic rats. This endothelial protective effect appears to be due to increases in eNOS activity and expression and decreases in cytokine and ROS generation[212-215]. Genistein also improves endothelium-dependent vasodilator response in healthy postmenopausal women, increases plasma nitrite/nitrate concentration but decreases plasma endothelin-1 levels[216]. In this regard, genistein may be useful for the treatment of endothelial dysfunction associated with atherosclerosis and hypertension.

Beta blockers

Some beta blockers, particularly the β1-selective beta blockers exert endothelial protective effects. Nebivolol, a β1-antagonist with β2,3-agonist property, improves endothelium-dependent vasodilator responses in patients with essential hypertension[217,218] and in smokers[219]. Nebivolol also improves endothelial function, which is associated with reduced vascular remodeling and expression of endothelin-1 and cytokines in rats with pulmonary hypertension and in endothelial cells taken from these rats[220]. The effect of Nebivolol on endothelial function appears to be mediated by increasing NO release and reducing prothrombotic blood levels of fibrinogen, homocysteine and plasminogen activator inhibitor-1, especially in smokers[218,219]. Carvedilol, a non-selective β1- and β2 antagonist with α-antagonist property, also improves endothelium-dependent responses in patients with essential hypertension but this seems to be related to its antioxidant capacity[218]. The combination of carvedilol with an ACE inhibitor produces more beneficial effect on endothelial function than each drug alone in hypertensive patients with obesity[221]. Thus, this type of beta blockers and its combination are suitable for the treatment of endothelial dysfunction associated with hypertension, atherosclerosis, and probably diabetes.

Dihydropyridine calcium channel blockers

Nicardipine and nifedipine protects against ROS-induced endothelial cell death and lose of glutathione in cultured cells[222]. Benidipine exerts an endothelial protective effect against OxLDL induced ROX generation in human endothelial cells[223]. Israpidine improves endothelial function in cholesterol-fed rabbit[224]. Thus, the endothelial protective effect of dihydropyridine calcium channel blockers is mainly mediated by their antioxidant actions related to reduction in lipid peroxidation and associated ROS generation[222,225]. In addition, some dihydropyridines such as, amlodipine, azelnidipine and nifedipine were shown to exert an antiinflammatory action as indicated by decreased C-reactive protein and interleukin-6 levels as well as leucocyte activation[226,227]. Amlodipine or in combination with an renin inhibitor improves endothelial dysfunction in hypertensive patients, which seems to be linked to its NO-releasing action and anti-inflammatory effect[181,228-230]. In addition, the combination of amlodipine with a statin induces more favorable vascular effects than each drug alone in rats with hypertension or diabetes[231,232]. Thus, in addition to hypertension, dihydropyridines may also be useful for the treatment of endothelial dysfunction in diabetes.

Phosphodiesterase-5 inhibitors

Phosphodiesterase-5 (PDE5) is a cytosolic enzyme localized in vascular smooth muscle, heart, skeletal muscle, platelet, placenta, brain, kidney, liver, pancreas, gastrointestinal tissues and lung[233]. In vasculature, the primary action of PDE5 is to degrade cGMP and thereby induces vasoconstriction. PDE5 inhibitors are a class of drugs used to improve erectile dysfunction. These drugs block PDE5-induced cGMP degradation, leading to tissue cGMP accumulation and vasodilation[234]. PDE5 inhibitors upregulate eNOS expression and thereby increase NO release[235,236], which may contribute to long-term vasodilator effects of PDE5 inhibitors. PDE5 inhibitors also exert other initially unexpected effects. For example, in mouse hind limb ischemia model, treatment with sildenafil not only improves blood flow recovery but also increases capillary density and endothelial progenitor cell mobilization[237]. In patients with vasculogenic erectile dysfunction, daily treatment with vardenafi reduces both arterial stiffness and plasma AM level[238]. These effects may also account

for the effects of chronic PDE5 inhibition. In addition to erectile dysfunction, PDE5 inhibitors can improve endothelial dysfunction in other circumstances. For example, PDE5 inhibition improves coronary and peripheral vascular endothelial function, and inhibits platelet activation in patients with coronary artery disease[239] or with congestive heart failure[240-242], and improves endothelium-dependent vasorelaxation in rats with experimental diabetes mellitus[243]. PDE5 inhibitors also improves erectile function in patients with systemic sclerosis and reduces plasma endothelin-1 concentration[244]. Similarly, PDE5 inhibitors improve Raynaud's phenomenon characterized by reduced blood flow to fingers and toes in response to cold and stress, probably through decreasing plasma endothelin-1 and improving microcirculation[245]. However, the mechanism underlying endothelin-1-reducing effect of PDE5 inhibitors remains to be determined.

Statins

Statins, inhibitors of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase are a class of drugs utilized to reduce hypercholesterolemia, especially LDL cholesterol. In 1994, pravastatin was shown to improve endothelium-dependent response of coronary and peripheral arteries in patients with hypercholesterolemia[246], which was confirmed later by other studies[247]. The beneficial effect of statins on endothelial function involves multiple mechanisms. Statins improving endothelial dysfunction is partly due to their lowering LDL cholesterol effect, while native LDL and OxLDL reduce eNOS expression[248,249] and increase levels of caveolin-1[250]. Statins also exert direct antioxidant effects on LDL to reduce electronegative form of LDL[251,252]. Statins increase NO bioavailability by activating eNOS *via* the PI3K/Akt signaling pathway[253], agonist-stimulated eNOS-hsp90 interaction[250], and BH4-mediated eNOS coupling. This latter was demonstrated in patients with atherosclerosis[254] and in rat model of insulin resistance of diabetes[231]. These studies showed that atorvastatin increased vascular BH_4 content and NO bioavailability and reduced O_2^- production *via* upregulating GTP-cyclohydrolase Ⅰ gene expression and activity. These effects occurred rapidly in patients with atherosclerosis and could be reversed by mevalonate, indicating a direct effect of vascular HMG-CoA reductase inhibition[254]. In addition, statins upregulate eNOS expression through enhancing eNOS mRNA stability. Indeed, statins increase eNOS mRNA polyadenylation through Rho-mediated changes in the actin cytoskeleton[255,256]. However, a study showed that statins can increase eNOS gene transcription by upregulating Kruppel-like factor 2 through inhibition of Rho pathway[257]. The effect of statins on eNOS expression may account for the long-term effect of stains on endothelial function. Statins also exerts antiinflammatory effects[258]. For example, atorvastatin treatment reduces proinflammatory cytokines (TNF-α, IL-1 and IL-6), intercellular adhesion molecules and C-reactive protein blood levels in hypercholesterolemic patients[259], while rapid withdrawal of statin treatment increases proinflammatory and prothrombotic biomarkers[260]. Statins were also shown vascular benefice in other inflammatory diseases such as rheumatoid arthritis[261]. Otherwise, statins increase circulating endothelial progenitor cells, likely through the PI3K/Akt pathway[262], which could contribute to long-term effects of statins on endothelial function.

Anther type of LDL-lowering drugs, the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, may be expected to improve endothelial function. In humans, PCSK9 mutation is closely correlated with LDL cholesterol levels and inhibition of PSCK9 with a monoclonal antibody reduces LDL cholesterol levels[263] and enhances the LDL cholesterol-lowering effect of atorvastatin[264]. Studies in cells and animals have shown that PCSK9 is associated with inflammation and endothelial cell apoptosis. In mice, systolic inflammation and OxLDL upregulate PCSK9, whereas PCSK9 interacts with macrophage, leading to NF-κB activation[265,266]. Knockdown of PCSK9 with PCSK9 siRNA or induction of gain of function mutant D374Y-PCSK9 reduces expression of stress-response genes and specific inflammation pathways, inflammation pathway activation and OxLDL-induced endothelial apoptosis[266-268]. Nonetheless, the effects of PCSK9 inhibition on human endothelial function are not yet explored.

Angiotensin-(1-7)

Angiotensin-(1-7) is a metabolite of angiotensin I under the action of various enzymes, including neutral endopeptidase, prolylendopeptidase, aminopeptidase A and neprilysin[36]. It can also be generated from angiotensin Ⅱ by prolylcarboxypeptidase[269] and carboxypeptidase (ACE2)[270]. In endothelial cells, angiotensin-(1-7) activates eNOS *via* the Mas/PI3K/Akt pathway and inhibits angiotensin Ⅱ-induced NAD(P)H oxidase activation[271,272]. Chronic treatment with angiotensin-(1-7) improves renal endothelial dysfunction associated with apolipoprotein E-deficiency[273] and diet-induced obesity in mice[274], which is likely mediated by increasing NO release[275] and eNOS expression[276,277]. Otherwise, angiotensin-(1-7) restores vascular ACE2-angiotensin-(1-7)-Mas receptor axis function that impairs ROS production by angiotensin AT1 receptor-activated NAD(P)H oxidases in hypertensive or diabetic rats[278,279]. Angiotensin-(1-7) restores NO/cGMP production and migration, decreases NADPH oxidase activity, and enhances survival and proliferation of endothelial progenitor cells isolated from the blood of diabetic patients in a Mas/PI3K/Akt-dependent manner[280]. Interestingly, overexpression of angiotensin-(1-7) gene restores the vasoreparative function of endothelial progenitor cells in mice[280]. Despite these encouraging results in cells and in animals, the information regarding the effects of angiotensin-(1-7) on human endothelial function

remains lacking.

Bradykinin

As discussed above, endogenous bradykinin exerts multiple actions that affect endothelial function. It is worth noting that bradykinin as an investigational drug protects against ROS- and toxin-induced microvascular endothelial cell death[281], and chronic treatment with bradykinin not only preserves eNOS expression in dogs with pacing-induced heart failure[101], but also upregulates eNOS and nNOS expression in vessels and in the heart of dogs with dystrophin-deficiency cardiomyopathy[19,282]. However, due to the very short half-life and implication of bradykinin in the inflammation[283] and cancers[284,285], the clinical use of bradykinin remains a challenge.

eNOS transcription enhancer

Interestingly, specific targeting eNOS transcription with a chemical compound, AVE3085, increases eNOS expression but reduces oxidative stress and platelet activation, which is associated with improved endothelium-dependent relaxation and cardiac function in animals with different experimental diseases[286-289]. This compound also prevents the inhibitory effect of ADMA on endothelium-dependent vasodilation in human internal thoracic artery rings and in pig coronary artery rings[290,291]. Thus, this compound showed a potential for the treatment of endothelial dysfunction although its effects in human clinical situations remains to be demonstrated.

I_f inhibitor, ivabradine

I_f current is an inward current carried by Na^+ and K^+, activated by hyperpolarization and conducted by hyperpolarization-activated cyclic nucleotide-gated channels (f-channels)[292]. I_f current participates in the spontaneous depolarization during Phase 4 of the action potential and plays a crucial role in the pacemaker activity of pacemaker cells located in the sinus node and atrioventricular node. Inhibition of this current by ivabradine slows down heart rate and exerts cardioprotective effects[293-296], which may involves pleiotropic actions of ivabradine[297]. Among them, beneficial effects of ivabradine on the endothelium-dependent vasodilation and on the expression of eNOS expression in both animals and humans have been reported[298-300]. Nevertheless, the effects of ivabradine on human endothelial dysfunction are controversial. Several studies did not observe significant improvement in flow-mediated vasodilation by ivabradine in patients with microvascular angina pectoris[301] or stable coronary heart disease[302] and in patients with type Ⅱ diabetes[303]. In addition, in patients with stable of coronary disease without heart failure, the additional ivabradine plus standard treatment did not improve outcome but was associated with increased frequency of atrial fibrillation, questioning the utility of this drug in the treatment of stable coronary disease[304].

Sphingosine-1-phosphate

Sphingosine-1-phosphate (S1P), a signaling sphingolipid formed by sphingosine kinase in the blood and in tissues, regulates different biological responses such as angiogenesis, vascular permeability and trafficking of T- and B-cells. S1P enhances endothelial barrier function[305,306], stimulates endothelial NO release through Akt-mediated phosphorylation of eNOS[307], and reconstitutes high density lipoproteins[308]. S1P also has antiinflammatory properties and exerts protective effect against endotoxin-induced lung injury[309,310]. Moreover, S1P exhibits a potent effect on the differentiation of adipose-derived stem cells into endothelial-like cells and upregulation of eNOS in these cells[311]. All of these properties of S1P may contribute to its endothelial protective effects. Interestingly, an orally active of S1P analogue, FTY720 also shows similar effects[312]. Thus, S1P and analogues may be used to improve endothelial function, especially in atherosclerosis and acute lung injury where presents an impairment of endothelial barrier function[313].

CONCLUSION

Endothelial dysfunction is a common mechanism involved in many cardiovascular diseases, although in some diseases such as atherosclerosis, endothelial dysfunction plays a critical role in the development of diseases, whereas in others such as essential hypertension and type Ⅱ diabetes, endothelial dysfunction generally occurs as a complication but thereafter contributes to the development and progression of organ damages. Clearly, multiple mechanisms such as inflammation, increased ROS and RNS, cellular apoptosis, increased vasoconstrictor production, decreased vasodilator production and vascular remodeling are involved in endothelial dysfunction and a specific pathology may involve more or less them as described above. However, a decreased NO bioavailability appears to play a central role because in many pathologies such as atherosclerosis, diabetes, essential and pulmonary hypertension and heart failure except for septic shock where there is a overproduction of NO, a reduction in NO bioavailability occurs sooner or later in response to different risk factors. This may explain the beneficial effects of some drugs in the treatment of a variety of cardiovascular disorders. It appears that a drug with endothelium-protective property may yield more therapeutic benefits than that without such feature. For this reason, the evaluation of endothelium-improving action may be helpful for the development of a novel cardiovascular drug. Moreover, due to the differences in risk factors contributing to the different cardiovascular diseases and the differences in mechanisms of action, treatment of endothelial dysfunction with drugs needs to be carried out according to specific mechanisms

underlying endothelial dysfunction of the disease.

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REVIEW

Therapeutic modification of arterial stiffness: An update and comprehensive review

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Abstract

Arterial stiffness has been recognized as a marker of cardiovascular disease and associated with long-term worse clinical outcomes in several populations. Age, hypertension, smoking, and dyslipidemia, known as traditional vascular risk factors, as well as diabetes, obesity, and systemic inflammation lead to both atherosclerosis and arterial stiffness. Targeting multiple modifiable risk factors has become the main therapeutic strategy to improve arterial stiffness in patients at high cardiovascular risk. Additionally to life style modifications, long-term ω-3 fatty acids (fish oil) supplementation in diet may improve arterial stiffness in the population with hypertension or metabolic syndrome. Pharmacological treatment such as renin-angiotensin-aldosterone system antagonists, metformin, and 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors were useful in individuals with hypertension and diabetes. In obese population with obstructive sleep apnea, weight reduction, aerobic exercise, and continuous positive airway pressure treatment may also improve arterial stiffness. In the populations with chronic inflammatory disease such as rheumatoid arthritis, a use of antibodies against tumor necrosis factor-alpha could work effectively. Other therapeutic options such as renal sympathetic nerve denervation for patients with resistant hypertension are investigated in many ongoing clinical trials. Therefore our comprehensive review provides knowledge in detail regarding many aspects of pathogenesis, measurement, and management of arterial stiffness in several populations, which would be helpful for physicians to make clinical decision.

Key words: Arterial stiffness; Cardio-ankle vascular index; Pulse-wave velocity; Renin-angiotensin-aldosterone system antagonist

Core tip: Arterial stiffness has been recognized as

a marker of cardiovascular disease and associated with long-term worse clinical outcomes in several populations. Age, hypertension, smoking, and dyslipidemia, known as traditional vascular risk factors, as well as diabetes, obesity, and systemic inflammation lead to both atherosclerosis and arterial stiffness. Targeting multiple modifiable risk factors has become the main therapeutic strategy to improve arterial stiffness in patients at high cardiovascular risk.

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INTRODUCTION

Arteries provide not only blood flow conduits from the heart to peripheral organs, but also play a major role in hemodynamic cushioning, buffering the forward propagating flow from the heart, and the backward resistance by the peripheral arterioles, which maximize cardiovascular efficiency. Arterial stiffness characterized by higher intravascular distending pressure has been recognized as a marker of cardiovascular disease (CVD) and associated with long-term prognosis in several populations[1-4]. A recent meta-analysis including 17 longitudinal studies demonstrated that aortic stiffness was an independent predictor of incident CVD and all-cause mortality in the general population[4]. Therefore, evidence-based approaches for improving arterial stiffness are of clinical importance to reduce the hazards of subsequent CVD. This review article will discuss the latest knowledge of the pathological backgrounds, the measurements, and the effects of pharmacological and non-pharmacological interventions for arterial stiffness.

The pathophysiology of arterial stiffness

As a major component of the circulatory system, the arterial system can be functionally and structurally divided into two sub-systems: (1) the large elastic, conducting arteries (*e.g.*, the aorta, the carotid arteries, and the iliac arteries), which store blood ejected from the heart during systole, and expel blood to the peripheral tissues during diastole, thereby ensuring a steady blood flow irrespective of cardiac cycles or concurrent blood pressure; (2) resistance muscular arteries, especially those of the lower limb (*e.g.*, femoral, popliteal, and posterior tibial arteries), which are capable of altering vascular smooth muscle tone, allowing them to modulate the velocity of pressure wave that is conducted to the resistance muscular arteries from the central aorta[5]. The sites of aortic flow reflection are not simply anatomically determined, but also subjected to systemically structural and functional control. For example, the site of reflection is more central in the case of hypertension, atheromatous arteries or increased sympathetic activity[6].

The pressure waveform recorded at any site of aorta is the summation of the forward-traveling waveform generated by cardiac pumping force and the backward traveling wave, the "echo" wave reflected at peripheral sites. The summation result determines the cardiac afterload during systolic phase and the augmented backward coronary perfusion pressure during diastolic phase. When the arteries are compliant and elastic, the reflected wave merges with the incident propagating wave during diastole, thus augmenting the diastolic blood pressure and enhancing coronary perfusion[7]. On the contrary, when arteries are stiffer, pulse wave velocity increases, and both the incident and the reflected wave travel faster; therefore, the reflected wave merges with the incident wave at systole and increase systolic pressure and cardiac afterload, while, concomitantly, losing the augmented diastolic perfusion pressure[7] (Figure 1). The added part on systolic pressure and cardiac afterload was named aortic augmentation index [AIx, (second/first systolic peak) $\times$ 100%][8]. In the long term, increasing pulsatility causes stretching of load-bearing elastic lamellae and mechanical stress on the wall leading to vascular structural changes and stiffening. Hence, the harm of arterial stiffness is two-sided, negatively affecting the heart and blood vessels[9] (Figure 2).

Factors affecting arterial stiffness

Age is a main determinant of stiffness in large elastic arteries[7,10]. The stiffness of these arteries increases significantly after the age of 55 years. Aging causes the degeneration and remodeling of elastic components of arterial wall. At the cellular-molecular level, an age-related decrease in intra-cellular magnesium concentration is associated with increases in stiffness[10].

Most traditional cardiovascular risk factors and CVD have an adverse effect on arterial stiffness, *via* endothelial dysfunction and adverse vascular remodeling. Hypertension, diabetes, dyslipidemia, and insulin resistance, which contribute to atherosclerosis, have been involved in the process of arterial stiffening. In essential hypertension, the elastic properties of large arteries are impaired, although it is not clear whether the disease itself alters the intrinsic elastic properties or this is the ultimate final effect of increase in distending pressure[11,12]. Distending pressure as estimated by 24-h pulse pressure was another major factor additionally to older age contributing to the occurrence of arterial stiffness[13]. In patients with diabetes or metabolic syndrome, arterial stiffening is consistently observed across all age groups, even in childhood[14]. Insulin resistance is dose-dependent and positively correlated with arterial stiffness[15-17]. Chronic hyperglycemia and hyper-insulinemia may increase local activity of renin-angiotensin-aldosterone system (RAAS) and expression of angiotensin type I receptor in vascular tissue and thus promote the development of arterial wall hypertrophy

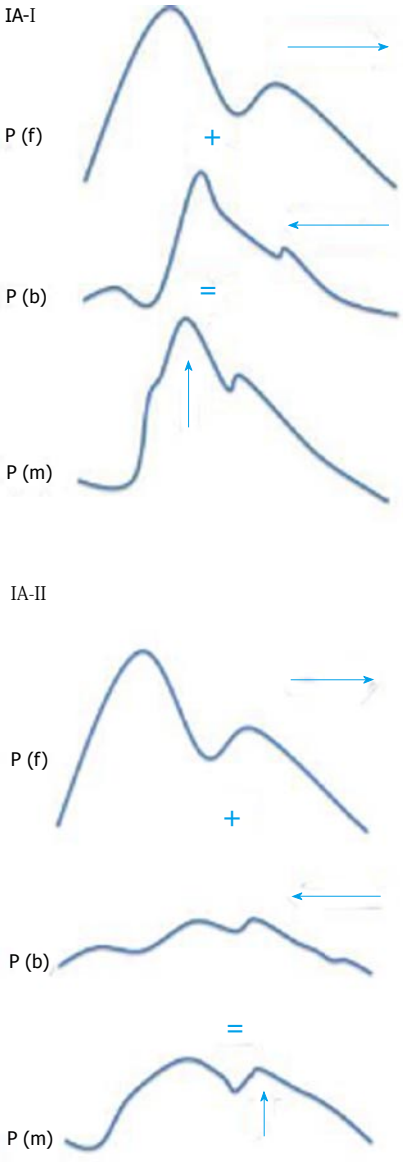

Figure 1 The central aortic pressure waveform is the summation of forward travelling wave, P (f) and the reflected backward-travelling wave, P (b). On the top graph IA-I, is an illustration of a stiff aorta or peripheral vasoconstriction, both P (f) and P (b) travel fast and the magnitude of the reflected wave is increased, thus augmenting the systolic pressure of summated central aortic pressure waveform, P (m). In graph IA-II, is another illustration of a distensible aorta or with vasodilatation. Length and thickness of horizontal arrows correspond to the waveform velocity and the magnitude of the reflected wave, respectively. Vertical arrows indicate point of merging of P (f) and P (b).

and fibrosis[18,19].

In addition hyperinsulinemia has proliferative effects, *via* unbalanced activities on growth-promoting mitogen activated kinase pathways and PI3-kinase-dependent signaling[20]. In pre-diabetic stage, impaired glucose tolerance enhances nonenzymatic glycation of proteins with covalent cross-linking of collagen and alters the mechanical properties of interstitial tissue of arterial wall[21,22].

Chronic kidney disease (CKD) is a well-known risk factor of arterial stiffness[23]. Several mechanisms have been proposed to explain the effect of CKD. For instance, upregulation of matrix metalloproteinases enhances collagen and elastin turnover through enzymatic cross-link degradation[24], causing weakening of the extracellular matrix[25]. Accumulation of advanced glycation end-products makes collagen stiffer as well[26]. In addition, CKD may cause endothelial dysfunction, which attributes to high oxidative stress, increased endothelin-1 concentrations and impairment of endothelial nitric oxide synthase and arterial relaxation[27]. Chronic inflammation and RAAS activation are also involved in the process of arterial stiffening in CKD[28,29]. CKD alters bone metabolism to promote vascular calcification by increasing osteoclast activity, fibroblast growth factor 23, osteoprotegerin which inhibit bone morphogenic proteins, and reducing pyrophosphate, Matrix G1a protein, and fetuin A levels[30].

Arterial elastic properties are impaired in young people with a family history of hypertension, diabetes or myocardial infarction[31]. It has been recognized that genetic factors may contribute to arterial stiffening as well. The latest advances in genome-wide association study have identified that some genetic variants and specific polymorphisms may affect arterial stiffness. The Framingham Heart Study showed that four regions of suggestive linkage were found in chromosomes 2, 7, 13, and 15 (LOD scores 2.0) for higher risk of arterial stiffness[32]. Potential candidate genes in these regions included the insulin-like growth factor-1 receptor, myocyte-specific enhancer factor 2A, chondroitin synthase (CHSY1), proprotein convertases (PACE4 and FURIN), b-adducin (ADD2), neurokinin-1 receptor (TACR1), a-2B adrenergic receptor (ADRA2B), and interleukin-6 (IL-6). Other candidate gene polymorphism, such as the renin-angiotensin-aldosterone genes, the Matrix and metalloproteinase genes, the endothelial cell-related genes, and the inflammatory genes, are all in undergoing investigations[33].

Lifestyle characteristics are important determinants of arterial stiffness. Cigarette smoking, including passive smoking and current smoking has an adverse impact on the arterial stiffness[34-36]. Elevated arterial stiffness has been found among patients with chronic obstructive pulmonary disease and inflammation, which are highly related to the adverse effect of smoking. Obesity, weight gain, lack of physical activity and high dietary intake of sodium chloride, which is associated with blood pressure elevation, can aggravate arterial stiffness[37-40]. Intake of caffeine, a neurotoxin has also been acknowledged of an unfavorable effect on arterial compliance[40]. Other risk factors such as chronic cytomegalovirus infection, has been known as a novel potential contributor to arterial stiffening[41]. Table 1 lists the main demographic, clinical and lifestyle characteristics that may influence arterial stiffness.

Measurement of arterial stiffness

A stiffer vessel will conduct the pulse wave faster than a

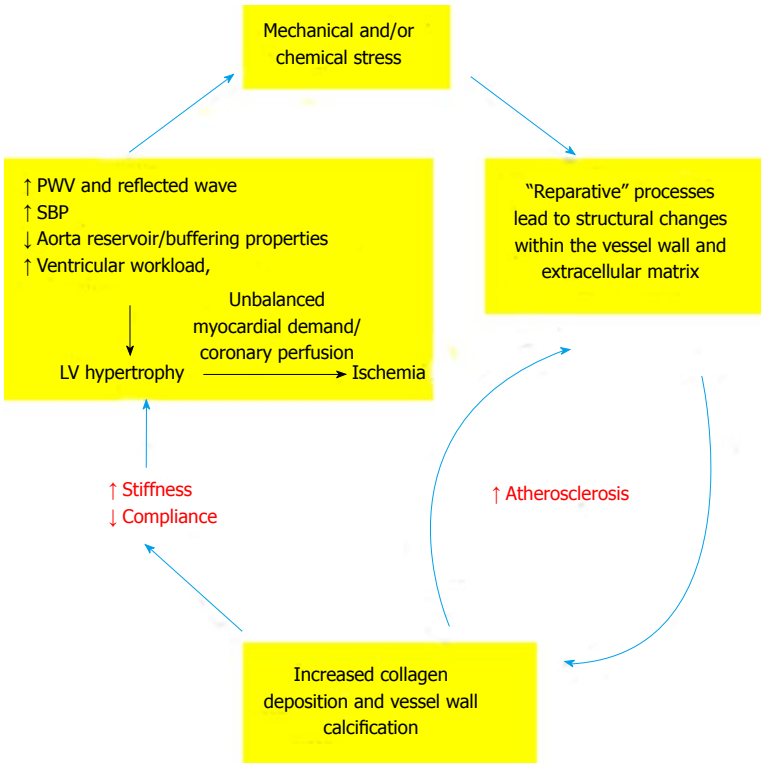

Figure 2 Aortic elastic properties may be altered by several processes, resulting in increased stiffness, decreased compliance, and encompassing the diseased ventricular-arterial coupling. Mechanical and chemical stress factors include hypertension, inflammation, advanced glycation end products, *etc.* LV: Ventricular; PWV: Pulse wave velocity; SBP: Systolic blood pressure.

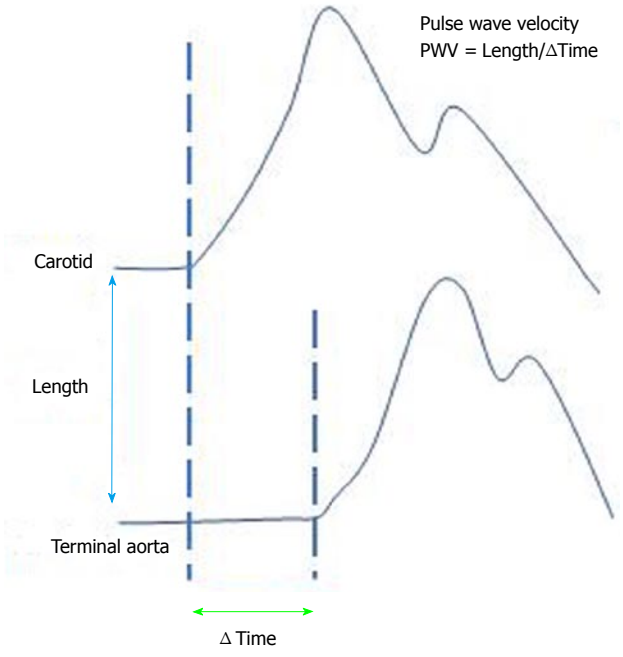

Figure 3 For practical purpose, femoral artery is counted as the terminal aorta. The measured distance is length. If ΔTime represents the time delay between the feet of the 2 waves, pulse wave velocity.

Table 1 Demographic, clinical, and lifestyle factors associated with arterial stiffness

Age[7]
Sex[110]
Established cardiovascular disease[3]
Potential risk factors for atherosclerosis
Hypertension[11]
Dyslipidemia[2]
Cigarette smoking[63]
Chronic obstructive pulmonary disease[111]
Diabetes[14]
Obesity[3]
Obstructive sleep apnea[106]
Menopause[110]
Polycystic ovarian syndrome[112]
Hypothyroidism[113]
Chronic kidney disease[23]
Endothelial dysfunction[27]
Systemic inflammation[99]
Cytomegalovirus infection[114]
Nutritional and lifestyle aspects
Caffeine[115]
Chronic alcohol consumption[116]
Sedentary lifestyle[58]
Resistance exercise training[61]
Genes variants
Genes of the Renin-Angiotensin-Aldosterone system[33]
Genes of the extracellular matrix proteins[33]

more distensible and compliant vessel. Arterial stiffness can be noninvasively evaluated by measuring pulse-wave velocity (PWV). The PWV is calculated by the distance (L) between the 2 vascular sites divided by the wave foot-to-foot time (ΔT) it takes for that forward wave to reach the end measuring point (Figure 3) Currently, PWV is the most validated measurement to noninvasively quantify arterial stiffness. It is considered the gold standard index to measure arterial stiffness, given its simplicity, reproducibility, accuracy, and strong prediction of adverse CVD events[42-44]. An increase in aortic PWV by 1 m/s corresponds to an age-, sex-, and risk factor-adjusted risk increase of 14%, 15% and 15% in total CVD events, CVD mortality, and all-cause mortality, respectively[5]. Nowadays, two kinds

of PWV were frequently used to evaluate arterial stiffness. Carotid-femoral PWV (cfPWV) measured by Doppler ultrasound is the most widely used measure of aortic stiffness and is regarded as the gold standard measure for evaluating arterial stiffness. Alternatively, brachial-ankle PWV (baPWV) measured by the Omron oscillometric/plethysmographic system has recently received attention because of its consistent association with CVD risk factors and its ease of use for large-scale population studies[42-44]. Based on the formula assumptions, cfPWV reflects the stiffness of descending aorta, while baPWV reflects the stiffness of both descending aorta and leg arteries. In a study conducted among healthy men aged 40-49, cfPWV strongly correlated with central PWV, and baPWV correlated with both central and peripheral PWVs[45]. The two indexes were highly correlated and the predictive values of these two PWVs were comparable[46]. Both cfPWV and baPWV have been reported to be independent predictors of subclinical coronary artery calcification, incident vascular events, incident heart failure, and all-cause mortality in the general population[47,48]. The main disadvantage of cfPWV is inevitably affected by blood pressure, which is an important confounder for CVD. In addition, cfPWV is often overestimated for the inaccurate measurement in the distance between the carotid and the femoral to measure the pulse wave[49]. Other methods for the PWV measurements include single-point, carotid–radial or femoral–tibial arterial segments. The predictive values of these more peripheral PWV measurements to incident vascular events remain unknown[50]. Aortic characteristic impedance standing for the minimal impedance for higher frequencies of pressure-and-flow harmonics and being proportional to PWV is an indirect technique, but this is rarely used alone now[51]. AIx, arterial wave reflection magnitude [(reflected/forward wave amplitude) × 100%], and pulse pressure amplification [(radial/aortic pulse pressure) × 100%], the analysis of pulse waveforms parameters of central arteries, have been associated with the development of end organ damage as well[52].

The stiffness parameter β is another measure of arterial stiffness. The equation for stiffness parameter β is ln(Ps/Pd) × D/ΔD, where Ps is the systolic blood pressure, Pd is the diastolic blood pressure, D is the diameter of the artery, and ΔD is the change in arterial diameter between Ps and Pd[53]. The stiffness parameter β is less affected by blood pressure; however it is limited by assessing a local segment of the artery, and becoming dependent on blood pressure for those with hypotension or moderate and severe hypertension[53]. Therefore, the cardio-ankle vascular index, CAVI, was developed to incorporate the stiffness parameter β[54]. The equation for CAVI is a $[(2\rho/\Delta P) \times \ln(Ps/Pd) \times PWV^2] + b$, where ρ is the blood viscosity, ΔP is Ps - Pd, PWV is the pulse wave velocity from the aortic origin to the ankle region *via* the femoral artery, and a and b are constants for converting a CAVI value to a value obtained by Hasegawa's method[55]. Theoretically, the CAVI is essentially intrinsic to the stiffness parameter β and thus less dependent of blood pressure than PWV. Table 2 summarizes the merits and disadvantages of different measurements of arterial stiffness.

Therapeutic modification of arterial stiffness

Lifestyle modification: Obesity is related to insulin resistance, hypertension, obstructive sleep apnea (OSA), and eventually arterial stiffness. A meta-analysis involving 20 studies (including 3 randomized controlled trials) revealed that modest weight loss (mean 8% of initial body weight) could improve PWV values by 32% in the collected 1259 participants[56]. In addition, weight reduction was found in association with decreased CAVI values in a cohort of 47 obese individuals in Japan[57]. Effects of exercise on arterial stiffness were extensively investigated. Physical activity was associated with 35% reduction in cardiovascular mortality and 33% reduction in all-cause mortality[58]. Almost 60% of the benefits are contributed by the reduction of body weight, blood pressure and serum lipids[59], and the other 40% may be explained by the improvement of vascular hemodynamics including endothelial function, arterial compliance and remodeling[60]. Whether mode and dose of exercise affecting arterial stiffness had been recently reviewed in a meta-analysis[61]. In total, forty-two studies and 1627 participants were included in the study, which concluded aerobic exercise, but not resistant exercise or combined aerobic and resistant exercise, improved PWV weighted mean difference (WMD): -0.63 m/s, 95%CI: -0.90 to -0.35, and AIx (WMD: -2.63%; 95%CI: -5.25 to -0.02). The benefits for improving arterial stiffness were greater in the peripheral index, baPWV (WMD: -1.01 m/s; 95%CI: -1.57 to -0.44) than in central index, cfPWV (WMD: -0.39 m/s; 95%CI: -0.52 to -0.27). There was dose-dependent relationship between exercise intensity (frequency of exercise sessions and absolute exercise intensity) and the improvement of AIx. Nevertheless, the exercise session duration was not significantly associated with the reduction of AIx[61]. In individuals with stiffer arteries (PWV ⩾ 8 m/s), aerobic exercise had a larger effect in reducing PWW. In addition, the benefits of aerobic exercise were documented in subpopulations with normal health, overweight/obese, pre-hypertension, hypertension, or CKD.

Smoking cessation has been proven to decrease aortic stiffness. In one 60 wk follow-up observational study, smoking cessation group had better arterial stiffness indices (central blood pressure, -7.1 ± 1.4 mmHg *vs* 1.2 ± 2.7 mmHg, $P < 0.01$; baPWV, -204 ± 64 cm/s *vs* -43 ± 72 cm/s, $P < 0.01$; reduced radial AIx, -6.4 ± 2.8% *vs* -1.0 ± 3.9%, $P < 0.01$)[62]. Another observational study also showed that smoking cessation was associated with improved arterial stiffness as evaluated by CAVI values[63]. Moreover, avoidance of second-hand smoke, such as workplace smoking bans,

Table 2 A summary of the advantages and disadvantages of different measurements for evaluating arterial stiffness

	Advantage	Disadvantage
cfPWV[42-44]	Reflects the stiffness of the descending aorta The gold standard measure for arterial stiffness	Largely affected by the change of BP Overestimated for the inaccurate measurement in the distance between the carotid and the femoral arteries
baPWV[116]	Reflects the stiffness of both the descending aorta and the leg artery High association with CV risk factors Ease of use for large-scale population studies	Largely affected by the change of BP Underestimates arterial stiffness in hypertensive patients with a history of cardiovascular events
hfPWV[117]	Strongly correlated with cfPWV moderately correlated with baPWV	Require a high level of proficiency in order to obtain accurate results
faPWV[117]	Moderately correlated with baPWV	The predictive value to incident vascular events remains unknown
pAIx[110]	Assessed non-invasively and peripherally, *e.g.*, carotid, and radial arteries Correlated well with the central AIx	Largely affected by the change of BP Not a valid surrogate of arterial compliance in the elderly and diabetic populations
The stiffness parameter β[53,54]	Independent of the change of BP	Assessing only a local segment of the artery Loss of the independence of BP for those with moderate to severe hypertension or hypotension
CAVI[118]	Independent of the change of BP A novel atherosclerotic index that incorporates PWV and BP measurements The coefficients of variation are small (< 4%), and does not require significant training	CAVI, as a cardiovascular risk marker has not to be investigated definitively in large prospective clinical trials

Ba: Brachial-ankle arteries; CAVI: Cardio-ankle vascular index; BP: Blood pressure; cf: Carotid-femoral arteries; fa: Femoral-ankle arteries; hf: Heart-femoral arteries; pAIx: Peripheral augmentation index; PWV: Pulse-wave velocity.

has been reported to improve PWV after introducing smoke-free workplaces[64].

Dietary and nutrient interventions: Several dietary modifications had been reported with beneficial effects on arterial stiffness. Among them, omega (ω)-3 fatty acids (fish oil) supplementation was mostly studied. In most of clinical trials, ω-3 fatty acids supplementation improved arterial stiffness, especially in the population with overweight, metabolic syndrome, diabetes or hypertension[65]. Aside from a study with acute ω-3 fatty acids administration in healthy participants, almost all ω-3 trials were long-term prescribed varying from 1.5 to 25 mo. In this acute fish-oil supplementation study, there were no immediate reductions in parameters of arterial stiffness[66]. The lowest daily dosage of long-chain polyunsaturated fatty acids (PUFAs) that documented an effect on arterial stiffness was 540 mg eicosapentaenoic acid (EPA) along with 360 mg docosahexaenoic acid (DHA) in overweight patients with hypertension[67]. Sjoberg *et al*[68] introduced 2, 4, and 6 g of fish oil supplementation per day into the diets of overweight or obese adults for 12 wk. Only the highest dose group (6 g of fish oil per day) revealed significant improvement in arterial distensibility, as measured by PWV. Among healthy subjects, Chong *et al*[69] reported a significant improvement in PWV and AIx immediately after a long chain ω-3 PUFA-rich meal containing 4.7 g of DHA and EPA. In a randomized controlled trial in Japan, highly purified EPA administration (1.8 g/d for 3 mo) significantly reduced both PWV and CAVI values in individuals with metabolic syndrome[70]. However, other two studies using smaller amount (1.7 g of EPA/ DHA per day for 12 wk and 1.8 g of EPA/ DHA per day for 12 mo) did not improve arterial stiffness among slightly overweight but relatively healthy subjects[71,72]. Accordingly, the benefits from ω-3 supplementation could be more evident using a comparable dose over a greater duration within an older age, more diseased populations.

Soy isoflavones was another nutrient, which has been studied frequently. Among five soy isoflavone interventional studies, four interventional studies showed an improvement in PWV or systemic arterial compliance in subjects taking soy isoflavone relative to their placebos[73-76], whereas one study reported no effect[77]. Notably, the majority of the soy interventions were conducted in postmenopausal women. In other studies with positive results, one study reported that consumption of alcoholic red wine might decrease AIx acutely relative to that after consumption of dealcoholized red wine[78], and a study showed that consumption of black tea flavonoids could reduce the digital volume pulse-stiffness index but not PWV[79]. Other dietary and nutritional interventions, nonetheless, reported no definite effect on arterial stiffness, such as garlic[80], conjugated linoleic acid[81], vitamins or folic acid[82-86] on PWV.

Among the minerals, salt plays a detrimental role. Consistent evidence suggest that 10-140 mmol sodium chloride supplementation per day would increase arterial stiffness in individuals with hypertension[87,88]. In a randomized clinical trial, salt reduction was associated with decreased pulse pressure across all ethnic groups including white, black and Asians, whereas PWV decreased only in blacks in response to salt reduction[87].

In addition, Gates *et al*[89] revealed that large elastic artery compliance was much improved in the older adults with systolic hypertension following only one-week of dietary sodium restriction.

Pharmacological therapy: Since blood pressure is the strongest modifiable factor directly leading to arterial stiffness, a number of clinical trials have been conducted to investigate the effect of antihypertensive medications on the change of arterial stiffness. Notably almost all classes of anti-hypertensive medications except diuretics and non-vasodilating beta-blockers such as atenolol could decrease arterial stiffness effectively[90,91]. Among all classes of anti-hypertensive medications, RAAS system antagonists have shown the best clinical results, probably due to their anti-fibrotic properties[91]. With regard to other modifiable risk factors, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) could decrease arterial stiffness by lowering low-density lipoprotein cholesterol concentrations, the effect of anti-inflammation, and stabilizing the atheroma plaques[92,93]. In patients with diabetes, glycemic control with oral anti-diabetic agents with metformin and glitazone were reported to improve arterial stiffness[94,95]. Using high dose of RAAS antagonists was extremely effective in attenuating the severity of arterial stiffness in diabetic patients with hypertension[96]. Notably, pharmacological modifications to these traditional vascular risk factors have been confirmed to improve arterial stiffness evaluated by PWV or CAVI[97]. In patients with chronic inflammatory disease such as rheumatoid arthritis, several anti-inflammatory agents have been tested, but until now, only antibodies against tumor necrosis factor-alpha have been shown to improve arterial stiffness, independently of adequate blood pressure control[98,99]. In menopausal women, although the effect of sex hormone replacement therapy on arterial stiffness is uncertain, one study showed that using raloxifene, a potent selective estrogen receptor modulator may lead to positive result[100]. The phosphate binder, sevelamer was found to improve arterial stiffening in patients with end-stage renal disease[101]. Alagebrium, an advanced glycation end-products crosslink breaker, has shown to improve arterial stiffness in animal studies despite the effect was missing in a small group of older individuals[102,103]. However, further clinical trials were not conducted because of financial problems of the developing company. Currently, some ongoing trials are conducted to evaluate the effect of antidiabetic pharmacological therapy including metformin and alogliptin, the dipeptidyl peptidase 4, on the improvement of arterial stiffness in obese children and adolescents, and in adult individuals with type 2 diabetes, respectively[104,105].

Device and interventional therapy: It is well known that OSA is related to obesity and correlated with several CVD risk factors, such as hypertension and metabolic syndrome, which contributes to adverse clinical outcomes. A meta-analysis involving 15 articles, investigated the effect of continuous positive airway pressure (CPAP) on arterial stiffness in 615 patients with OSA. A significant improvement of all indices of arterial stiffness was observed after CPAP treatment (SMD = -0.74; 95%CI: -1.08 to -0.41). Neither the proportion of compliance nor the duration of CPAP use altered the outcomes after CPAP treatment[106].

Enhanced external counterpulsation (EECP), using pneumonic cuffs over the legs to inflate and deflate according to the cardiac cycle, is a non-invasive modality for treatment of symptomatic patients with coronary artery disease not amenable to revascularization procedures. In a randomized clinical trials conducted in 42 patients with coronary artery disease, central arterial stiffness and AIx were reduced following 17- and 35-sessions respectively, as well as peripheral arterial stiffness was reduced following 35 sessions in the EECP treatment group as compared with the placebo[107].

Since autonomic nervous system is involved in the pathogenesis of hypertension, its modification such as renal sympathetic denervation, and baroreflex activation therapy could attenuate arterial stiffness by improving arterial stiffness indices and central hemodynamics in patients with resistant hypertension[108,109]. However, these studies were conducted in patients with resistant hypertension, and the result may not be simply extrapolated to all the patients with arterial stiffness.

CONCLUSION

Arterial stiffness has been recognized as a marker of CVD and associated with long-term prognosis in several populations. Older age, hypertension, cigarette smoking, and dyslipidemia, known as traditional vascular risk factors, as well as diabetes, obesity, and systemic inflammation contribute to arterial stiffness. Targeting multiple modifiable risk factors has become the main therapeutic strategy to improve arterial stiffness in patients at high cardiovascular risk. Additionally to life style modifications, long-term ω-3 fatty acids intake in diet may improve arterial stiffness in the population with hypertension or metabolic syndrome. Pharmacological treatment such as RAAS antagonists, metformin, and HMG-CoA reductase inhibitors were useful in individuals with hypertension or diabetes. In obese people with OSA, weight reduction, aerobic exercise, and CPAP treatment may improve arterial stiffness as well. In specific populations such as with chronic inflammatory disease, a use of antibodies against tumor necrosis factor-alpha could work effectively. Other therapeutic options such as renal sympathetic nerve denervation for patients with resistant hypertension remains under investigated clinically. Therefore this comprehensive review provides knowledge in detail regarding the aspect of pathogenesis, measurement, and management of arterial stiffness in several populations, which

would be helpful for physicians to make clinical decision.

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MINIREVIEWS

Visualization of catheter ablation for atrial fibrillation: Impact of devices and anatomy

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Abstract

Endocardial access to the left atrium is commonly achieved to treat patients with atrial fibrillation, using different device delivery systems for cardiac ablation. But the large variation in human anatomy presses the limits of existing medical devices. In this unique study, we directly visualized the device-tissue interface in fresh reanimated human hearts using Visible Heart® methodologies. Our goal was to better understand any opportunities to improve therapeutic approaches. The visual images obtained in this study (also featured in this article) allow a more intimate grasp of the key steps required in various ablation procedures, as well as some limitations of current device designs. These images show the potential risks of conducting transseptal punctures and the difficulties of placing catheter tips in certain scenarios (*e.g.*, when creating circumferential lesions); they also demonstrate potential problems that could occur while attempting to place catheter tips on such anatomies like the mitral isthmus. In our analysis of these images, we focus on where enhancements are needed to refine device functionality.

Key words: Atrial fibrillation; Cryogenic catheter ablation; Radiofrequency ablation; Transseptal puncture

Core tip: Visible Heart® methodologies are utilized to directly visualize a functional human heart anatomy and key steps in the cardiac ablation procedure to emphasize limitations of current device delivery systems. Specifically, these images illustrate potential risks of transseptal punctures as well as the challenges faced by clinicians when placing catheter tips in certain scenarios, considering the wide variation in human anatomy. The focus is on where enhancements are

needed to refine device functionality and improve therapeutic approaches.

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INTRODUCTION

For many years, ablation (either radiofrequency or cryogenic) has been used to treat patients with atrial fibrillation (AF)[1-3]. But variations in cardiac anatomy have consistently influenced therapeutic success[4-8]. Different medical device designs have been developed for creating effective lesions in such varied anatomic structures[9-12]. However, in order to apply therapies for left atrium (LA) targets, navigation is first required into the right atrium (RA) and then across the septum.

Ablating the anatomic locations within the left heart was initially made feasible by a modified Cox's maze procedure[13-16]. In such a procedure, each step requires an intimate understanding of the endocardial anatomy[17]. Importantly, the inappropriate placement of devices in any ablation procedure can result in significant unintended consequences, including the creation of ineffective lesions (no transmurality), the need for subsequent ablation procedures, and/or cardiac tamponade during transseptal punctures[18-20]. In an effort to reduce the incidence of such unintended consequences, ablation is commonly performed with the assistance of imaging tools such as fluoroscopy or echocardiography. Imaging tools not only help eliminate unintended consequences such as perforation, but also help ensure occlusion of pulmonary veins (PVs). In addition, the use of fluoroscopy, angiography, and noncontact mapping has improved the quality of the images[21]. However, no imaging method allows one to directly visualize the device-tissue interface or to take into consideration the impact of accuracy on heart rhythm[21,22].

In this unique study, we used Visible Heart® methods to directly visualize the device-tissue interface in fresh human hearts reanimated in a clear Krebs-Henseleit buffer (Sigma-Aldrich Corporation, St. Louis, MO, United States), as previously described[22,23]. Our goal was to better understand any opportunities to improve therapeutic approaches during the key steps of various ablations procedures. The visual images obtained in our study (and featured in this article) allow a more intimate grasp of the steps required as well as any limitations of current device designs.

In particular, the images reveal the interaction of ablation technology with human tissue, providing a sense of the spatial relationship between the device and anatomic structures. In our analysis of these images, we focused on where enhancements are needed to refine device functionality. For purposes of analysis, we separated the key steps of ablation procedures into 3 distinct image sets, based on the device used and the anatomy: (1) navigating the RA; (2) conducting transseptal punctures from the RA to the LA; and (3) creating lesions and reaching the key anatomic locations in the LA with different types of ablation devices. Delineating the limitations of current devices and pinpointing the major anatomic challenges should prove to be of great importance for both practicing physicians and medical device designers[23].

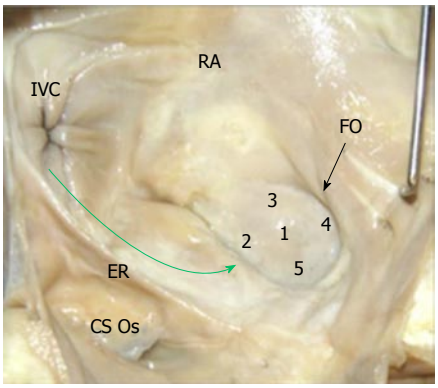

Figure 1 Fossa ovalis and transseptal punctures. All of the 5 locations shown on the fossa ovalis (black arrow) are transseptal puncture possibilities, and the path of device delivery to the fossa ovalis is shown (green arrow). CS Os: Coronary sinus ostium; ER: Eustachian ridge; FO: Fossa ovalis; IVC: Inferior vena cava; RA: Right atrium.

NAVIGATING THE RA

Success in navigating the RA has been limited, given the challenging anatomies of key RA structures combined with the limitations of current device designs. Endocardial cardiac ablations of the atria commonly originate *via* access from the inferior vena cava (IVC). An introducer, at the groin, is inserted into the femoral vein and then advanced into the RA. The IVC serves as a low-pressure return path of deoxygenated blood to the RA. Thus, the IVC is a suitable starting point for endocardial procedures because it eliminates risks associated with device introduction. Key ablation procedure structures in the RA include the fossa ovalis (FO), coronary sinus (CS), and right atrial appendage (RAA).

FOSSA OVALIS

The FO serves as the access point for ablation of the LA. As devices enter the RA, the Eustachian valve of the IVC forms a bridge between the IVC and the Eustachian ridge (ER) (Figure 1).

The FO is also a structure that causes devices to bind or become lodged, and device tips can catch on the compliant membrane of the valve[24-26]. Because the

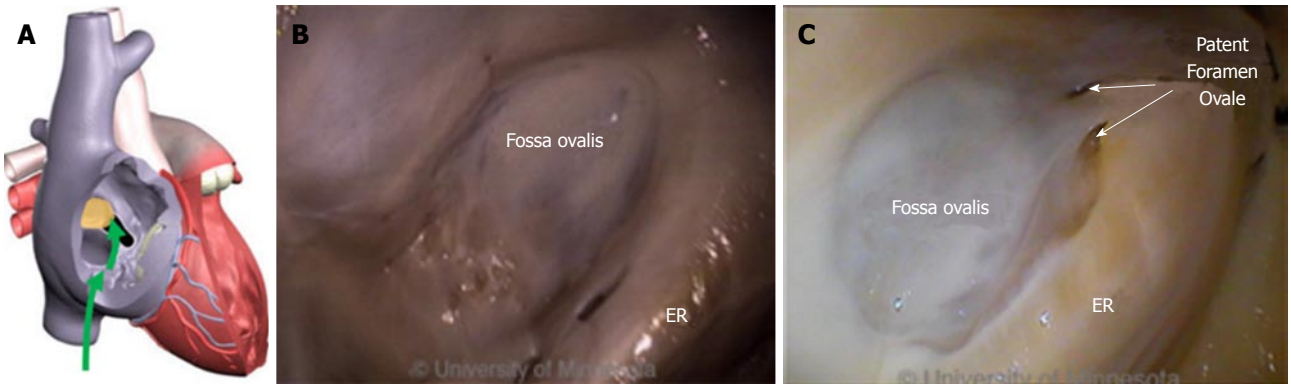

Figure 2 Anatomy of fossa ovalis and Eustachian ridge related to transseptal punctures. A: Inferior vena cava approach to transseptal punctures (green arrows); B: Image of fossa ovalis and Eustachian ridge (ER); C: Image of a patent foramen ovale in the fossa ovalis (two white arrows), with the ER adjacent to the fossa ovalis.

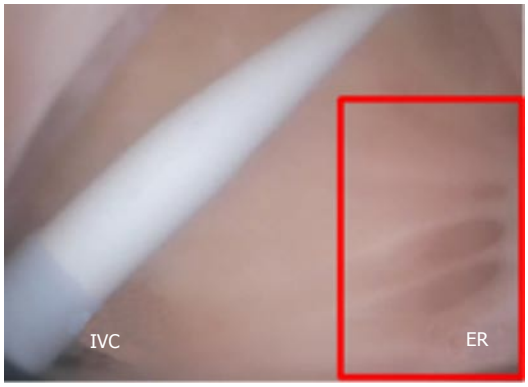

Figure 3 Catheter dilator and sheath in the right atrium. Pectinated muscles border the Eustachian ridge (ER, red rectangle). IVC: Inferior vena cava.

FO and the IVC are located on the superior aspect of the ER, the ER can serve as a guide to facilitate device delivery to the FO, allowing the device to glide along the valve and onto the ER (Figure 2B and C).

The ER is a prominent rise between the FO and the CS ostium (Os)[27-29]. The superior and posterior margins of the FO are enfolded to produce the prominent muscular protrusion on the endocardial surface. The FO lies next to the aorta, in some cases making transseptal punctures difficult[30]. Bordered by septal tissue and the ER, the FO is typically slightly recessed (Figure 2B and C). These structures can either facilitate or inhibit the operation of a medical device, either directing it in the intended direction or preventing it from being placed in the desired location.

Current catheter delivery systems often face challenges in reaching the FO and conducting transseptal punctures. Pectinated muscles adjoin the ER, which itself is pronounced and moves with each contraction. The pectinated muscles adjacent to the FO are composed of undulations that are capable of restraining the tip of a dilator or catheter (Figure 3). Dilator tip dimensions are sized to only allow a transseptal needle to pass. This small tip size also increases the chance of binding in these muscles if the tip is placed incorrectly.

During transseptal punctures, the FO is manipulated extensively. Both its size and thickness contribute to changes in the amount of compliance when force is applied (Figure 4). A large amount of compliance in the FO, coupled with a lack of compliance in the much thicker septal wall, can result in concentration of the transseptal force on the FO.

Dilator tips enable practitioners to confirm anatomic location by tenting the FO. Once tenting is achieved, a transseptal needle can be advanced through the FO (Figure 5A and B). The large amount of tenting that is usually required and the compliance of the membrane draw into question how much force the FO is able to tolerate before the procedure fails. Though necessary to perform transseptal punctures, FO tenting-combined with excess extension of the transseptal needle tip into the LA - can result in cardiac tamponade.

The very close proximity of the FO to both the right superior pulmonary vein and the right inferior pulmonary vein makes it challenging to reorient device tips after transseptal punctures (Figure 6).

Devices whose total deflection is limited, or whose deflection is located more proximally in the shaft, result in tip changes that make it nearly impossible to orient the device in a way that facilitates catheter introduction into the right PVs. Consequently, the FO needs to be manipulated more. Additionally, an incorrect puncture site location can increase the difficulty of introducing a catheter into the right PVs.

Once a transseptal puncture is complete, the tissue is stretched over the outside diameter of the dilator and onto the outside diameter of the sheath (Figure 5B). This transfer of force, the overall diameter of the sheath, and manipulation of the device in the LA can all contribute to the possibility of tearing the FO. As the sheath is deflected and the device is introduced into the LA, the resulting forces on the sheath push and pull the FO. If these forces become excessive, the FO can tear (Figure 7).

This step in the procedure prompts additional consideration of the use of the transseptal needle in the

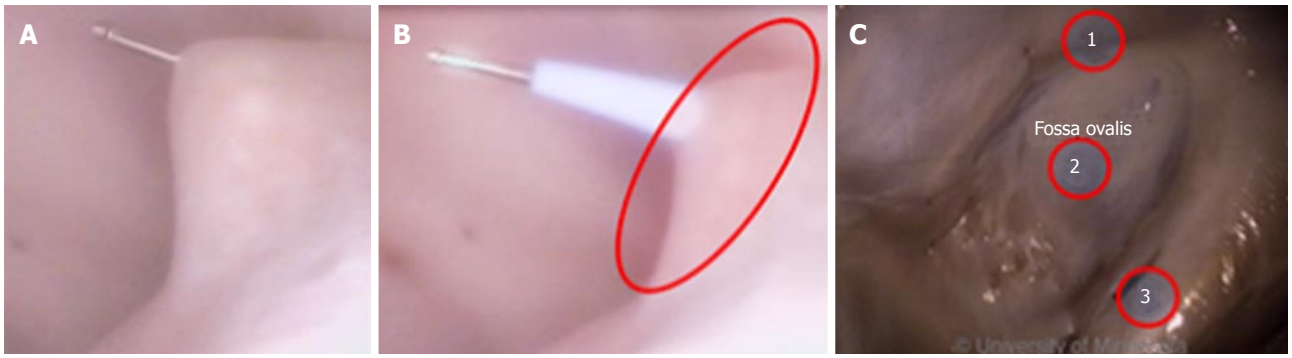

Figure 4 Fossa ovalis manipulation during transseptal punctures. A: Deformation of the fossa ovalis at the point of needle puncture in the left atrium; B: Transseptal puncture at the time of dilator insertion, with deformation of the fossa ovalis (red oval) and a tight fit between the dilator and the fossa ovalis; C: Image of the septal ridge (red circle 1) around the fossa ovalis, the thin and highly compliant nature of the fossa ovalis (red circle 2), and the Eustachian ridge (red circle 3).

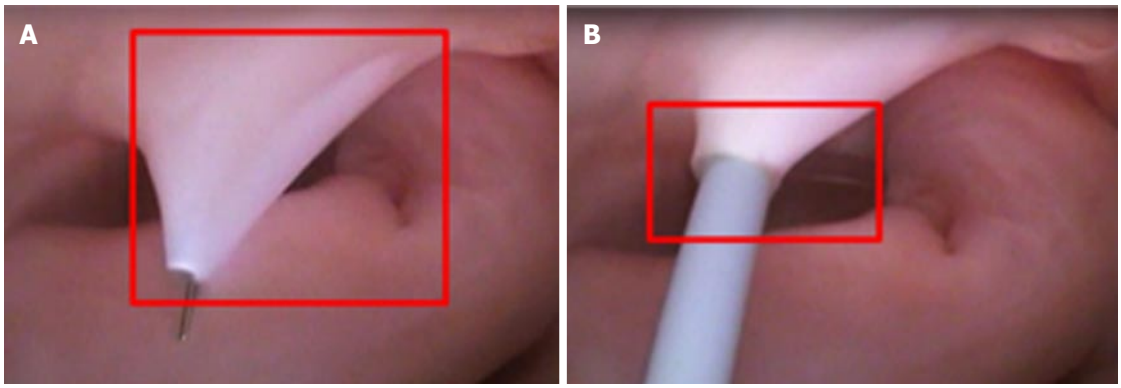

Figure 5 Tenting of the fossa ovalis. A: View from the left atrium of the fossa ovalis at the point of needle puncture (red square); B: Simultaneous view from the right atrium at the point of needle puncture (red rectangle).

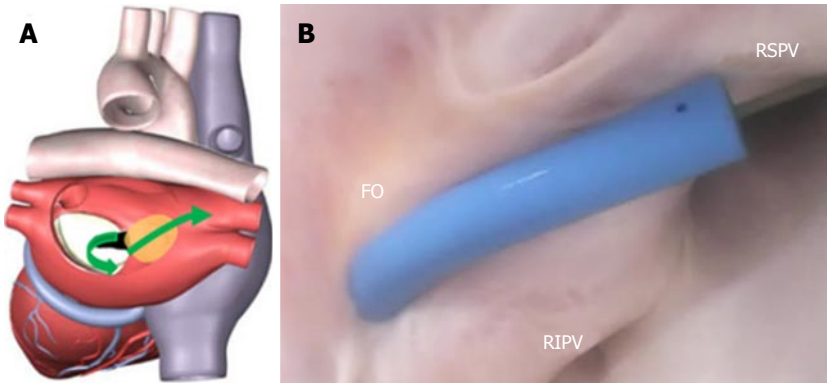

Figure 6 Fossa ovalis anatomy and device delivery for transseptal punctures. A: Path of device delivery to the pulmonary vein originating from the fossa ovalis (green arrows); B: Left atrial sheath placement after a transseptal puncture, with a guidewire introduced into the right superior pulmonary vein (RSPV). FO: Fossa ovalis; RIPV: Right inferior pulmonary vein.

LA. The transseptal needle extends beyond the tip of the dilator. The amount of extension is dictated by the interference fit of the diameter on the needle shaft to the internal diameter reducer in the dilator tip. Given the large amount of needle extension and the relative thickness of the FO, future device designs must improve the needle tip to reduce the risk of cardiac tamponade, while still preserving the ability to achieve successful punctures. Clearly, anatomic variations can have an impact on the ability to conduct transseptal punctures as well as possible complications.

Such variability in anatomic structures - combined with current device limitations in sheath size and in needle, length, and deflection capabilities - will require continued advancements in order to decrease the risk to patients. Device developers must continue to collaborate with electrophysiologists. A partnership between engineers and health care providers is critical

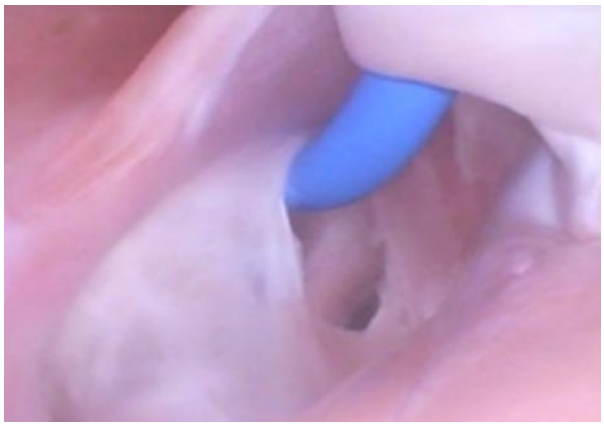

Figure 7 Tearing of the fossa ovalis. Example of a potential complication as a result of catheter navigation performed in a swine, showing the fossa ovalis tearing due to excessive deflection force from a delivery sheath attempting to navigate into a pulmonary vein.

for improving patient outcomes.

CORONARY SINUS

Arrhythmia ablation procedures commonly involve the CS[6,31]. Its ostium is located on the opposing side of the ER. In addition, the thebesian valve is located at the CS ostium (Figure 8). Inferior to the CS ostium, anatomic structures can be of various shapes and sizes[32].

Clearly, anatomic factors can increase the complexity of device delivery. The CS ostium resides in a deep pocket that is bordered by the ER, making catheter tip placement challenging. The location of the CS ostium relative to the IVC, along with the size of the ostium, can also present challenges.

To enter the CS, devices must have a high degree of deflection; furthermore, the region of deflection must have a small radius. With devices whose deflection is located more proximally in a stiff shaft and whose diameter is 8-Fr or larger, it will be more difficult to orient the tip so that it aligns with the CS ostium (Figure 9). Further design work is needed to develop devices that can deflect in a small radius, allowing the catheter tip to be oriented in such a way that it can align with the CS ostium.

RIGHT ATRIAL APPENDAGE

Right atrial ablation is required in instances of AF in which the cycle length recorded in the RAA is shorter than is the cycle length recorded in the left atrial appendage (LAA). The RAA location near the ostium of the IVC prompts the need to deflect devices (Figure 10A). The appendage can be a large structure; it is composed of very thin tissue as well as pectinated muscle and a sagittal band (Figure 10B and C).

Given the thin tissue of these anatomic structures, devices need to have very smooth tips that do not focus force into a point. With devices that have a rigid shaft, the risk of perforating the RAA is greater, because of the higher transfer of force. In contrast, with devices that have a compliant tip at the distal end, the tip can bend, thereby lessening the chance of perforating the RAA.

ABLATING LEFT ATRIAL STRUCTURES: PV, MI, AND LAA ROOFLINE

The LA has a venous component, along with a vestibule and an appendage. The additional 4 venous orifices serve as corners to the atrium. The vestibule surrounds the mitral ostium. The LAA is typically a small extension that originates adjacent to the mitral valve annulus and the left PVs.

In the atrial areas, the anterior wall behind the aorta is commonly thin and can be torn during transseptal punctures[33]. The thicker parts of the LA are on the superior wall[34]. The ostium of the right PVs are adjacent to the plane of the atrial septum. The tissue that makes this transition is smooth. The target of PV isolation is a muscular sleeve that extends into each vein and ends inside the sleeve; the role of the sleeve has been reviewed in other studies[35,36]. The organization of electrical activity from the PV is well known[37,38].

The smooth wall of the LA facilitates a uniform drag of the catheter tip against the tissue. The size of the LA is conducive to catheter tip placement against the tissue surface[27]. But the formation of a small gap is possible; complex cardiac navigation systems do provide some guidance as to gap location, yet it might not be sufficient.

LA ablation can occur in a number of different locations and can be prompted by continuous electrical activity, with a minimum duration of 100 ms[39] and either fractionated or fragmented electrical activity[40].

LEFT ATRIAL PULMONARY VEINS

Pulmonary vein isolation is currently considered as a key step in treating patients with all forms of AF. Of note, the muscle sleeves in the ostial opening of all 4 PVs emit ectopic beats. Electrical isolation of each vein is now the standard of care for treating AF, using either cryogenic or radiofrequency ablation[28-30]. In electrical isolation procedures, both ablation and diagnostic devices are used around and inside the PVs including guidewires, balloons, diagnostic catheters, and focal ablation catheters.

The ostia of the right PVs are adjacent to the FO. The ostial opening of the PV is a smooth surface. The close proximity of the PV ostia to the FO, and the sharp angle between them, make it difficult to orient a catheter through the puncture site and into the PV (Figure 11).

The ostia can comprise ridges and are adjacent to each other on opposing sides of the atrium. The shape and orientation of the PV can vary; other anatomic

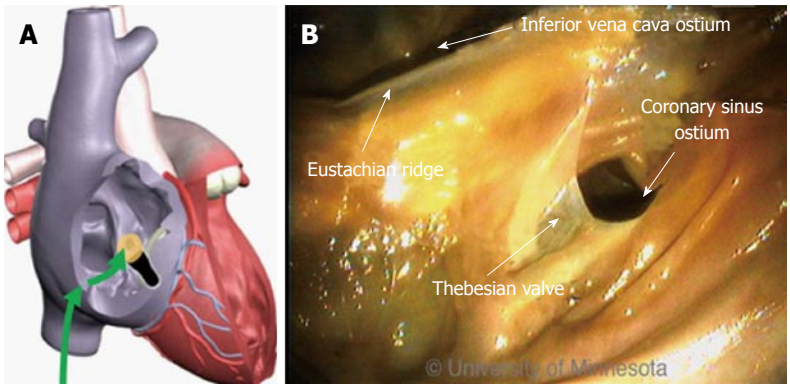

Figure 8 Coronary sinus and ablation procedures. A: Device approach originating from the inferior vena cava into the coronary sinus ostium (green arrows); B: Regional anatomy in the area of the coronary sinus is bordered by the thebesian valve, including the inferior vena cava catheter introduction point.

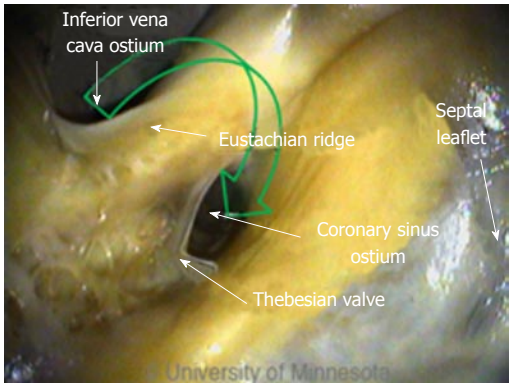

Figure 9 Deflection of devices in the coronary sinus. Green arrow depicts the device approach originating from the inferior vena cava; devices in this region require a high degree of deflection.

structures can be atypical. Variations can include differences in ostial size and the existence of a common shared ostium. All of these factors can affect the effectiveness of the devices used to electrically isolate the tissue.

The PVs interface with guidewires, sheaths, balloons, and focal ablation catheters. The location of the transseptal puncture can have a dramatic effect on the ability to place the catheter tip in the ostium of the PV, especially because the distance from the FO to the ostium is short. In addition, the orientation of the opening of the PV is directed in a way that can result in the need to twist the guidewire to allow it to migrate inside the PV (Figure 12A). This anatomic orientation of the FO and the PV illustrates the importance of a sheath that has a small radius of deflection at the tip in order to facilitate guidewire insertion.

Catheter placement into the PV is also affected by the contour of the catheter tip. The angle of the catheter's approach might require anatomic guidance to properly position the tip (Figure 12B). This device-tissue interface shows the importance of a smooth, contoured tip.

The complete insertion of the ablation catheter is affected by its size and by the size of the atrium. The proximity of the FO to the PV can limit the ability to have both the sheath and the catheter in the chamber (Figure 12C). Limiting the distance of the therapeutic region of the catheter would provide greater latitude for use of the entire system in the atrium. Operation of these devices on the right side of the atrium is one of the more challenging steps of the procedure.

Performing the same steps on the left side of the atrium requires different device performance. The orientation of the FO to the left PV ostia (as compared with the right side of the atrium) is more conducive to device delivery. Guidewire introduction is typically facilitated by the nearly linear orientation of the FO to the PV ostium (Figure 13A), which allows the guidewire to be placed and lodged in the PV (Figure 13B).

The alignment of the FO and left PVs allows for easy catheter introduction into the LA and sufficient room to operate the device, thereby reducing the stress on the FO and lessening the demands on the sheath (Figure 13C). For balloon-based devices, which require more area to operate than do focal ablation catheters, the alignment of the FO and left PVs is of particular importance.

Once the catheter is delivered into the PVs, therapy delivery remains challenging. For example, balloon-based therapeutic devices are larger, with only a limited amount of deflection ability, so they require more room to operate. Given the orientation of the transseptal puncture to the ostium of the PV, the balloon is able to fully occlude the vein. However, uniform cooling may not be achieved, because the balloon's orientation is limited by the FO's orientation to the PV's muscular sleeve (Figure 14).

These anatomic challenges accentuate the importance of having an acute distal deflection segment on the ablation device, in order to improve catheter tip orientation to, and alignment with, the PV ostium. Such challenges also jeopardize the ability of the sheath to maintain its placement in the LA. Decreasing the length of the distance between the distal tip of the sheath and the proximal end of the balloon would allow more sheath to be retained in the LA. The sheath must have a very distal deflection control with an acute radius of deflection. The smooth wall of the atrium facilitates placement of the ablation catheter.

If additional lesions are necessary beyond PV isolation, they can be created in the LA in the form of a linear lesion along the roofline or a mitral isthmus (MI)

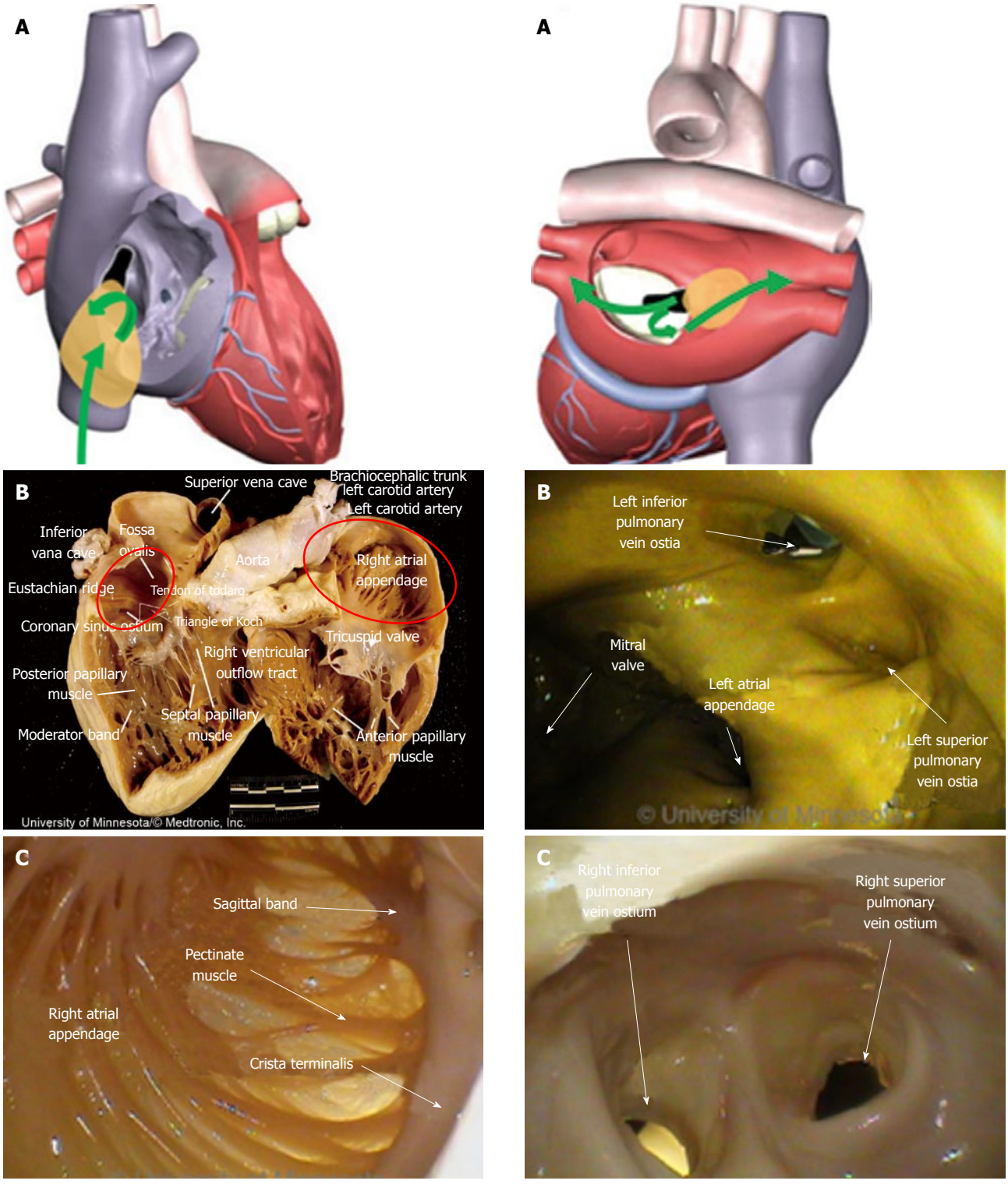

Figure 10 Right atrial appendage and transseptal punctures. A: Approach to the right atrial appendage through the inferior vena cava (green arrows); B: Image shows the large size of the right atrial appendage (red oval, right side) and pectinated muscles (red oval, left side); C: Image of the pectinated muscles and thin tissue.

Figure 11 Pulmonary veins and ablation devices. A: Directions of device introduction originating from the fossa ovalis into the left atrial pulmonary anatomy (green arrows); B: Image shows the left pulmonary vein ostium; C: Image of the right pulmonary vein.

lesion, or *via* ablation of the LAA.

LEFT ATRIAL APPENDAGE

Many times, the LAA is a site for the deposition of thrombus. A stroke is a possibility if the thrombus is able to dislodge and travel to a part of the vasculature that supplies blood to the brain. The LAA is oriented on the opposing side of the LA from the FO, making device delivery less challenging (Figure 15A).

For achieving and retaining device placement, the

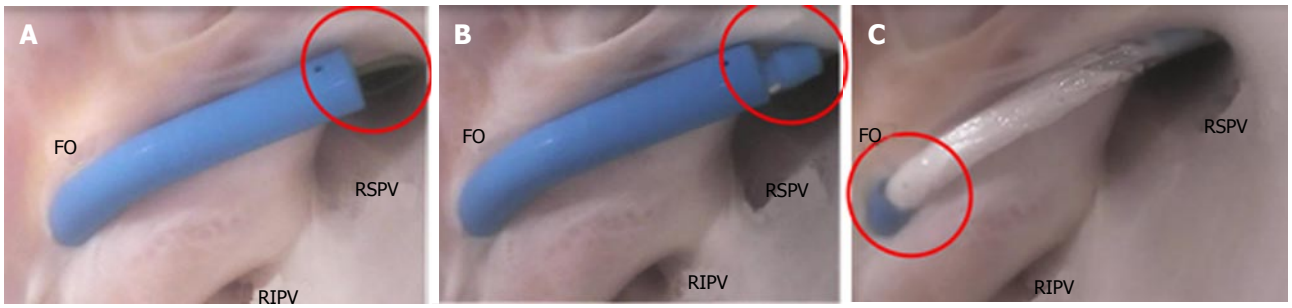

Figure 12 Device placement in the right pulmonary veins. A: Catheter sheath and the use of a guidewire for placement into the right superior pulmonary vein (RSPV, red oval); B: Introduction of the catheter tip at the pulmonary vein ostium (red oval); C: Retraction of the catheter sheath to the fossa ovalis (FO) and the introduction of a balloon catheter (red oval). RIPV: Right inferior pulmonary vein.

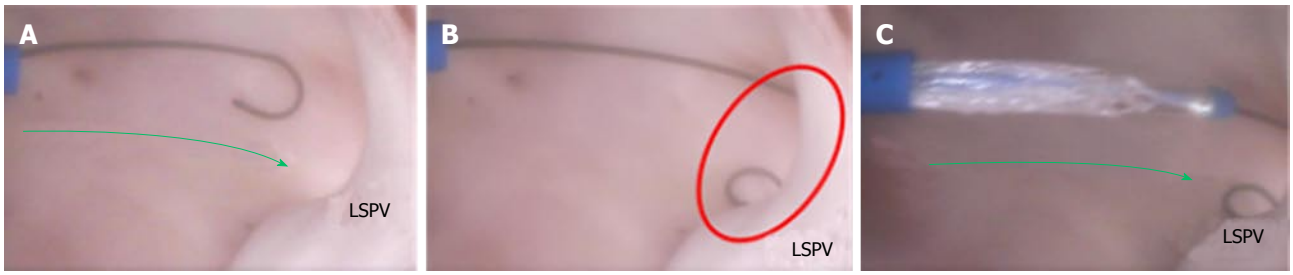

Figure 13 Device placements in the left pulmonary veins. A: Catheter sheath and the use of a guidewire for placement into the left superior pulmonary vein (LSPV, green arrow); B: Placement of the guidewire into the pulmonary vein ostium (red oval); C: Introduction of the balloon catheter across the left atrium (green arrow).

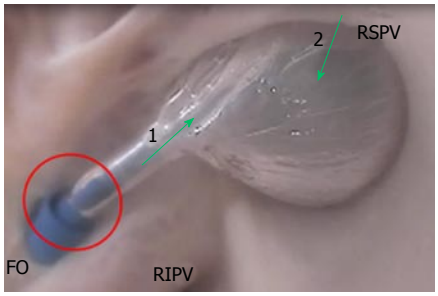

Figure 14 Balloon-based left atrial right superior pulmonary vein occlusion. The sheath is retracted to accommodate the ablation catheter (red circle). The catheter orientation (green arrow 1) is not aligned with the pulmonary vein ostium orientation (green arrow 2). FO: Fossa ovalis; RIPV: Right inferior pulmonary vein; RSPV: Right superior pulmonary vein.

opening of the LAA can be challenging. The ability to place a focal ablation device at the ostial opening is complicated by the presence of prominent ridges in the ostial area of the LAA. Focal devices for performing point-by-point ablation around the opening are difficult to operate. Alternatively, devices that encircle the LAA, or that occlude it, preclude the need to create point-by-point lesions and remove the complexity of attempting to place a catheter tip on a ridge structure.

Devices that deploy into the LAA and then place the therapeutic region at the opening are able to encircle the opening (Figure 16). Focal ablation devices need the ability to apply sufficient force on the tissue for lesion generation, without slipping into the pectinated muscles of the LAA interior.

Dynamically shaped ablation devices that could occlude the LAA would have an advantage, as they might be able to maintain position and sufficient force for lesion generation.

MITRAL ISTHMUS AND ROOFLINE ABLATION

The MI is a region of tissue that borders the annulus of the mitral valve and the LAA, and then rises over the ridge toward the left PV (Figure 17A). This is a common area to create a contiguous lesion in which it helps to terminate conduction patterns in patients with AF in whom PV isolation is not sufficient[27,31,32].

The creation of the MI or roofline linear lesion is affected by even a minor amount of anatomic movement of the MI with each contraction, making catheter tip placement on the ridge difficult. Any anatomic movement changes the force on the catheter tip and can contribute, at times, to a temporary loss of sufficient contact for lesion creation. The ability to maintain tissue contact is a byproduct of the amount of force on the catheter tip. For MI linear lesion creation, given the orientation of the FO in relation to the mitral valve annulus (MVA), the catheter tip must be able to reach to the MVA, and the deflection must be able to place force on the catheter tip (Figure 17B). The presence of a ridge is an additional complicating factor; the shape of the ridge can be pointed, making it difficult for the catheter to be placed on it.

To ensure necessary contact when creating a linear lesion, a focal catheter may be used against a

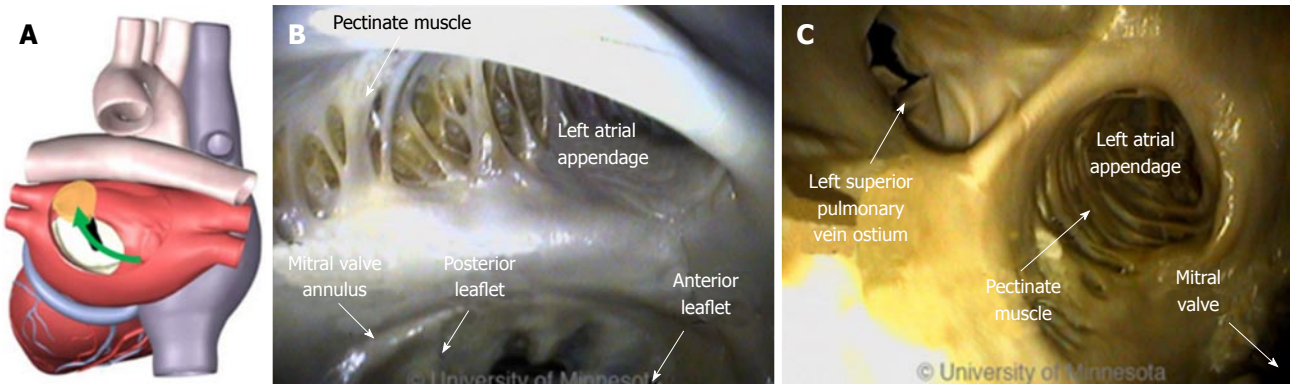

Figure 15 Left atrial appendage and device delivery. A: Approach to the left atrial appendage from the fossa ovalis (green arrow); B: Position of the left atrial appendage relative to the mitral valve annulus and the presence of pectinated muscle; C: Position of the left atrial appendage relative to both the mitral valve and the left superior pulmonary vein.

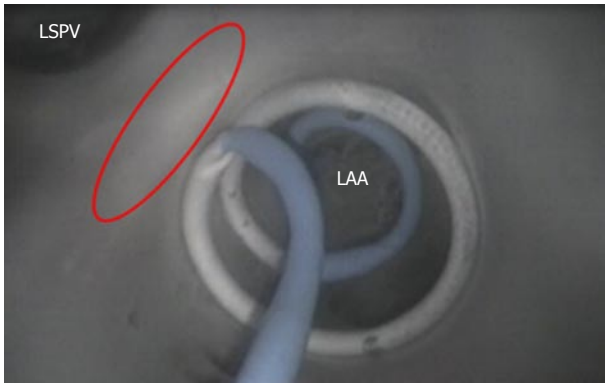

Figure 16 Device encircling the left atrial appendage. Image shows the catheter shaft circulating around the left atrial appendage (LAA) ostium; the red oval depicts the ridge between the LAA and left superior pulmonary vein (LSPV).

supporting structure, such as another catheter (Figure 18A) or the wall of the atrium (Figure 18B).

A focal ablation catheter has the advantage of adaptability. This device design could be extended to include repositioning of electrodes, softening of the tip, and better deflection capabilities - all of which could widen application across an array of atrial anatomies, resulting in an improvement in energy delivery.

CONCLUSION

Understanding how ablation devices interface with tissue and anatomic structures can make a crucial difference in their therapeutic application. Anatomic structures vary from person to person. Within each person, the endocardial surface changes shape with each heartbeat and can prompt catheter migration, making it difficult to know exactly where the device was placed and what is happening at the device-tissue interface. By using Visible Heart® methods to directly visualize the device-tissue interface in fresh reanimated human hearts, we assembled and analyzed an array of illuminating images, providing a critical aid to clinicians and medical device designers alike. For examples of

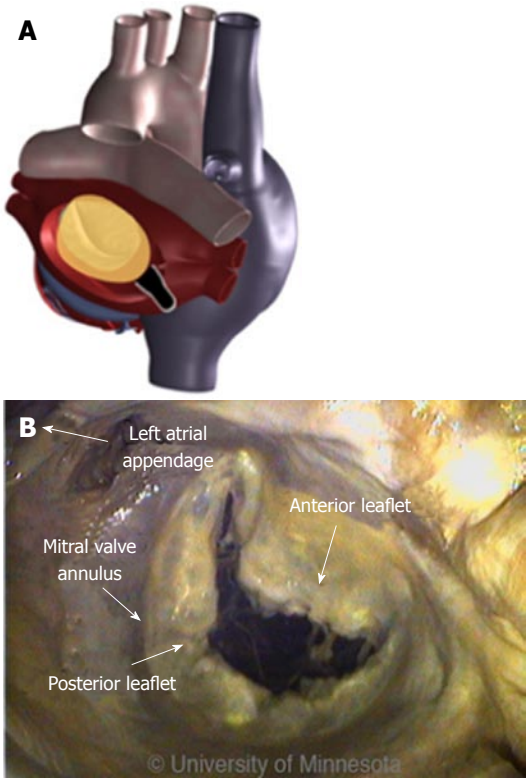

Figure 17 Mitral isthmus and roofline ablation. A: Diagram shows the mitral valve in the left atrium; B: Various structures, including the mitral valve annulus, which serve as the starting point for creating a linear lesion in the mitral isthmus.

functional anatomies of the human heart, refer to the free-access website, "The Atlas of Human Cardiac Anatomy" (www.vhlab.umn.edu/atlas).

The tools that have traditionally been used to treat patients with AF have numerous limitations, all of which lengthen ablation procedure time and increase the likelihood of disease recurrence. Future research in this field needs to focus on reducing the risks of transseptal procedures, increasing catheter mobility, enhancing the anatomic precision of catheter tip placement, and improving imaging capabilities. Studies must investigate

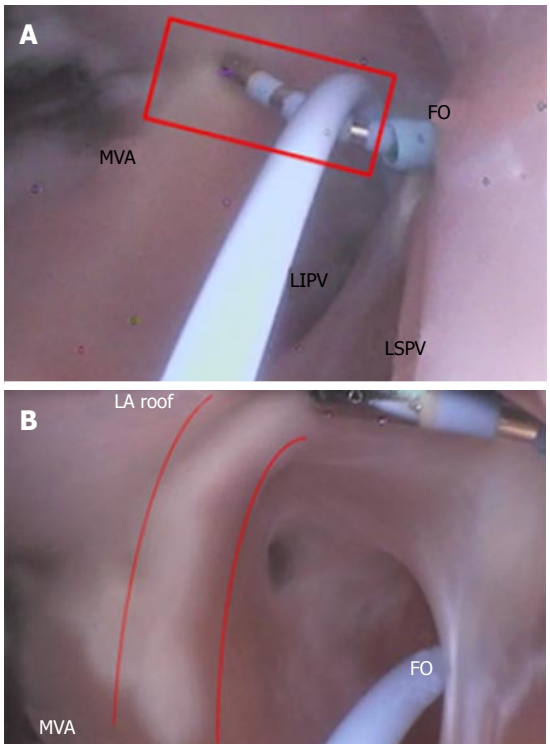

Figure 18 Focal ablation catheter. A: Mitral isthmus lesion creation originating at the mitral valve annulus (MVA, red rectangle); B: Creation of a roofline linear lesion (red curved lines). FO: Fossa ovalis; LA: Left atrial; LIPV: Left inferior pulmonary vein; LSPV: Left superior pulmonary vein.

methods for improving transseptal punctures, reaching targeted anatomies with therapeutic devices, and assessing the effectiveness and quality of lesions at the point of their creation.

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MINIREVIEWS

Coarctation of the aorta: Management from infancy to adulthood

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Abstract

Coarctation of the aorta is a relatively common form of congenital heart disease, with an estimated incidence of approximately 3 cases per 10000 births. Coarctation is a heterogeneous lesion which may present across all age ranges, with varying clinical symptoms, in isolation, or in association with other cardiac defects. The first surgical repair of aortic coarctation was described in 1944, and since that time, several other surgical techniques have been developed and modified. Additionally, transcatheter balloon angioplasty and endovascular stent placement offer less invasive approaches for the treatment of coarctation of the aorta for some patients. While overall morbidity and mortality rates are low for patients undergoing intervention for coarctation, both surgical and transcatheter interventions are not free from adverse outcomes. Therefore, patients must be followed closely over their lifetime for complications such as recoarctation, aortic aneurysm, persistent hypertension, and changes in any associated cardiac defects. Considerable effort has been expended investigating the utility and outcomes of various treatment approaches for aortic coarctation, which are heavily influenced by a patient's anatomy, size, age, and clinical course. Here we review indications for intervention, describe and compare surgical and transcatheter techniques for management of coarctation, and explore the associated outcomes in both children and adults.

Key words: Coarctation of the aorta; Cardiac surgery; Cardiac catheterization; Balloon angioplasty; Stents

Core tip: This review explores both surgical and transcatheter approaches for the treatment of coarctation of the aorta and examines outcomes of these techniques in children and adults.

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INTRODUCTION

Coarctation of the aorta was first described by Morgagni in 1760[1], and in its simplest form refers to congenital narrowing of the proximal thoracic aorta. While aortic coarctation most commonly occurs as a discrete stenosis in the juxtaductal position, it may also be associated with long segment narrowing, hypoplasia of the transverse aortic arch, or stenosis of the abdominal aorta[2-4]. Coarctation of the aorta accounts for 5%-7% of all congenital heart disease[5], with an incidence of approximately 3 cases per 10000 births[6]. Coarctation may be seen in isolation or with additional cardiac lesions, such as bicuspid aortic valve, ventricular septal defect, patent ductus arteriosus, transposition of the great arteries, atrioventricular canal defects, or left-sided obstructive heart defects, including hypoplastic left heart syndrome[7-11]. Crafoord was the first to perform a successful surgical repair of aortic coarctation in 1944[12]. Since then, various surgical and transcatheter approaches have been developed, allowing patients to have significantly improved outcomes. Here, we briefly review the presentation and diagnosis of aortic coarctation and then focus on surgical and transcatheter approaches with their most recent associated outcomes.

DIAGNOSIS

Clinical presentation

Coarctation can present at any age. Neonates with ductal dependent or "critical coarctation" often present with heart failure, acidosis, and shock following closure of the ductus arteriosus. Without prompt medical resuscitation and surgical intervention, death may occur rapidly[13,14]. Prenatal diagnosis can prevent these sequelae by allowing for intervention before ductal closure. However, prenatal diagnosis of coarctation is challenging due to the presence of the ductus arteriosus and limited blood flow across the aortic isthmus in utero[13]. In the United States, fewer than 1 in 4 patients with isolated coarctation requiring neonatal intervention are diagnosed prenatally[15,16]. Moreover, approximately 30% of neonates with coarctation remain undiagnosed upon discharge after delivery[17]. For these reasons, many physicians advocate for newborn pulse oximetry screening programs, which increase the likelihood of detecting lesions like coarctation before ductal closure[18,19]. Additionally, coarctation must be suspected in infants with other left-sided obstructive heart lesions and may be diagnosed in infants with chromosomal defects, especially those with Turner syndrome and Jacobsen syndrome[20].

Patients with less severe coarctation may not be diagnosed until later in childhood when a murmur is heard or hypertension noted. In these patients, collateral vessels develop from the internal thoracic and subclavian arteries, thyrocervical trunks, and vertebral and anterior spinal arteries to supply blood to the descending aorta[21,22]. For those who enter adulthood undiagnosed, hypertension is the most common presenting symptom[23]. Others may complain of frequent headaches or claudication of the lower extremities with exertion. In these patients, the most telling exam findings suggestive of coarctation are diminished and/or delayed lower extremity pulses and a systolic pressure gradient between the upper and lower extremities[13,23]. However, for patients with extensive collateral blood flow, femoral pulses and lower extremity blood pressures may only be minimally diminished[24].

Evaluation

Chest X-ray is often nonspecific in young patients. In older patients, an anterior-posterior film may show indentation of the aorta at the site of coarctation with pre- and post-stenotic dilation of the aorta, creating the classic "3 sign". Notching of the posterior fourth to eighth ribs due to dilated intercostal arteries may also be seen in older patients[24,25]. Electrocardiogram is typically normal in infants, but in older children and adults, left ventricular hypertrophy is common due to ventricular pressure overload[24].

Transthoracic echocardiography can assess the presence and severity of aortic coarctation and any associated cardiac defects and is the diagnostic gold standard in neonates and infants (Figure 1). Although transthoracic echocardiogram remains the initial test of choice for coarctation, in larger children and adults[24], echocardiographic windows may be suboptimal. When this is the case, a computed tomography scan or magnetic resonance imaging (MRI) provides excellent anatomic detail at the site of coarctation, and these modalities are commonly used to create three-dimensional images for interventional planning (Figure 2). MRI has the additional benefit of defining and quantifying collateral vessel flow. Although cardiac catheterization was frequently used for diagnosis of coarctation in the past, it is now typically reserved for therapeutic intervention or in those cases where hemodynamic data is additive to the diagnostic evaluation[24,25].

TREATMENT

Without intervention, the outcome for patients with coarctation of the aorta is poor. In his classic 1970 natural history study, Campbell examined autopsy and clinical records of 465 patients with coarctation who survived beyond one year of age. The mean age of death was 34 years, with 75% mortality at 43 years of age. Causes of death included congestive heart failure (26%), aortic rupture (21%), bacterial endocarditis (18%), and intracranial hemorrhage (12%)[26]. Fortunately, several treatment options are now available, including surgical and transcatheter interventions. Guidelines regarding indications for

Table 1 Notable studies and guideline statements in the treatment and outcome of coarctation in adults and children

Ref.	*n*	Follow-up	Outcome
Cowley *et al*[60]	36	Mean 14 yr	Randomized trial comparing BA and surgery for native coarctation in children. Aortic aneurysm developed in 35% of BA patients and none of the surgical patients
Carr[81]	846	Mean 36 mo for catheter-based group and 7.8 yr for surgical group	Meta-analysis comparing catheter *vs* surgical intervention for adults with coarctation. Higher risk of restenosis and need for reintervention found in catheter-based group
Forbes *et al*[68]	578	Median 12 mo	Retrospective multicenter analysis at intermediate follow-up after stent placement for coarctation. Exceeding a balloon:coarct ratio of 3.5 and prestent BA increased risk of aortic wall injury
Warnes *et al*[24]	-	-	ACC/AHA guidelines for management of coarctation in adults
Holzer *et al*[67]	302	3-60 mo	Prospective analysis of acute, intermediate, and long-term follow-up after stent placement for coarctation using CCISC registry. At long-term follow-up, recoarctation in 20% of patients, 4% required unplanned reintervention, and 1% had aortic wall injury
Feltes *et al*[27]	-	-	AHA guidelines for transcatheter intervention in children with coarctation
Forbes *et al*[69]	350	Mean 1.7 yr	Multicenter observational study comparing surgery, BA, and stent placement for native coarctation in children using CCISC registry. Significantly lower acute complication rates in stent group but higher planned reintervention rates. Hemodynamic and arch imaging outcomes superior in stent and surgical patients compared to BA group
Harris *et al*[55]	130	3-60 mo	Prospective, multicenter analysis of short and intermediate outcomes for BA in native and recurrent coarctation in children. Trend toward increased acute aortic wall injury and restenosis in native coarctation patients
Sohrabi *et al*[75]	120	Mean 31.1 mo	Randomized clinical trial comparing covered and bare CP stents for native coarctation in adolescents and adults. Trend of increased rates of restenosis and lower rates of pseudoaneurysm in bare stent group
Meadows *et al*[70]	105	2 yr	Prospective, multicenter, single-arm study assessing safety and efficacy of CP stent in children and adults with coarctation. Two year follow-up of 86% showed 23 fractured stents with no significant clinical effects, 6 aortic aneurysms, 19 repeat catheter interventions, and no surgical interventions

BA: Balloon angioplasty; ACC: American College of Cardiology; AHA: American Heart Association; CCISC: Congenital Cardiovascular Interventional Study Consortium; CP: Cheatham platinum.

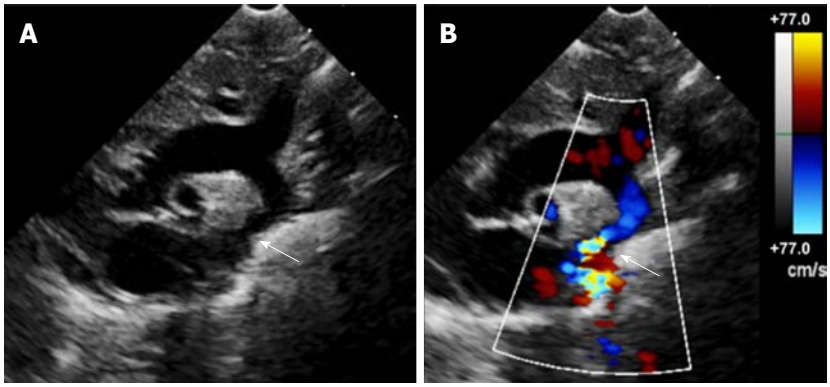

Figure 1 Echocardiogram of coarctation. A: Two-dimensional transthoracic echocardiogram image obtained from the suprasternal notch in an 11-day-old infant demonstrating discrete coarctation (arrow); B: Color Doppler of the same image with aliasing of flow at the site of coarctation (arrow).

intervention exist for both children and adults with coarctation (Tables 1 and 2), which include a peak-to-peak gradient ⩾ 20 mmHg or lesser gradients when there is significant anatomic evidence of narrowing on imaging with extensive collateral flow[24,27]. Other factors that may be considered include the presence of systemic hypertension, additional cardiac defects and/or single ventricle physiology, left ventricular hypertrophy, or elevated left ventricular end diastolic pressure[24,27-29].

Surgical repair

The first surgical technique described for coarctation of the aorta was resection with end-to-end anastomosis by Crafoord in 1944[30] (Figure 3A). Early studies showed recoarctation rates in over half of patients with this technique, and the use of a circumferential suture line was thought to be a major contributor[31,32].

Vosschulte described prosthetic patch aortoplasty as an alternative technique for coarctation repair in 1961. In this approach, the ductal tissue is ligated and divided, a longitudinal incision across the coarctation is made, and a prosthetic patch is sutured in place to enlarge the stenotic region (Figure 3B). This technique can be applied to longer segments of coarctation, avoids a circumferential suture line, and minimizes aortic mobilization and ligation of intercostal arteries[33]. While recoarctation rates of 5%-12%[34] were lower compared to the resection and end-to-end anastomosis technique, aortic aneurysm was a long-term concern, with rates between 18%-51% of patients[35-38]. Using

Table 2 Executive summary on the diagnosis and treatment of coarctation in children and adults

Diagnosis
Accounts for 5%-7% of congenital heart disease diagnoses
Neonates often present with heart failure, acidosis, and shock with critical coarctation
Less severe coarctation often detected during evaluation for hypertension or murmur in the older child or adult
Diminished or delayed lower extremity pulses and a systolic pressure gradient between the upper and lower extremities are the most useful exam findings
Transthoracic echocardiogram is initial test of choice; CT and MRI useful if echocardiogram inconclusive and for surgical planning
Treatment
Surgical repair
Extended end-to-end anastomosis typically preferred surgical method, as it avoids prosthetic material, allows resection of the coarctation, and has a wider incision that is less prone to restenosis
Surgical repair typically preferred over transcatheter approaches in the infant and young child with native coarctation, patients requiring repair of associated cardiac defects, or in those with complex coarctation anatomy
Balloon angioplasty
Often the preferred intervention for recurrent coarctation
Concern for recoarctation and aneurysm formation in native coarctation
Endovascular stent
Provides structural support and decreased rates of aortic wall injury and aneurysm compared to balloon angioplasty
Covered stents may protect against shear stress and subsequent restenosis, though care must be taken to avoid overlying vital branch vessels
Use of stents in small children controversial due to need for large sheath size and limitations in accommodating for somatic growth
Patient follow-up
Lifelong follow-up with at least annual cardiology visits and repeat imaging every 5 yr to assess coarctation site
High suspicion and aggressive treatment of baseline and exercise-induced hypertension
Future perspectives
Further long-term data analysis needed to determine optimal intervention based on patient anatomy, size, and age

CT: Computed tomography; MRI: Magnetic resonance imaging.

more distensible polytetralfluroethylene patch material instead of Dacron was initially promising[34] but still showed a 7% risk for aortic aneurysm and a 25% risk of recoarctation[39].

Subclavian flap aortoplasty was a modified approach reported by Waldhausen and Nahrwold in 1966. Here, the left subclavian artery is ligated and divided, and a longitudinal incision down the proximal left subclavian artery is extended beyond the area of coarctation. The proximal left subclavian stump is then folded down to enlarge the area of coarctation (Figure 3C). This technique avoids a circumferential suture line and the use of prosthetic material, which may allow for improved growth, and it can be used for repair of long segment coarctation[40,41]. Although still occasionally used by surgeons, one of the main reservations of this approach has been the need to sacrifice the left subclavian artery. This can create a subclavian steal phenomenon, with retrograde flow down the vertebral artery, and it has been associated with decreased length and muscle bulk of the left upper extremity, as well as claudication with exercise[42,43].

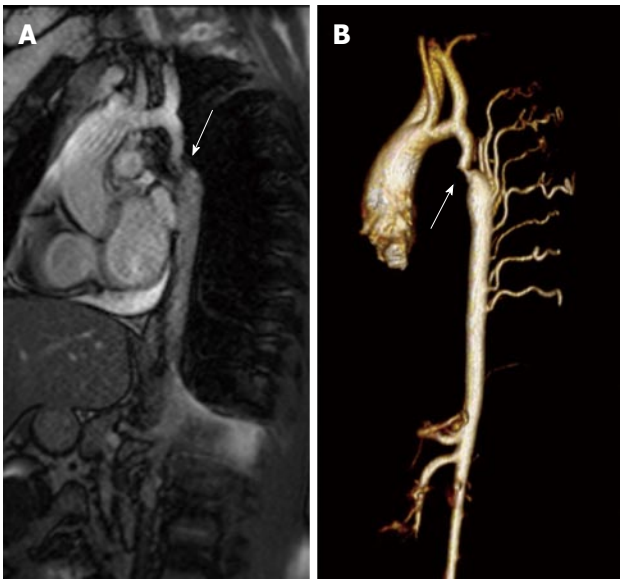

Figure 2 Magnetic resonance imaging of coarctation. A: Magnetic resonance image (steady-state free precession) in a sagittal projection demonstrating transverse arch hypoplasia and long segment coarctation of the aorta distal to the left subclavian artery (arrow) in a 12-year-old male; B: Three-dimensional reconstruction of a gated contrasted angiogram for the same patient, which demonstrates transverse arch hypoplasia, coarctation at the aorta at the distal transverse aortic arch and isthmus (arrow), and dilated intercostal arteries (collaterals).

Coarctation resection and replacement with an interposition graft was described by Gross[44] in 1951. After aortic cross clamp, the stenotic tissue is excised, and either a homograft or Dacron tube graft is sutured into the aorta. This approach is rarely used in the current era, as it is not ideal for pediatric patients due to growth limitations. However, occasionally it is an appropriate technique for adult patients with coarctation, especially those with aneurysm, long segment coarctation, or recoarctation after primary repair[45].

In 1977, Amato *et al*[46] described a modification to Crafoord's resection and end-to-end anastomosis technique, where a broader, longitudinal incision and anastomosis are created across the proximal aorta (Figure 3D). The extended end-to-end anastomosis still avoids the use of prosthetic material and allows resection of the coarctation and residual ductal tissue, but the wider incision is less prone to restenosis and enables enlargement of the transverse aorta, which is particularly helpful in neonates[46,47]. In the present era, extended end-to-end anastomosis is typically the preferred technique for surgical repair, especially in small children, due to low mortality rates and low rates of restenosis, ranging between 4%-11%[47-50].

Balloon angioplasty: Native coarctation

Surgical therapy was the only treatment option for coarctation until 1982, when the use of balloon angioplasty was described by Lock *et al*[51]. Several studies since then have shown balloon angioplasty to be a relatively effective acute intervention for native

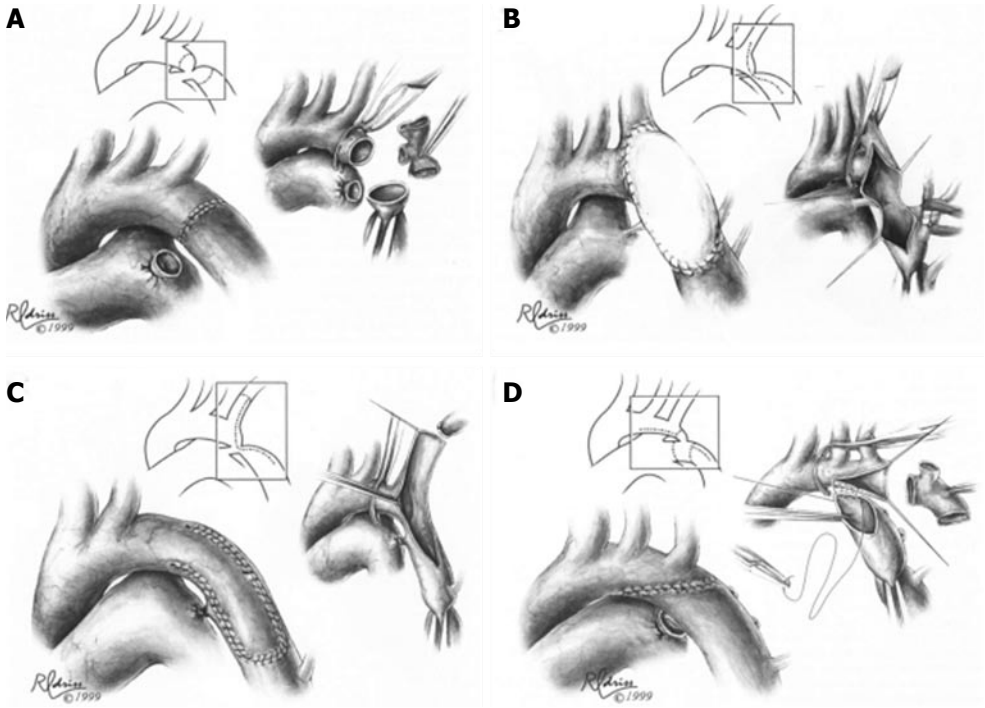

Figure 3 Surgical techniques in coarctation repair. A: Resection and simple end-to-end anastomosis. The coarctation is resected, and an end-to-end, circumferential anastomosis is created; B: Patch aortoplasty. An incision is extended across the coarctation, and a patch is sutured in place to enlarge the stenotic region; C: Subclavian flap aortoplasty. The left subclavian artery is ligated and divided. A longitudinal incision is extended from the proximal left subclavian artery beyond the area of coarctation, and the proximal left subclavian stump is folded down to enlarge the area of coarctation; D: Resection with extended end-to-end anastomosis. The coarctation is resected using a broad, longitudinal incision, and an oblique anastomosis is constructed between the undersurface of the transverse arch and the descending thoracic aorta. Figures adapted and reprinted with permission from the *Journal of Cardiac Surgery*[30].

coarctation, with rates of recoarctation ranging from 8%-32%[52-54]. In a report from the prospective, multicenter Congenital Cardiovascular Interventional Study Consortium (CCISC), 34 patients undergoing balloon angioplasty for native coarctation had adequate intermediate follow-up data at 18 to 60 mo post-intervention. In these patients, the rate of recoarctation was 15%[55]. A second concern with native coarctation angioplasty is aneurysm formation. Histologic and intravascular ultrasound studies have demonstrated the mechanism of angioplasty involves tearing of the intima and media[56-59]. Although some of these tears may heal, disruption of vascular integrity is believed to contribute to a relatively high incidence of aneurysm formation. This was demonstrated in a single center randomized trial comparing balloon angioplasty *vs* surgical repair of coarctation in older children (ages 3 years to 10 years). In this study with mean follow-up of 14 years after intervention, 35% of the balloon angioplasty patients developed aneurysm, compared to none of the surgical patients[60]. Similarly, the 2014 CCISC observational study showed 24% of patients with native coarctation developed aortic aneurysm at intermediate follow-up after balloon angioplasty[55].

Balloon angioplasty: Recurrent coarctation

In contrast to native coarctation, balloon angioplasty is often the preferred intervention for recurrent coarctation in children[27]. Acute success rates for this procedure range from 80%-93%[61]. Reported rates of aortic wall injury are low (1%-2%), and the longer term risk of aneurysm is believed to be ameliorated by scar tissue at the site of the recoarctation, which limits the degree of vascular disruption. However recoarctation rates remain a concern, with a broad range between 6%-53% described[62,63].

Likely the most fragile patient population to develop recurrent coarctation is children with hypoplastic left heart syndrome or other single right ventricle lesions. These patients are at risk for significant morbidity and mortality with recoarctation due to exacerbation of atrioventricular valve regurgitation and ventricular dysfunction[64]. The Pediatric Heart Network Single Ventricle Reconstruction trial was a large, multicenter, prospective study examining the outcome of infants with single right ventricle lesions after randomization to either right ventricle-pulmonary artery shunt or a modified Blalock-Taussig shunt at the time of Norwood procedure[65]. The incidence and timing of intervention for recoarctation in the first 12 mo after randomization was assessed, and 97 of 549 patients (18%) underwent intervention for recoarctation, which was most commonly performed at the time of pre-stage II cardiac catheterization by balloon angioplasty. Balloon angioplasty achieved adequate short term results, but 39% of patients required reintervention for recoarctation, compared to 5% of the patients who underwent surgical reintervention. Reassuringly,

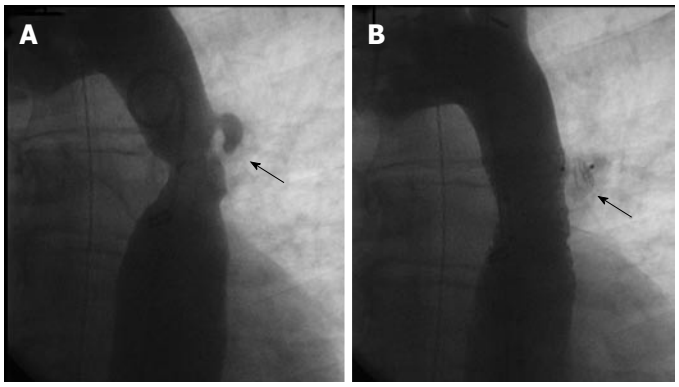

Figure 4 Endovascular stent placement for coarctation. A: Angiogram (LAO 30°, caudal 30°) demonstrating a discrete coarctation and intercostal aneurysm (arrow) in a 45-year-old male; B: Angiogram in the same projection after endovascular bare metal stent placement showing no significant residual stenosis. The intercostal aneurysm was successfully occluded with an Amplatzer Vascular Plug II (arrow).

with catheter or surgical intervention, the presence of recoarctation did not affect survival rates in this tenuous patient population[64].

Endovascular stent placement

In 1991, the use of endovascular stents for the treatment of coarctation was first reported[66], adding another dimension to the utility of transcatheter treatment for coarctation. Endovascular stents are inserted using balloon catheters but do not require overdilation of the vessel wall. Stents also offer structural support, thereby decreasing the rates of aortic wall injury and restenosis observed with balloon angioplasty alone[5] (Figure 4). Several studies have assessed the utility of endovascular stent placement in the treatment of coarctation[67]. Retrospective analysis of acute procedural data from 17 institutions from 1989 to 2005 showed successful stent placement without a significant residual gradient or serious complication in 553 of 565 (97.9%) patients. The overall complication rate was 14.3%, with aortic wall complications (aneurysm, intimal tear, or dissection) contributing 3.9%[68]. In a subsequent study, acute and long-term data regarding endovascular stent placement for coarctation were obtained from the CCISC. Here, the acute results of stent placement were successful without a significant blood pressure gradient or need for reintervention in 249/260 (96%) of cases. During follow-up spanning between 3 to 60 mo, recoarctation was seen in 20% of the 164 patients with follow-up imaging, and 4% of patients required unplanned repeat interventions. Aortic wall complications consisting of dissection or aneurysm were seen in 1% of the 302 total cases[67].

In a multicenter observational study comparing the outcomes of surgical, balloon angioplasty alone, and endovascular stent placement for coarctation using the CCISC registry, patients undergoing stent placement had significantly lower complication rates compared to balloon angioplasty or surgical patients. There was no significant difference among treatment groups for unplanned reintervention rates (4%-7%) at a mean of 1.7 years of follow-up, but those who underwent stent placement were more likely to undergo planned reintervention. Aortic wall injury was more likely to occur in patients who underwent balloon angioplasty alone, at a rate of 9.8% compared to none in the endovascular stent group[69]. In an attempt to more rigorously assess the effectiveness and safety of endovascular stent placement *vs* surgical repair, a Cochrane review was attempted in 2012, but it was determined that there was insufficient data available to identify the best treatment modality[5].

The Coarctation of the Aorta Stent Trial (COAST) has been an influential prospective study examining the effectiveness and safety of endovascular stent placement in children and adults with coarctation. Currently, no endovascular stent has been granted FDA approval for use in the aorta, and initially biliary stents were used off-label for treatment of coarctation. NuMED (Hopkinton, NY) created the Cheatham Platinum (CP) stent for specific use in the aorta, and in 2007, the COAST trial was designed as a prospective, multicenter, single-arm clinical study to assess the safety and efficacy of this stent for the treatment of coarctation[6].

Results for up to two years from CP stent placement are currently available from the COAST trial. CP stent implantation was attempted in 105 patients ranging from 8 to 52 years of age. All but one implantation was successfully completed, with no significant adverse events acutely. No patient had a significant gradient between the ascending and descending aorta after stent placement, and 99% had a gradient < 20 mmHg at one month. There was 89% follow-up at one year after stent placement and 86% follow-up at two years. At two years, 90% of patients maintained a blood pressure gradient < 20 mmHg between the upper and lower extremities. To date, 23 fractured stents have been identified, though none have led to decreased stent integrity, stent migration, aortic wall injury, or hemodynamic obstruction. Aortic aneurysm was diagnosed in 6 patients, one of which resolved without intervention. No patient has required surgical reintervention, and a total of 19 patients have undergone repeat transcatheter intervention, either due to aortic wall injury or dilation of the initial stent[70].

Thus far, the CP stent is felt to be a safe and effective treatment option for coarctation in older children and adults with native or recurrent coarctation. Stent fracture is common but thus far has been clinically insignificant. Reintervention is also common, either due

to planned dilation of smaller stents or aortic wall injury. Follow-up for the COAST trial is planned for up to 60 mo after stent placement and will provide further insight regarding these issues[6].

The use of endovascular stents in small children remains controversial due to the challenges in accommodating for significant somatic growth and the requirement for relatively larger sheath sizes. The ideal stent would be deployed through a small sheath but retain the ability to be dilated to an adult vessel diameter. This technology does not yet exist and still would require multiple interventions for stent dilation throughout a patient's lifetime. Additionally, neonates often have transverse arch hypoplasia, which does not easily lend itself to endovascular stent placement. Some small, single center studies have had positive short-term results with endovascular stent placement in young children, but further follow-up and investigation is needed[71,72].

Covered endovascular stents represent the latest transcatheter innovation and were first used for the treatment of coarctation in 1999. The fabric within the stent provides additional structural support, creates a protective barrier at the site of stent placement, and can help decrease shear stress. All of these factors theoretically reduce the risk of acute vascular trauma as well as longer term aneurysm formation. When aortic aneurysm or stent fracture occurs with bare stent placement, covered stents are often used as a rescue therapy. Covered stents may also be the initial transcatheter intervention of choice, especially in the setting of complex anatomy of the coarctation or in older patients with more friable and calcified aortic wall tissue. However, covered stents require larger sheath sizes, which limits their use in small children. Additionally, care must be taken to avoid stent occlusion of significant aortic branches, including paraspinal branches off of the descending aorta, which can be difficult to identify[73].

Initial smaller studies examining the use of covered stent placement in aortic coarctation were promising, with no reported aneurysms[73,74]. More recently, a randomized clinical trial was performed comparing bare CP stent *vs* covered CP stent placement in 120 patients. Both groups had no acute procedural complications, and at an average follow-up of 31.1 mo, the bare CP stent group had a statistically nonsignificant increase in the rate of recoarctation (6.7% *vs* 0%) and a nonsignificant lower incidence of pseudoaneurysm (0% *vs* 3.3%), compared to the covered CP stent cohort. The two cases of pseudoaneurysm in the covered stent group occurred at the proximal end of the stent, and both were successfully treated with a second covered stent with no further complications[75]. Further investigation into the safety and efficacy of covered stents is on-going with the COAST Ⅱ trial. This trial was initiated in 2010, hoping to provide information that will support FDA premarket approval of the covered CP stent in preventing aortic wall injury in high risk patients with coarctation as well as treatment of existing aortic wall injury related to complications from previous interventions for coarctation. Results are expected in the near future, but at this time, covered CP stents are not available for the treatment of coarctation in the United States outside of use in the COAST Ⅱ trial[70].

MANAGEMENT ALGORITHM

With many different options, deciding on the optimal treatment strategy for coarctation can be complicated, and there is no comprehensive evidence-based standard of care or algorithm. Guidelines from the American College of Cardiology and the American Heart Association provide some insight, but the level of evidence supporting these recommendations is suboptimal (Level B or C for all recommendations)[24,27]. In general, management is dictated by the age at presentation, complexity of the coarctation, and whether or not the coarctation represents a native *vs* recurrent obstruction. For the infant or young child presenting with native coarctation, most centers prefer surgical repair due to the long-term risk of aneurysm after balloon angioplasty, the need for redilation with stent placement, and the limitations imposed by small arteries unable to accommodate larger sheath sizes[60]. However, balloon angioplasty can be considered as a palliative strategy to stabilize neonates presenting in extremis and considered too sick for immediate surgical intervention[27]. Surgical repair may also be more appropriate in patients with complex coarctation anatomy, including those with transverse arch obstruction, tortuous segments of recoarctation, distortion of adjacent arterial branches, or when repair of associated cardiac defects is required[24].

In the older child, adolescent, or adult presenting with a simple, juxtaductal, native coarctation, stent placement is considered a reasonable approach, offering a less invasive alternative to surgical intervention and good long-term outcomes[24,27,76]. Only stents expandable to an adult size should be used, in an effort to avoid later surgical intervention[27].

For recurrent coarctation in the younger child, it is reasonable to consider initial balloon angioplasty, as aneurysm is less of a long-term concern than with native coarctation[27]. Balloon angioplasty is variably successful, and surgical reintervention may be required when there is incomplete relief of obstruction[55]. Stent placement can also be considered for recoarctation in older children and adolescents when the stent can be dilated to near adult size, thus avoiding the need for multiple redilations[27].

PATIENT FOLLOW-UP

Patients with repaired or unrepaired coarctation must be followed by a cardiologist throughout their lifetime. For those who have undergone repair, this follow-up should be at least annually, with specific attention paid to baseline or exercise-induced hypertension[24].

Hypertension is endemic in patients with aortic coarctation, even if no residual coarctation exists, and it must be appropriately treated[77,78]. The etiology of such high rates of baseline and exercise-induced hypertension remains unclear but may be due to any combination of underlying arteriopathy, decreased aortic wall compliance, abnormal streaming of blood flow, or renal abnormalities[77].

Additionally, imaging of the repaired coarctation should be performed at least every 5 years, or sooner based on original anatomy and symptoms, to assess the coarctation repair site for complications like aortic aneurysm or recurrent stenosis[24]. For repaired patients with a normal upper to lower extremity blood pressure gradient, normotension at rest and with exercise, and no evidence of aneurysm or associated heart defects, exercise is encouraged, and only activities with a large static component should be avoided[79]. Finally, per the 2007 American Heart Association guidelines, endocarditis prophylaxis is not routinely recommended beyond the first six months after surgical or transcatheter intervention, barring a previous history of infectious endocarditis[80].

CONCLUSION

In the seventy years since the first description of surgical intervention for aortic coarctation by Crafoord, tremendous progress has been made in the treatment and outcomes for these patients. Modifications of various surgical techniques have led to low mortality and morbidity rates, even in the smallest patients. Transcatheter balloon angioplasty and subsequently endovascular stent placement have expanded treatment options and provided less invasive approaches for repair in some patients. Still, both surgical and transcatheter approaches retain risks for adverse events and subsequent patient morbidity. As examined in this review, much effort has been spent investigating the intervention which yields the best patient outcomes, but further long-term data assessment is needed. The aortic coarctation patient population is a fascinating and heterogeneous one, and considering an individual patient's clinical presentation, anatomy, size, and age will most certainly continue to heavily influence treatment approach.

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Neoatherosclerosis: Coronary stents seal atherosclerotic lesions but result in making a new problem of atherosclerosis

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Abstract

Chronic inflammation of the native vessel wall with infiltration of lipid-laden foamy macrophages through impaired endothelium results in atherosclerosis. Percutaneous coronary intervention, including metallic stent implantation, is now widely utilized for the treatment of atherosclerotic lesions of the coronary artery. Bare-metal stents and the subsequently developed drug-eluting stents seal the atherosclerosis and resolve lumen stenosis or obstruction of the epicardial coronary artery and myocardial ischemia. After stent implantation, neointima proliferates within the stented segment. Chronic inflammation caused by a foreign body reaction to the implanted stent and subsequent neovascularization, which is characterized by the continuous recruitment of macrophages into the vessel, result in the transformation of the usual neointima into an atheromatous neointima. Neointima with an atherosclerotic appearance, such as that caused by thin-cap fibroatheromas, is now recognized as neoatherosclerosis, which can sometimes cause in-stent restenosis and acute thrombotic occlusion originating from the stent segment following disruption of the atheroma. Neoatherosclerosis is emerging as a new coronary stent-associated problem that has not yet been resolved. In this review article, we will discuss possible mechanisms, clinical challenges, and the future outlook of neoatherosclerosis.

Key words: Neoatherosclerosis; Percutaneous coronary intervention; Drug-eluting stent; Atherosclerosis

Core tip: Percutaneous coronary intervention, including metallic stent implantation, causes chronic inflammation of the coronary artery and neovascularization, which involves the continuous recruitment of macrophages

into the vessel. The phenomenon of stent neointima transformation from normal neointima to atherosclerotic lesions is now recognized as neoatherosclerosis, which causes in-stent restenosis and acute thrombotic occlusion. Neoatherosclerosis is now emerging as a new atherosclerosis-related problem that has not yet been solved. In this review, we will discuss possible mechanisms, clinical challenges, and the future outlook of neoatherosclerosis.

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INTRODUCTION

Atherosclerosis is caused by chronic inflammation at the site of damaged vascular endothelium and lipid-laden foamy macrophages derived from infiltration of monocytes into the arterial wall, and it results in coronary stenosis and thrombotic obstruction after atherosclerotic plaque disruption[1]. Percutaneous coronary intervention (PCI) is now widely accepted worldwide for the treatment of coronary artery disease due to atherosclerosis. In 1977, PCI by plain old balloon angioplasty (POBA) was performed for the first time by Gruntzig[2] to treat angina pectoris. In 1986, Sigwart *et al*[3] implanted a self-expandable stainless-steel stent to prevent acute occlusion and chronic restenosis caused by intimal dissection after balloon dilatation and elastic recoil of the coronary artery, respectively. In 1994, randomized clinical trials showed that bare-metal stent (BMS) implantation was superior to POBA with regard to short-term procedural success and long-term arterial patency[4,5]. However, in-stent restenosis (ISR) occurred in approximately 20%-30% of cases, causing the long-term failure of PCI that was bestowed the title of the "Achilles' heel" of PCI. According to pathological investigations, the primary pathogenesis of ISR is neointimal hyperplasia due to migration and proliferation of vascular smooth muscle cells (VSMCs) from the media. In the 2000s, the drug-eluting stent (DES) was introduced to prevent inhibition of neointimal hyperplasia and ISR of the BMS. Application of the DES to coronary artery disease has dramatically reduced the incidence of ISR in the clinical setting[6,7]. The so-called "first-generation DESs" were composed of a stainless steel stent platform and was coated with durable polymer-releasing anti-proliferative drugs. Although the first-generation DES, the sirolimus-eluting stent (SES) and paclitaxel-eluting stent (PES), decreased ISR, they are associated with a steady increase in very late stent thrombosis (VLST; > 1 year post-stent implantation) due to delayed re-endothelialization or a hypersensitivity reaction to the stent polymer[8]. Therefore, the next-generation DES were developed with new technology; specifically, the main feature of these DES was the inclusion of a biocompatible or biodegradable polymer to reduce vessel inflammation and a thin stent strut for normalization of rheological flow around the strut to diminish thrombogenicity. The second-generation DES, namely, zotarolimus-eluting stents, everolimus-eluting stents, and biodegradable polymer-coated biolimus-eluting stents, showed reduced incidences of VLST[9-11]. Nevertheless, the placement of second-generation DES was found to cause acute coronary syndrome originating from the stent segment[12].

Although metallic coronary stents, BMS, and DES resolve the problem of coronary lumen stenosis or occlusion in the acute phase after their implantation, they potentially cause new problems in the chronic phase, such as late ISR and VLST. It is now understood that some of these phenomena arise from the new pathogenic concept of "neoatherosclerosis", which is defined as the phenomenon of the transformation of stent neointima from normal neointima to an atherosclerotic lesion. We will review basic and clinical studies concerning topical problems of neoatherosclerosis that are associated with coronary stenting.

VASCULAR RESPONSE AFTER PCI

Mechanical injury of the vessel wall cannot be avoided by PCI, such as balloon dilatation and stent implantation. PCI procedures cause denudation of the coronary artery endothelium, resulting in exposure of the myointima, fissures in the atheromatous plaque, and overstretching of the circumferential vessel layers[13]. The endothelium regulates vascular tone, controls inflammation, maintains lipid and tissue-fluid homeostasis, and possesses antithrombotic properties[14]. The vascular endothelium protects against thrombus formation and blood coagulation through its production of nitric oxide, prostacyclin, tissue plasminogen activator, heparin-like molecules, tissue-factor pathway inhibitor, thrombomodulin, and other molecules[14]. Perturbation of the normal endothelium function by PCI is related to the pathogenesis of atherosclerosis and results in accelerated formation of atheromatous lesions[13]. Incomplete re-endothelialization in the coronary vascular wall induces thrombotic events after stent implantation in the early, late (> 1 mo, ⩽ 1 year post-stenting), or very late phase[15]. Denudation of the endothelium after PCI causes VSMCs to be exposed to blood flow directly, which modulates the proliferation and viability of the VSMCs[16,17]. Although BMS implantation is superior to POBA with respect to procedural success and long-term target lesion patency[4,5], dysfunction of the regenerated endothelium is more pronounced after stent implantation than after ballooning[18]. Any interventional procedure, even POBA, causes denudation of the endothelium and is associated with the same risk of very late thrombosis as BMS[19],

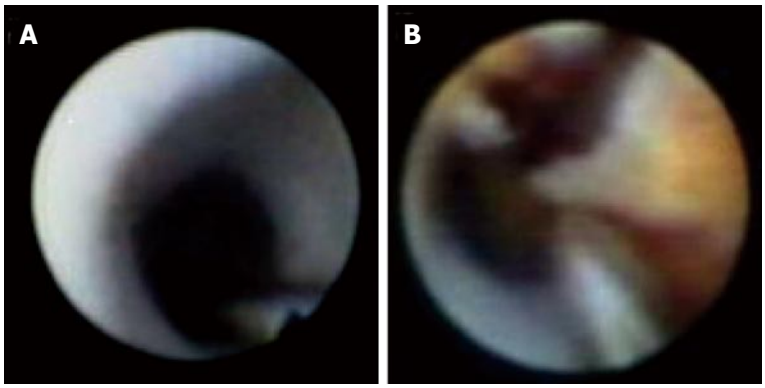

Figure 1 Coronary angioscopy images of ordinary neointima and neoatherosclerosis. A: Coronary angioscopy reveals ordinary neointima as a white and smooth membranous structure; B: The neointima appears as atheromatous and yellow, occasionally disrupted with thrombus formation.

and the regenerated endothelial cells are not structurally and functionally normal[20]. Stent implantation into the vessel leads to perturbations in blood flow, and flow reversal and disturbed shear stress around the stent strut promote vascular inflammation and injury[21,22]. The thickness of the stent struts determines the size of blood flow recirculation, which is associated with thrombogenicity within the stent segment[23]. Compared with the BMS, the first-generation DES, namely, SES and PES, which incorporated anti-proliferative drugs and durable polymers, were associated with dramatic reductions in the proliferation of the neointimal hyperplasia and ISR[6,7]. However, an increased risk of VLST was observed for these first-generation DES compared with BMS[24,25]. Autopsy studies showed that a lack of re-endothelialization with > 30% of the stent strut uncovered per cross-section was a strong predictor of late stent thrombosis (LST) and VLST[26]. Moreover, the polymer-induced type Ⅳ hypersensitivity reaction is one of the mechanisms of LST or VLST associated with SES. In contrast, excessive fibrin deposition and consequent stent mal-apposition (detachment of the stent struts from the coronary arterial wall) are associated with thromboses in the case of PES[26,27]. The new stent technology of second-generation DES involved minimization of vessel injury and normalization of micro-rheology around the stent strut, thinner struts, and the use of a biocompatible or biodegradable polymer[28]. The pathophysiology of LST and VLST is multifactorial, as mentioned above. However, other mechanisms are possibly linked to stent thrombosis. LST or VLST after placement of BMS and DES is an unresolved problem, and the new pathological concept of neoatherosclerosis is another mechanism of stent failure. It is understood that the pathophysiology and development of neoatherosclerosis differ between BMS and DES.

NEOATHEROSCLEROSIS IN BMS

Neointimal hyperplasia associated with BMS was considered to be stable, with peaks at 6 mo and 1 year after stenting during a 3-year follow-up[29]. However, extended follow-up of BMS showed that late luminal re-narrowing beyond 4 years was common[30]. Moreover, one-third of patients implanted with BMS who had restenosis presented with acute myocardial infarction or unstable angina 5 years after the index procedure that was not clinically benign[31]. Some reports have documented the occurrence of ACS due to the disruption of neoatherosclerosis after BMS implantation[32].

The findings of a histopathological study suggested the mechanism of the catastrophic late events after BMS implantation[33]. This study, which assessed nineteen stented coronary arteries obtained from 19 patients autopsied after non-cardiac death 2-7 years post-BMS implantation, showed that after more than 4 years of stenting, there was prominent infiltration of lipid-laden macrophages with strong collagen-degrading matrix metalloproteinase expressing ruptured and vulnerable plaque accompanied by thrombi around the struts evoked by remarkable foreign-body inflammation[33]. Regenerated endothelium after PCI forms poor endothelial cell junctions and expresses reduced numbers of antithrombotic molecules and nitric oxide, which contributes to neoatherosclerosis[15,18,34]. Neoatherosclerosis is now recognized as chronic inflammation in the vessel wall caused by the stent itself and subsequent neo-vessel formation, which causes continuous recruitment of macrophages and forms unstable lesions called thin-cap fibroatheroma (TCFA) that contribute to disruption of neointima and thrombus formation, leading to VLST[15].

Serial angioscopic observation at baseline, 6 to 12 mo, and ⩾ 4 years after BMS implantation revealed changes in the smooth white intima characterized by atheromatous yellow plaque with vulnerable features, such as surface disruption and thrombus formation, during the study period (Figure 1)[35]. In addition, the atheromatous transformation was correlated with ISR[35]. Optical coherence tomography (OCT) is a near-infrared light-based imaging modality with high-resolution that can accurately characterize tissue components *in vivo*[36]. Although there are no data regarding the angioscopic findings and histopathologic correlation in intimal tissue, an OCT study showed that the angioscopic yellow neointima likely corresponds to foamy macrophages infiltrating into the fibrous cap and underlying lipid accumulation, as well as that

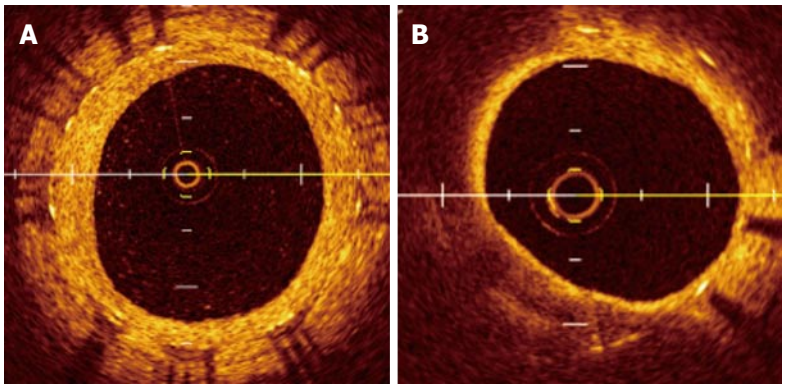

Figure 2 Optical coherence tomography images of common neointima (A) and neoatherosclerosis (B). A: Common neointima is recognized by its high-signal intensity and homogeneous region inside stent struts; B: The neointima has a diffuse border and marked attenuation.

the intensity of yellow likely signifies the thickness of the fibrous cap and amount of necrotic core[37]. OCT observation of BMS segments was performed in the early phase (< 6 mo) and late phase (≥ 5 years) after BMS implantation[38]. The normal neointima proliferated homogeneously, and the lipid-laden intima was not observed in the early phase. In the late phase, the lipid-laden intima was found in 67% of cases (Figure 2)[38]. Additionally, pathological characteristics, such as intimal disruption and thrombus formation, appeared (38% and 52% of cases, respectively). There was a similar incidence of peri-stent neovascularization in the 2 phases. However, the location of neovascularization was different between the two phases. Intra-intima neovascularization was more prevalent in the late phase than the early phase (62% and 0%, respectively; $P < 0.01$) and in segments with lipid-laden intima compared with non-lipidic segments (79% and 29%, respectively; $P = 0.026$)[38]. There are few reports showing that neoatherosclerosis of BMS increases ACS, clinically diagnosed as VLST. Therefore, further careful follow-up of neoatherosclerosis after BMS implantation is needed.

NEOATHEROSCLEROSIS IN DES

Chronic inflammation and insufficient functional endothelialization induce neoatherosclerosis inside both BMS and DES, causing ISR and thrombosis in the late phase[39]. In intravascular ultrasound (IVUS) analyses of VLST, neointimal rupture was observed within the stent segment in 43.5% of the DES and all of the BMS[40]. OCT also indicated that ruptured atherosclerosis and thrombosis in BMS and DES was the most common mechanism of definite VLST presenting as myocardial infarction with ST-segment elevation[41].

Pathological analysis of human coronary arteries with stented segments showed that unstable lesions, such as TCFA or intimal rupture, were associated with shorter implant durations for first-generation DES (1.5 ± 0.4 years) compared with BMS (6.1 ± 1.5 years). These results indicate that neoatherosclerosis in first-generation DES is more frequent and occurs earlier than that in BMS[39]. Pathology of second-generation everolimus-eluting cobalt chromium stents implanted < 3 years showed less uncovered strut area and milder inflammation compared with first-generation DES. However, neoatherosclerotic changes were confirmed even in second-generation DES, and there was no significant difference in neoatherosclerosis between first-generation DES and second-generation DES[42]. Neoatherosclerosis occurs more rapidly in DES than BMS, possibly because the eluted drug prevents endothelial cell proliferation, viability, and migration, which allows infiltration of lipid-laden foamy macrophage into the vessel, thereby accelerating atherosclerotic changes[43-46].

In first-generation DES, angioscopic follow-up of SES at baseline, 6 mo, and 2 years after implantation showed that neointimal growth inside the SES progressed heterogeneously, uncovered struts persisted in 20% of the patients for up to 2 years, and subclinical thrombus formation was not a rare phenomenon[47]. Although uncovered stent struts on angioscopic images do not correspond to incomplete re-endothelialization, uncovered struts may play a role in promoting atherosclerosis. An angioscopic follow-up study demonstrated that the neointima at baseline changed into a lipid-rich atherosclerotic and yellow neointima at 10 mo, with intramural thrombi being more frequently detected on newly formed yellow neointima[48]. Serial angioscopic findings up to 2 years after SES implantation showed that neointimal coverage was completed by 3 to 6 mo in BMS, whereas SES demonstrated the presence of thrombi and yellow plaques as long as 2 years after implantation[49]. The long-term vascular response was evaluated by serial angioscopic follow-up at 2 and 5 years after SES implantation, and incomplete neointimal stent coverage and the prevalence of latent thrombus within the SES segments did not decrease from 2 to 5 years[50].

In-stent neoatherosclerosis was recognized as an important mechanism of DES failure, especially late after implantation, regardless of its generation[51]. OCT was performed on a total of 50 lesions with angiographic in-stent restenosis (30 stable and 20 unstable angina patients, median follow-up time of 32 mo). Patients with unstable angina had a thinner fibrous cap and a higher incidence of TCFA, including intimal rupture and thrombi, than those with stable angina[51]. A direct comparison of the characteristics of neointimal

Table 1 Summary of each type of stent

Stent type	BMS	First-generation DES	Second-generation DES	BRS
Strut thickness	Thick	Thick	Thin	Thick
Incorporated drug	None	Rapamycin derivatives/ paclitaxel	Rapamycin derivatives	None/ rapamaycin derivatives
Polymer	None	Durable	Durable/ biodegradable	None/ biodegradable
Inflammation	Not available Foreign-body inflammatory reaction[33]	Strong	Slightly	Slightly[65]
Onset of neoathero-sclerosis	After 4 yr[39]	SES 70 d[42] PES 120 d[42]	CoCr EES 270 d[42]	Not available

Data modified from Inoue *et al*[33], Nakazawa *et al*[39], Otsuka *et al*[65]. DES: Drug-eluting stent; BRS: Bio-resorbable scaffold; SES: Sirolimus-eluting stent; PES: Paclitaxel-eluting stent.

hyperplasia and its time course between BMS and DES using OCT showed that lipid-rich neoatherosclerosis develops within stent segments earlier (< 9 mo) in DES than in BMS (≥ 48 mo), and the majority of ISR lesions developed lipid-laden neointima in both groups by 48 mo[52]. Morphological analysis of first-generation DES-ISR by OCT revealed that early (< 1 year) ISR showed a speckled pattern; in contrast, very late ISR (> 3 years) exhibited a pattern more similar to that of TCFA[53]. Angiographic and integrated backscatter IVUS analysis of ISR lesions after SES and BMS implantation showed that focal angiographic restenosis was predominantly present in the SES group, whereas diffuse restenosis was more common in the BMS group. The neointimal tissue in SES-related ISR lesions consisted of a significantly larger percentage of lipid tissue and a smaller percentage of fibrous tissue compared with that in BMS-related ISR lesions[54]. Characterization of neointimal tissue approximately 9 mo after DES implantation by OCT revealed that heterogeneous lesion type can be helpful in predicting outcomes regardless of DES generation[55]. Second-generation 40 zotarolimus, 36 everolimus, and 35 biolimus stents were not more protective against neoatherosclerosis compared with the first-generation 65 SES and 36 PES[12]. Table 1 summarizes the characteristics of each type of stent with regard to neoatherosclerosis. Taken together, continuous follow-up is required to clarify clinical events after DES regardless of its generation.

CONFRONTING NEOATHEROSCLEROSIS

There are drugs and mechanical interventions available to treat neoatherosclerosis. In the clinical setting, univariate analysis revealed that smoking and angiotensin-converting enzyme inhibitor or angiotensin Ⅱ inhibitor usage were associated with the presence of neoatherosclerosis[56]. Chronic kidney disease and > 70 mg/dL of low-density cholesterol at OCT follow-up were independent predictors of neoatherosclerosis[12]. Whether interventions addressing these risk factors and aggressive lipid-lowering therapy can improve neoatherosclerosis should be assessed in prospective trials.

Regarding PCI, OCT observation at 9 mo following treatment for DES-ISR using a paclitaxel-coated balloon to avoid repeated stenting showed a heterogeneous pattern in the neointima with speckled structures consistent with macrophage infiltration and a lipid pool consistent with neoatherosclerosis, indicating insufficient treatment of DES-ISR[57]. New stent technologies that accelerate endothelial healing through the use of a thinner stent strut, biodegradable polymer with contraluminal drug coating (Synergy™; Boston Scientific, Ultimaster™; Terumo), or luminal surface coating with CD34 antibody (COMBO™; Orbusneich Medical Technologies) to capture endothelial precursor cells, rendering the stents free from neoatherosclerosis, are expected in future clinical trials[58].

Complete bio-absorption of the vascular scaffold [bio-resorbable scaffolds (BRS)] a few years after implantation, which potentially reduces late adverse events such as VLST provoked by neoatherosclerosis in the stent caused by the permanent presence of a polymer and metallic artificial implant[59,60], can restore endothelial function[61,62]. IVUS analysis of ABSORB BVS revealed a significant plaque media reduction without a significant change in the vessel wall area (plaque regression)[61]. Nevertheless, unresolved problems remain regarding BRS. If overstretched, BRS can lose their radial strength, leading to stent fracture[63]. BRS demonstrated a higher probability of procedural side branch occlusion in small side branches compared with everolimus-eluting metallic stents[64]. Moreover, most of the data on BRS use are derived from relatively small and non-randomized studies with short or mid-term follow-up, and further studies are warranted to determine the real-world efficacy and safety of BRS[59]. The features of each stent are summarized in Table 1.

CONCLUSION

Cardiologists have been combatting coronary atherosclerosis through stent implantation and preventive medicine. Neoatherosclerosis is now emerging as a new problem that has not yet been solved. Although coronary stenting resolves the problem of atherosclerotic lesion-induced myocardial ischemia, it results in a new problem of neoatherosclerosis. New stent technology or drugs may solve this problem in the future.

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MINIREVIEWS

Epigenetic regulation in cardiac fibrosis

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Abstract

Cardiac fibrosis represents an adoptive response in the heart exposed to various stress cues. While resolution of the fibrogenic response heralds normalization of heart function, persistent fibrogenesis is usually associated with progressive loss of heart function and eventually heart failure. Cardiac fibrosis is regulated by a myriad of factors that converge on the transcription of genes encoding extracellular matrix proteins, a process the epigenetic machinery plays a pivotal role. In this mini-review, we summarize recent advances regarding the epigenetic regulation of cardiac fibrosis focusing on the role of histone and DNA modifications and non-coding RNAs.

Key words: Cardiac fibrosis; Epigenetics; Endothelial cell; Fibroblast

Core tip: Cardiac fibrosis contributes to the increased accidence of sudden cardiac death, heart failure and arrhythmia. The molecular mechanisms underlying cardiac fibrosis remain obscure. Seminal studies have revealed complex pathways associated with cardiac fibrosis. How histone/DNA modifying enzymes and microRNAs fine-tune these events are actively pursued by investigators. This review provides an overview on recent advances regarding the epigenetic regulation of fibrosis.

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INTRODUCTION

The term "epigenetics" was coined in 1953 by Waddington CH and the following decades have witnessed great progress achieved in this field[1]. By consensus epigenetics is defined as stably inheritable phenotypes stemmed from changes of chromatin

without alterations in primary DNA sequences[2]. The nucleosome, the fundamental unit of eukaryotic chromatin, is composed of an octamer of four core histones (H2A, H2B, H3, H4) surrounding 147 bp of DNA. The amino-terminal tails of histones serve as a platform for diverse posttranslational modifications including methylation[3], acetylation[4,5], ubiquitination[6,7], O-linked N-acetylglucosamine (GlcNAc)[6], phosphorylation[5] and sumoylation[8] on specific residues catalyzed by histone-modifying enzymes. These covalent modifications are dynamic[7] and modulate gene regulation in a combinatorial manner upon exposure to different stimulus[5,9,10]. Histone modifications manipulate gene activation/repression by influencing the accessibility of transcriptional factors to chromatin or by recruiting and/or occluding of non-histone proteins, mostly co-factors, in contrast to promoter CpG island methylation for gene silencing[11]. Proper function of the epigenetic machinery, or lack thereof, is implicated in mammalian development[12], carcinogenesis[4] and cardiovascular diseases (CVDs).

Fibrosis or scarring in different organs, including the lungs[13], the kidneys[14], the liver[15], and the heart, is characterized by deposition of extracellular matrix (ECM) components, such as collagens, laminins and fibronectin, caused by diverse insults. Fibrosis can be deemed as erroneous ECM "turnover", *i.e.*, imbalance between ECM production (increased) and ECM degradation (reduced). Collagen is the most abundant component of the ECM in the heart including five types (types Ⅰ, Ⅲ, Ⅳ, Ⅴ and Ⅵ) identified in the myocardium. Among these, types Ⅳ and Ⅴ collagens are components of the basement membrane, while types Ⅰ and Ⅲ collagen are the main constituents of the ECM[16,17]. A number of different cell types in the heart are responsible for collagen synthesis: All cardiac collagen types are produced by fibroblasts, whereas endothelial cells synthesize all types except type Ⅵ. Degradation of collagen is mediated by both intracellular and extracellular pathways, the latter involving matrix metalloproteinase (MMPs) and tissue inhibitors of MMPs (TIMPs)[18].

Fibrosis is an evolutionarily conserved process that serves to facilitate host defense and wound healing. Deregulated fibrosis, however, is invariably associated with loss of organ function. For instance, cardiac fibrosis is correlated with elevated mortality in dilated cardiomyopathy[19], which is the most common cardiomyopathy globally and directly correlates with sudden cardiac death, heart failure and arrhythmia[20-22]. Despite numerous progress made in identifying molecular mechanisms and/or factors that contribute to hypertrophy over the past decades, the mechanistic underpinnings of cardiac fibrosis is poorly understood. Although an extensive body of evidence suggests that cardiac fibroblast may participate in the pathogenesis of cardiac fibrosis, other cell types involved remain to be determined, especially endothelial cells and macrophages[23-26]. This review summarizes our current understanding of the involvement of epigenetic machinery in cardiac fibrosis and attempts to identify some of the previously unaddressed questions that require further investigation. We only briefly discuss the pathways and transcriptional factors involved in cardiac hypertrophy because models used to study cardiac hypertrophy and fibrosis often overlap and excellent reviews on cardiac hypotrophy are available elsewhere[27,28].

SIGNALING CASCADE IN CARDIAC FIBROSIS

Cardiac fibrosis usually appears in patients with hypertrophic cardiomyopathy, hypertension and/or diabetes mellitus, suggesting that cardiac fibrosis may be secondary to these conditions[29-33]. Myocardial infarction (MI), aging, and mutation in cardiac fatal genes such as Mhy7, Troponin T and BNP can also trigger cardiac fibrosis[34-38]. Studies in animal models have revealed a convoluted network of signaling cascades and transcriptional factors. A body of evidence suggests that the calcineurin–nuclear factor of activated T cells (NFAT) circuit, the β-adrenergic–receptor signaling pathway, and the IGF-Akt signaling pathway all contribute to cardiac fibrosis by modulating the activities of such transcription factors as serum response factor, myocyte enhancer factor (MEF), and kruppel-like factor during development and in response to pathophysiological stimuli[29,30,39-43]. Meanwhile, evidence from different groups shows that extracellular-regulated kinases Erk1 and Erk2 (Erk1/2), downstream effectors of the mitogen-activated protein kinase cascades, play a prominent role in cardiac hypertrophy and fibrosis. ERK activation mediated by auto-phosphorylation at Thr188 enhances TAC-induced cardiac hypertrophy and fibrosis[26,39,40].

TGF-β is believed to play the most central role in cardiac fibrosis based on the fact that TGF-β is activated in different models of cardiac fibrosis, which in turn facilitates the synthesis of ECM proteins and contributes to endothelial-mesenchymal transition (EndMT)[33,44-47]. Meanwhile, TGF-β represses ECM degradation by suppressing the expression of MMPs[48] and by augmenting the levels of protease inhibitors such as plasminogen activator inhibitors and TIMPs[44,49]. TGF-β drives fibrotic process by binding to the heterodimeric membrane receptor, which results in phosphorylation and subsequently nuclear translocation of SMAD family of transcription factors[50]. Thus, inhibition of the specific cellular receptors, kinases and other mediators involved in the activation of TGF-β pathway may provide effective therapeutic targets for cardiac fibrosis.

HISTONE MODIFYING ENZYMES IN CARDIAC FIBROSIS

Numerous enzymes that catalyze specific residues

of core histones have been implicated in cardiac hypertrophy and fibrosis. For instance, p300, a histone acetyltransferase, accelerates left ventricular remodeling after MI[9,10]. Inactivation of Ezh2, the catalytic subunit of the Polycomb repressor complex 2 responsible for histone H3K27 methylation (H3K27me3), induces cardiac fibrosis[3,51]. These histone modifying enzymes influence cardiac fibrosis *via* the interaction with sequence-specific transcriptional factors to manipulate fibrosis-associated gene activation or repression. For example, p300 and GATA-4 synergistically activate GATA-4-dependent transcription of the *ET-1* and *ANF* genes[10] and Ezh2-mediated H3K27me3 on the promoter zones directly represses fetal gene expression[51].

Trivedi *et al*[52] show that the mice deficient in Hdac2, a class Ⅰ histone deacetylase (HDAC), are resistant to isoproterenol-induced cardiac hypertrophy and fibrosis. Mechanistically, Hdac2 deletion leads to the derepression of inositol polyphosphate-5-phosphatase f (Inpp5f). Consequently, glycogen synthase kinase 3β (GSK3β) is constitutively activation thereby causing the inactivation of cardiac fetal genes[52]. However, the authors did not address whether fibrosis is independent of GSK3β or GSK3β is responsible for both cardiac hypertrophy and fibrosis. Olson and colleagues report that class Ⅱ HDACs interact with MEF2 and repress its activity, acting as signal-responsive repressors of transcription of cardiac fetal genes[53]. This observation is verified by several complementary studies. First, inhibition of class Ⅰ and Ⅱ HDACs by trichostatin A (TSA) protects the mammalian heart from pressure overload-induced cardiac fibrosis and attenuates hypertrophy-associated protein expression[51]. Zhang *et al*[53] show that calmodulin binding transcription activator 2 (CAMTA2), transcriptional coactivator for Nkx2-5, is repressed by an interaction with class II HDAC. Activation of PKC/PKD signaling leads to phosphorylation of class Ⅱ HDACs, creating docking sites for 14-3-3 proteins to exclude HDACs from the nucleus and relieving the inhibition of CAMTA2, which proceeds to activate cardiac hypertrophy and fibrosis[54]. Recently, our laboratory has identified a histone H3K4 trimethylation-dependent pathway that contributes to cardiac fibrosis. Specifically, we have discovered that SET1, an H3K4me3 modifying enzyme, induces the transcription of endothelin (ET-1) in vascular endothelial cells. Once released into the circulation, ET-1 then serves as an angiocrine factor to induce cardiac fibrosis in response to chronic angiotensin Ⅱ infusion or mechanic stretch[55].

Histone modifying enzymes can communicate with each other or other branches of the epigenetic machinery to modulate cardiac fibrosis. A study by Eom *et al*[56] further highlights the role of crosstalk between HDACs and HATs and post-translational modifications of these proteins in cardiac hypertrophy and fibrosis. These authors propose that the acetylation status of HDAC2 and by extension its activity in regulating cardiac fibrosis is controlled by p300/CBP-associated factor and HDAC5[56]. Weng *et al*[57] have found that the H3K4 methyltransferase complex (COMPASS) can forge a dialogue with chromatin remodeling proteins BRG1 and BRM to transactivate ET-1, which in turn invokes a pro-fibrogenic response in the heart; depletion of either COMPASS or BRG1/BRM alleviates Ang Ⅱ-induced cardiac fibrosis in mice[57].

Overall, although there is abundant evidence supporting a role for histone modifying enzymes in cardiac fibrosis, the dataset appears to be fragmental with many outstanding issues awaiting resolution. For instance, what is the genome-wide role for any given histone modifying enzyme in cardiac fibrosis? How are different histone modifying enzymes are recruited to the chromatin? Is there a unique histone signature that defines cardiac fibrosis? How to differentiate histone modifications and non-histone protein modifications? These lingering questions will have to be addressed in future studies.

MICRORNA INVOLVED IN CARDIAC FIBROSIS

MicroRNAs (miRNAs), usually 20-30 nucleotide in length, are one major form of small non-coding regulatory RNAs that also include short interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs)[58]. In general, miRNAs act to silence gene expression by targeting specific mRNA at the posttranscriptional level. MiRNA expression profiles are widely used in cancer classification, diagnosis, therapy and prognosis[59], but mounting evidence shows that circulatory miRNAs, such as miR-29a and miR-21, may also be used as a diagnostic marker for cardiac fibrosis[60,61]. Numerous studies aimed to investigate the potential impact of miRNAs in the heart have demonstrated a key role for miRNAs in cardiac fibrosis in response to multiple injury stimulus.

It has been demonstrated that mice depleted of miR-212/132[62], miR-25[61,63], or miR-29[61] are protected from pressure-overload-induced cardiac fibrosis while miR-101[64] and miR-24[65] regulate fibrosis after MI. Knockdown of miR-133a[66] and cardiac-specific overexpression of miR-195 induces spontaneous cardiac hypertrophy and fibrosis. Thum *et al*[26] have shown that miR-21 silencing in fibroblasts decreases ERK-MAP kinase activity and curbs interstitial fibrosis. Follow-up studies have shown several different but not mutually exclusive mechanisms underlying the pro-fibrotic effect of miR-21. For instance, Roy *et al*[67] have found that miR-21 regulates fibroblast MMP-2 *via* targeting phosphatase and tensin homologue (PTEN). Alternatively, miR-21 also partly influences TGF-β-mediated EndMT *via* the PTEN/Akt pathway[68]. Conceivably, miR-21 might elicit a range of different pathways responsible for cardiac fibrosis at multiple levels. Cardiac-specific miR-208, transcribed from the a-myosin heavy chain (*a-MHC*) gene locus, regulates

stress-dependent fibrosis by negatively modulating expression of thyroid hormone receptor associated protein 1[69]. The role of miR-208 in cardiac fibrosis is further supported by the observation that inhibition of miR-208 by antisense oligonucleotide improves cardiac function and attenuates remodeling[70].

Sometimes miRNAs and their targets form feedback (forward or backward) loops to manipulate downstream pathophysiological events. For instance, da Costa Martins *et al*[41] have reported that pressure overload activates the calcineurin/NFAT axis to stimulate the expression of miR-199b. MiR-199b, once transcribed, targets dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a (Dyrk1a), which activates NFAT by phosphorylating and thereby excluding NFAT from the nucleus. Conceivably, reduced levels of Dyrk1a as a result of miR-199b activation will release NFAT from the cytoplasm, which will lead to increased expression of miR-199b[41].

Cardiac- and skeletal muscle–enriched miR-22 regulates cardiomyocyte hypertrophy and cardiac fibrosis in response to stress *via* targeting Sirt1 and Hdac4[71], supporting the possibility that microRNAs could communicate with other epigenetic factors by directly influencing their abundances. Meanwhile, miRNAs could also suppress fibrotic genes transcription. MiR-133 and miR-30 could reduce production of collagens by directly down-regulating connective tissue growth factor (CTGF) through specific binding to its 3′ untranslated region (3′-UTR)[72]. MiR-101a can restrain interstitial fibrosis in post-infarct rats by targeting c-Fos to repress downstream effectors of TGF[64]. Intriguingly, miR-18/19 and miR-34a dampen age-related cardiac remodeling by negatively regulating the CTGF and thrombospondin-1[73] expression and directly targeting protein phosphatase 1 nuclear-targeting subunit[38], respectively.

DNA METHYLATION IN CARDIAC FIBROSIS

Patterns of mammalian DNA methylation vary in time and space. Similar to histone modifications, levels of DNA methylation are dependent on the balance of methyltransferases (DNMTs) and demethylases. In general, DNA methylation modulates gene transcription *via* changing chromatin conformation and/or influencing the interplay between DNA and proteins[74,75]. Based on the structural and functional differences, the enzymes responsible for DNA methylation identified so far include two categories: DNMT1 and DNMT3a/3b. DNMT1 is responsible for maintenance of DNA methylation using hemimethylated DNA strand as substrate[76], while DNMT3a/3b catalyze de novo DNA methylation operating on two un-methylated "clean" DNA strands[77].

A recent investigation by Xu *et al*[78] showed that TGF-β induces aberrant methylation of RASAL1 (a Ras-GTPase) promoter and subsequently down-regulation of RASAL1, resulting in elevated Ras-GTP activity to enhance EndMT and cardiac fibrosis. Mechanistically, this process is associated with ten eleven translocation family enzyme (TET3)-mediated RASAL1 promoter hydroxymethylation (or demethylation) and reversal of EndMT[78]. A recent study indicates that mice with cardiac-specific knockout of DNMT3b, predominantly expressed in the heart, exhibit extensive interstitial fibrosis and myo-sarcomeric disarray[79]. Further exploration suggests that dysregulation of DNA methylation-induced alternatively spliced myh7 transcript may be accountable for these phenotypes, which is similar to the aforementioned effects of miR-208 derived from myh6[60].

Methylation of DNA is not an isolated event but instead forges crosstalk with non-coding RNAs and histone modifications. For instance, Wang *et al*[80] show that lysine demethylase (LSD1) interacts and demethylates DNMT1 to increase DNMT1 stability, indicating that LSD1 may coordinately modulate histone and DNA methylation by acting directly on both histones and Dnmt1. Meanwhile, DNMT3a/b are recruited to tri-methylated H3-K9 positions *via* interacting with heterochromatin protein 1 (HP1a)[81], synergistically silencing transcription at the pericentric satellite repeats[82]. Whether these interactions and/or cooperations function in the heart remain elusive. Dakhlallah *et al*[83] demonstrated that in lung fibroblasts from patients with idiopathic pulmonary fibrosis, there was a negative correlation between increased DNA methylation-induced repression of miR-17-92 cluster and DNMT1 expression. Several miRNAs from the miR-17-92 cluster, most prominently miR-19b, directly regulated DNMT1 expression by targeting seed sequences in the 3-UTR in a negative feedback loop. To further study whether this system function *in vivo*, Dakhlallah *et al*[83] use a classical murine model of pulmonary fibrosis. After the initiation of fibrosis, treatment with 5-aza-2-deoxycytidine in bleomycin-challenged mice alleviated lung fibrosis by decreasing *DNMT-1* gene expression while restoring miR-17-92 cluster expression[83]. These results are consistent with findings from Bechtel *et al*[84] that long-term TGFβ1 exposure induced RASAL1 hypermethylation depends on DNMT1, which is intimately linked to the perpetuation of kidney fibroblast activation and renal fibrosis. More importantly, 5-aza-2-deoxycytidine attenuated folic acid-evoked renal fibrosis by reducing DNMT1–induced methylation of RASAL1[84]. In the heart, whether miRNAs regulate DMNTs in a similar fashion needs to be addressed in the future study.

FUTURE DIRECTIONS IN CARDIAC FIBROSIS

The past two decades have seen a sea of groundbreaking discoveries in epigenetics fueling the research on CVD[85-88]. This mini-review only provides a snapshot

of how research on cardiac fibrosis has benefitted from epigenetic theories and tools. Many of the factors discussed here are enzymes, the activities of which can be manipulated *via* small-molecule compounds for therapeutic interventions. For instance, HDAC inhibitors have been successfully used to treat certain forms of cancer in the clinic[89,90]. The recent elucidation of the human functional genome has re-affirmed the notion that epigenetic regulation is the bedrock of human diseases[91]. In perspective, continued effort in investigating the epigenetic mechanisms underlying cardiac fibrosis will eventually bring cure to this debilitating pathology.

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ORIGINAL ARTICLE

Retrospective Study

Short and long term outcomes of 200 patients supported by continuous-flow left ventricular assist devices

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Abstract

AIM: To study the institutional experience over 8 years with 200 continuous-flow (CF) - left ventricular assist devices (LVAD).

METHODS: We evaluated our institution's LVAD database and analyzed all patients who received a CF LVAD as a bridge to transplant (BTT) or destination therapy from March 2006 until June 2014. We identified 200 patients, of which 179 were implanted with a HeartMate II device (Thoratec Corp., Pleasanton, CA) and 21 received a Heartware HVAD (HeartWare Inc., Framingham, MA).

RESULTS: The mean age of our LVAD recipients was 59.3 years (range 17-81), 76% (152/200) were males, and 49% were implanted for the indication of BTT. The survival rate for our LVAD patients at 30 d, 6 mo, 12 mo, 2 years, 3 years, and 4 years was 94%, 86%, 78%, 71%, 62% and 45% respectively. The mean duration of LVAD support was 581 d (range 2-2595 d). Gastrointestinal bleeding (was the most common adverse event (43/200, 21%), followed by right ventricular failure (38/200, 19%), stroke (31/200, 15%), re exploration for bleeding (31/200, 15%),

ventilator dependent respiratory failure (19/200, 9%) and pneumonia (15/200, 7%). Our driveline infection rate was 7%. Pump thrombosis occurred in 6% of patients. Device exchanged was needed in 6% of patients. On multivariate analysis, preoperative liver dysfunction, ventilator dependent respiratory failure, tracheostomy and right ventricular failure requiring right ventricular assist device support were significant predictors of post LVAD survival.

CONCLUSION: Short and long term survival for patients on LVAD support are excellent, although outcomes still remain inferior compared to heart transplantation. The incidence of driveline infections, pump thrombosis and pump exchange have declined significantly in recent years.

Key words: Left ventricular assist device; Outcomes; Heart failure; Continuous-flow

Core tip: In this paper, we report our experience over the last 8 years with implanting continuous-flow left ventricular assist devices (LVADs). The aim of this analysis is to identify common occurring complications after LVAD implantation and identify areas for potential improvement in both patient management and selection. This is the largest single institutional LVAD experience that has been published, to the best of our knowledge.

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INTRODUCTION

Continuous-flow left ventricular assist devices (CF LVADs) are now the standard treatment for patients with end stage heart failure refractory to medical management[1-3]. The shortage of heart donors and the overall minimal therapeutic impact of heart transplantation on advanced heart failure have certainly accelerated the recent advances made in LVAD technology. In 2001, the landmark REMATCH trial demonstrated superiority of the pulsatile-flow HeartMate XVE *vs* best medical management, although these devices were still limited by their large size, reduced durability, significant and frequent postoperative complications[4]. Newer generation CF LVAD has by and large overcome most of the limitations of the pulsatile devices. Following the HeartMate Ⅱ (HM Ⅱ) trial[1], continuous flow devices were approved by the FDA, initially for bridge to transplantion (BTT) and subsequently for destination therapy (DT). Increasing clinical implementation and a multidisciplinary approach between cardiac surgeons and cardiologists to postoperative LVAD therapy have in recent years further improved LVAD outcomes. Despite these significant advances, LVAD implantations are still associated with significant morbidity, especially in the early postoperative period[5,6]. Improvements are still required if LVADs are to become a plausible alternative to heart transplantation or a therapeutic option for less sick patients in earlier stages of heart failure. The aim of our study was to investigate our institution's 8-year experience with CF LVADs and to analyze short and long term results with a goal to identify areas of improvement.

MATERIALS AND METHODS

This retrospective study was approved by our health system's Institutional Review Board. We reviewed our institution's LVAD dataset and analyzed all patients who received a CF LVAD as a BTT or DT from March 2006 until June 2014. We identified 200 patients, of which 179 were implanted with a HeartMate II device (Thoratec Corp., Pleasanton, CA) and 21 received a Heartware HVAD (HeartWare Inc., Framingham, MA).

Patient data

Multiple patient comorbidities from our LVAD database were analyzed. Pre and postoperative hemodynamic measurements were also evaluated. Finally we examined post LVAD related complications. We defined ventilator dependent respiratory failure (VDRF) as inability to extubate after 7 d right ventricular (RV) failure was considered for patients who needed a RVAD or who required inotropes in excess of two weeks in order to support the RV. Defining acute renal failure, was based on the RIFLE criteria (two fold increase in creatinine or a decline in glomerular filtration rate by half.

Statistical analysis

Patient data were compared between patients who received LVAD as DT or BTT using chi-squared tests for nominal data and Wilcoxon two-sample tests for continuous variables. Nominal data were reported as count and percent whereas as mean and standard deviations were calculated for continuous variables. For counts that were not large, the fisher exact tests were utilized. Kaplan Meier curves were used to generate estimates of survival and Cox proportional hazards models were used to assess the various covariates effect on survival. A backward stepwise routine was used to generate the most parsimonious model where all variables included were significant. Statistical significance was considered $P < 0.05$. SAS 9.2 was utilized for our analysis.

Table 1 Patient demographics and comorbidities

Variable	Total (n = 200)	BTT (n = 98)	DT (n = 102)	P value
Age (yr)	54.3 ± 12.5	50.1 ± 12.8	58.4 ± 10.7	0.001
Gender				
Female	24% (48/200)	25.5% (25/98)	22.8% (23/102)	
Male	76% (151/200)	74.5% (73/98)	76.5% (78/102)	0.652
Race				
AA	46% (92/200)	39.8% (39/98)	52% (53/102)	0.375
Caucasian	54% (108/200)	54.1% (53/98)	42.4% (47/102)	
Etiology of heart failure				
ICM	52% (104/200)	29% (28/98)	74.5% (76/102)	0.001
NIDCM	48% (96/200)	51% (50/98)	45.1% (46/102)	
BSA	1.97 ± 0.27	1.96 ± 0.27	1.98 ± 0.28	0.667
BMI	28.3 ± 5.5	28.1 ± 4.3	28.5 ± 6.5	0.763
Albumin (g/dL)	4.14 ± 10.03	3.19 ± 0.51	5.06 ± 14.05	0.015
DM	46% (92/200)	38.8% (38/98)	52.9% (54/102)	0.038
HTN	83% (166/200)	79.6% (78/98)	86.2% (88/102)	0.153
CRI	40% (81/200)	29.6% (29/98)	51% (52/102)	0.002
Dialysis	2.5% (5/200)	3.1% (3/98)	1.8% (2/102)	0.680
COPD	15.5% (31/200)	15.3% (15/98)	15.7% (16/102)	0.917
PVD	12% (23/200)	7.1% (7/98)	15.7% (16/102)	0.055
Vented	12% (25/200)	9.2% (9/98)	15.7% (16/102)	0.134
Previous cardiac surgery	32% (63/200)	20.4% (20/98)	42% (43/102)	0.001
Creatinine (mg/dL)	1.42 ± 0.62	1.43 ± 0.58	1.42 ± 0.65	0.869
AST (U/L)	48.3 ± 82.8	58.0 ± 106.8	38.9 ± 45.7	0.212
ALT (U/L)	46.5 ± 78.5	59.8 ± 99.4	33.5 ± 47.3	0.002
CPB time (min)	113.5 ± 46.1	109.5 ± 46.0	117.8 ± 46.1	0.178
XCL time (min)	71 ± 30.6	85.2 ± 33.7	51.7 ± 26.0	0.054
MCS at time of VAD	18% (36/200)	24% (23/98)	13% (13/102)	0.051
On inotropes at time of VAD	75%(150/200)	81% (80/98)	69% (70/102)	0.036
Pre VAD CVP (mmHg)	11.8 ± 6.4	11.6 ± 6.4	12.0 ± 6.4	0.653
Pre VAD PAPs (mmHg)	51.4 ± 14.2	50.5 ± 14.5	52.3 ± 13.8	0.412
Pre VAD PAPd (mmHg)	24.5 ± 9.2	24.4 ± 9.8	24.7 ± 8.5	0.682
Pre Vad CI (L/min per square metre)	1.85 ± 0.51	1.87 ± 0.54	1.83 ± 0.47	0.961
Pre VAD PCWP (mmHg)	23.0 ± 9.6	22.7 ± 9.8	23.4 ± 9.4	0.463
Blood transfusions	23% (46/200)	18% (18/98)	27% (28/102)	0.250
Concomitant cardiac procedure	19% (39/200)	23% (23/98)	15% (16/102)	0.137

BTT: Bridge to transplant; DT: Destination therapy; ICM: Ischemic cardiomyopathy; NIDCM: Non ischemic dilated cardiomyopathy; BSA: Body surface area; BMI: Body mass index; DM: Diabetes mellitus; HTN: Hypertension; CRI: Chronic renal insufficiency; COPD: Chronic obstructive pulmonary disease; PVD: Peripheral vascular disease; AST: Aspartate transaminase; ALT: Alanine aminotransferase; CPB: Cardiopulmonary bypass; XCl: Cross clamp; MCS: Mechanical circulatory support; VAD: Ventricular assist device; CVP: Central venous pressure; PAP: Pulmonary artery systolic pressure; PAPd: Pulmonary artery diastolic pressure; CI: Cardiac index; PCWP: Pulmonary capillary wedge pressure.

RESULTS

Preoperative patient demographics and operative characteristics

The mean age of our LVAD recipients was 59.3 years (range 17-81), 76% (152/200) were males and 24% females (48/200). BTT was the indication for LVAD implantation in 49% of patients (98/200) and DT in 51% (102/200) of patients. Additional patient demographics and comorbidities are presented in Table 1. In terms of operative characteristics, 31% of patients had undergone previous median sternotomy, the average cardiopulmonary bypass time was 113 min, cross clamp time (when used) was 71 min, and 19% of patients underwent a concomitant procedure at the time of LVAD implantation. In our cohort, 18% were on some type of mechanical circulatory support (MCS) at the time of LVAD insertion. Types of pre CF LVAD MCS included intraortic balloon pumps (23/36, 63%), pulsatile flow HeartMate XVE (5/36, 15%), CentriMag devices (5/36, 15%), Impella (2/26, 8%) and AbioMed support (1/36, 3%). BTT patients were significantly younger, had worse pre LVAD liver function and albumin, whereas DT patients were more likely to be diabetic, to have PVD, CRI and to have undergone previous cardiac surgery. Pre-LVAD inotropic support or MCS was more likely in the BTT patients (Table 1).

Duration of support, heart transplant and survival rates

The mean duration of LVAD support was 581 d (range 2-2595 d) (Table 2). A 56-year-old male, who received a CF LVAD for DT, is our longest survivor having been on LVAD therapy for just over 7 years. Overall, 27% of LVAD recipients and 46% of the BTT patient underwent heart transplantation (Table 2). At 2 years, the survival rate for our heart transplant recipients was 95% (52/55) which was significantly superior to the 2 year 71% survival rate for DT patients (P = 0.02). The survival rate at 30 d, 6 mo, 12 mo, 2 years, 3 years and 4 years was 94%, 86%, 78%, 71%, 62% and 45%

Table 2 Postoperative outcomes

Variable	Total (n = 200)	BTT (n = 98)	DT (n = 102)	P value
Postoperative ICU stay (d)	195 10.7 ± 10.4	95 10.2 ± 7.7	100 11.2 ± 12.5	0.833
Overall length of stay (d)	198 21.4 ± 14.3	98 20.8 ± 12.9	100 22.1 ± 15.6	0.517
Readmitted within 30 d	26.5% (53/200)	26.0 (25/96)	27% (28/102)	0.725
Reexploration for bleeding	15% (31/200)	10% (10/98)	5% (6/102)	0.040
DL infection	7% (15/200)	9% (9/98)	5% (6/102)	0.386
Pocket infection	1% (2/200)	1% (1/98)	1% (1/102)	0.493
Pneumonia	7% (15/200)	9% (9/98)	5% (6/102)	0.375
Hemorrhagic stroke	10% (21/200)	9% (9/98)	11% (12/102)	0.432
Emboli stroke	5% (10/200)	6% (6/98)	3% (4/102)	0.493
VDRF	9% (19/200)	10% (10/98)	8% (9/102)	0.774
Tracheostomy	2% (5/200)	1% (1/98)	3% (4/102)	0.369
Dialysis	2% (5/200)	3% (3/98)	1% (2/102)	0.680
GIB	21% (43/200)	17% (17/98)	25% (26/102)	0.289
Reoperation for AI	2% (4/200)	4% (4/98)	0% (0/102)	0.058
RV failure	19% (38/200)	15% (15/98)	22% (23/102)	0.192
RV failure requiring milrinone	13% (26/200)	9% (9/98)	16% (17/102)	0.103
RV failure requiring RVAD	6% (12/200)	6% (6/98)	5% (6/102)	0.803
Heart transplant	27% (55/200)	45% (45/98)	10% (10/102)	0.001
Duration of support (d)	581.0 ± 517.9	554.8 ± 535.0	606.4 ± 502.1	0.253

ICU: Intensive care unit; DL: Driveline; VDRF: Ventilator dependent respiratory failure; GIB: Gastrointestinal bleeding; AI: Aortic insufficiency; RV: Right ventricular; RVAD: Right ventricular assist device.

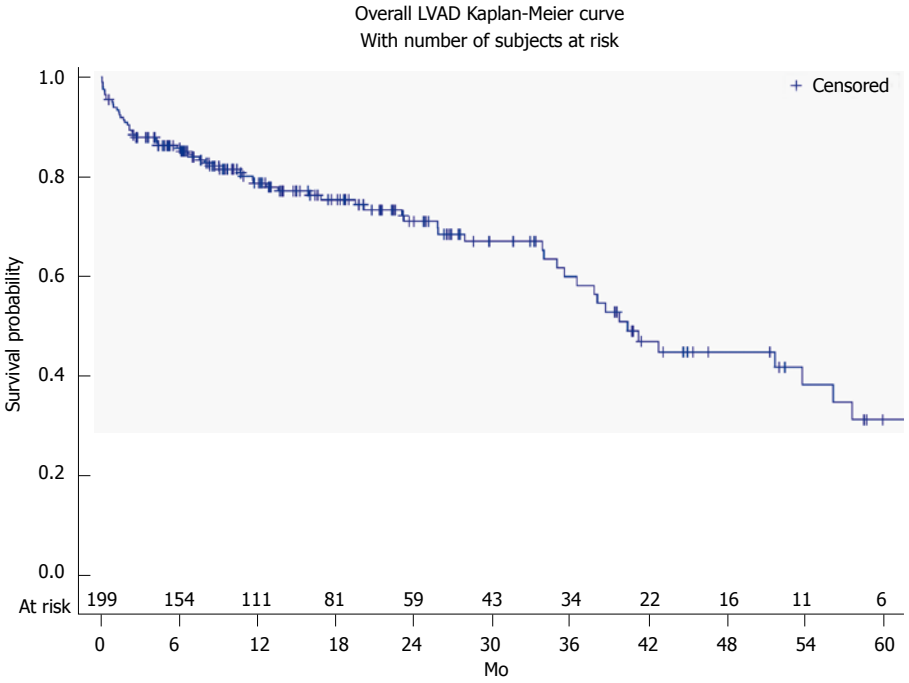

Figure 1 Kaplan-Meier survival curve for all patients receiving continuous-flow left ventricular assist devices. LVAD: Left ventricular assist device.

respectively (Figure 1). Survival rates were similar for BTT and DT patients (P = 0.566). Survival at 1 mo, 6 mo, 1 year, 2 years, 3 years and 4 years for the BTT patients was 93%, 87%, 70%, 70%, 63% and 52% respectively whereas for the DT group survival was 95%, 85%, 78%, 71%, 58% and 40% respectively (Figure 2). Competing outcomes of BTT *vs* DT patients is demonstrated in Table 3.

Causes of death

Since implanting our first CF LVAD in 2006, a total of 63 patients have died. Causes of death included: stroke (20/63, 32% of which 15 /63, 24% were hemorrhagic and 5/63, 8% were ischemic, range 2-654 d postoperatively, median 35 d), sepsis (17/63, 27%, range 5-320 d postoperatively, median 47 d), multi-organ failure (15/63, 24%, range 4-211 d median 35 d), right ventricular failure (6/63, range 2-139 d, median 10 d), refractory arrhythmia (2/63, 3%, at 64 and 128 d after LVAD implantation), bowel perforation (1/63, 1.5%, on postoperative day-11 and day-13), disconnection from the power source (1/63, 1.5%, 14

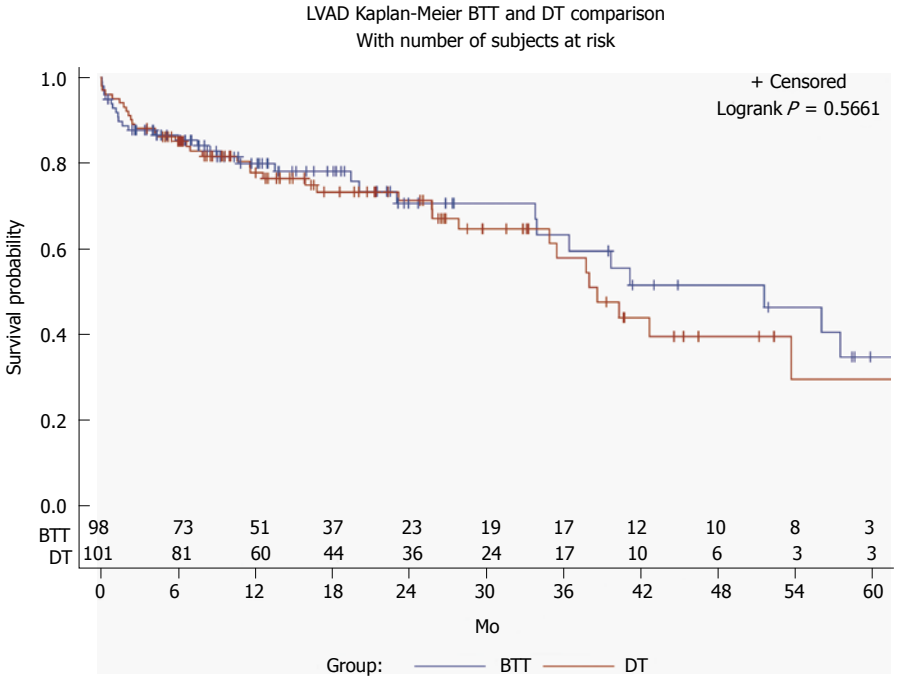

Figure 2 Comparison of Kaplan-Meier survival between bridge to transplan and destination therapy patients. LVAD: Left ventricular assist device; BTT: Bridge to transplan; DT: Destination therapy.

Table 3 Outcomes for bridge to transplan and destination therapy patients

	Variable	Patients (%)
BTT	Died	28.6 (28/98)
	Ongoing	25.5 (25/98)
	Transplant	45.9 (45/98)
DT	Died	34.3 (35/102)
	Ongoing	54.8 (56/102)
	Transplant	9.8 (10/102)

mo after implantation), and pump thrombosis (1/63, 1.5%, 18 mo after implantation).

Postoperative LVAD complications

Post LVAD complications are listed in Table 2. GIB was the most common adverse event (43/200, 21%), followed by RV failure (38/200, 19%), stroke (31/200, 15%), re exploration for bleeding (31/200, 15%), VDRF (19/200, 9%) and pneumonia (15/200, 7%). Our driveline infection rate was 7%. Pump thrombosis occurred in 6% of patients. Device exchanged was needed in 6% of patients, of which 77% (10/13) were for pump thrombosis and 13% (3/13) for severe driveline and pocket infections. No differences were noted between BTT and DT patients in terms of adverse events.

Length of ICU and hospital stay, and early readmissions

The average length of hospital stay (LOS) for our LVAD patients was 21 d, of which 11 d were spent in the intensive care unit (ICU). Readmissions within 30 d of index hospitalization discharge occurred in 27% of patients. No differences were observed between the BTT and DT patients in terms of LOS, ICU stay and readmission (Table 2). The most common cause of 30 d readmission were cardiac related (chest pain, SOB/heart failure, arrhythmia), gastrointestinal bleeding (GIB) (25%), infections 12% (pneumonia, wound/driveline infections, UTI) and stroke 8%.

Hemodymanic measurements pre LVAD and post LVAD at 6 mo

Hemodynamic measurements prior to LVAD implantation and after 6 mo of LVAD therapy are demonstrated in Table 4. Significant improvement was noted for all indices and measurements, which confirmed adequate LV decompression and improvement in RV function.

Predictors of survival

Univariate analysis showed that pre-LVAD renal (HR = 1.56; 95%CI: 1.11-2.21, P = 0.012) and hepatic function (HR = 1.03; 95%CI: 1.01-1.05, P = 0.004), length of ICU stay (HR = 1.34; 95%CI: 1.12-1.61, P = 0.001), the occurrence of VDRF (HR = 4.66; 95%CI: 2.51-8.67, P = 0.001), the need for tracheostomy (HR = 15.18; 95%CI: 5.56-41.4, P = 0.001) and the occurrence of post LVAD RV failure that required RVAD support (HR = 5.81; 95%CI: 2.84-11.9, P = 0.001) were significant predictors of survival. Variables with a P < 0.25 were included in a cox regression model. On multivariate analysis, pre LVAD liver function, VDRF, tracheostomy and implantation of a RVAD for RV failure still predicted survival (Table 5).

DISCUSSION

Continuous flow LVADs have now become an efficient

Table 4 Hemodymanic measurements pre and post left ventricular assist device at 6 mo

Variables	Pre VAD	Post VAD	*P* value
CVP (mmHg)	12 ± 6	8 ± 4.5	0.001
PAPs (mmHg)	53.52 ± 13.76	36.03 ± 11.85	0.001
PAPd (mmHg)	26.15 ± 9.50	16.11 ± 6.24	0.001
CI (L/min per square meter)	1.78 ± 0.39	2.52 ± 0.60	0.001
PCWP (mmHg)	25.09 ± 10.05	11.93 ± 7.84	0.001
LVEDD (mm)	71.70 ± 13.61	57.45 ± 15.3	0.001
LVEF (%)	16 ± 7.90	21 ± 9.00	0.017

VAD: Ventricular assist device; CVP: Central venous pressure; PAP: Pulmonary artery systolic pressure; PAPd: Pulmonary artery diastolic pressure; CI: Cardiac index; PCWP: Pulmonary capillary wedge pressure; LVEDD: Left ventricular end diastolic diameter; LVEF: Left ventricular ejection fraction.

Table 5 Multiple cox proportional hazard models

Variable	HR 95%CI	*P* value	Backwards stepwise model
Albumin	0.64 (0.27, 1.52)	0.310	
Length of stay	0.85 (0.62, 1.17)	0.319	
CPB time	1.05 (0.98, 1.14)	0.175	
CRI	1.13 (0.44, 2.91)	0.804	
PVD	0.95 (0.30, 3.03)	0.931	
Vented	0.93 (0.17, 4.97)	0.929	
Creatinine	0.77 (0.37, 1.63)	0.495	
PreVAD AST	1.03 (1.00, 1.07)	0.072	1.03 (1.01, 1.05) 0.01
PreVAD ALT	1.02 (1.00, 1.05)	0.064	1.02 (1.01, 1.04) 0.02
Blood transfusion	1.19 (0.45, 3.14)	0.732	
ICU stay	0.80 (0.52, 1.24)	0.320	
Reexploration	1.70 (0.50, 5.79)	0.794	
VDRF	4.92 (1.62, 14.93)	0.005	3.05 (1.41, 6.59) 0.005
Tracheostomy	5.53 (0.65, 46.78)	0.116	4.54 (1.35, 15.32) 0.015
RV failure	0.45 (0.09, 2.26)	0.330	
RVAD	8.90 (1.30, 61.06)	0.066	3.64 (1.59, 8.36) 0.002
Age	1.02 (0.95, 1.12)	0.176	
Gender	0.75 (0.66, 1.56)	0.321	
Resternotomy	1.31 (0.83, 4.55)	0.673	
Etiology of heart failure	1.23 (0.59, 4.08)	0.512	

CPB: Cardiopulmonary bypass; CRI: Chronic renal insufficiency; PVD: Peripheral vascular disease; AST: Aspartate transaminase; ALT: Alanine aminotransferase; ICU: Intensive care unit; VDRF: Ventilator dependent respiratory failure; RV: Right ventricular; RVAD: Right ventricular assist device.

treatment for patients with end stage heart failure for the indication of BTT or DT, with excellent short and long term survival, as demonstrated in this study. Our analysis showed that after CF LVAD implantation, survival at 30 d was 94%, at 1 year 78%, at 2 year 71%, and at 4 years 45%. Our longest survivor has been on LVAD therapy for over 7 years. Although these results by far surpass outcomes of patients with advanced heart failure on medical therapy, they are still inferior to heart transplantation which remains the gold standard for treating ESHF[7]. At 2 years the survival rate for our heart transplant recipients was 95% (52/55), which was superior to the 2 year 71% survival rate for DT patients (P = 0.02). Apart from improvement in survival, LVAD patients benefit from improved peripheral perfusion which certainly enhances quality of life. As demonstrated in our hemodynamic and ECHO measurements, 6 mo of LV therapy is associated with adequate LV decompression, significant improvement in RV function and in end organ perfusion. This is achieved with close postoperative surveillance and by obtaining regular echocardiograms to assess for aortic ejection, LV decompression, positions of the inteventricular septum, right ventricular function, and for residual mitral and tricuspid regurgitation[5]. We aim to maintain a flow index (CI) > 2.2 L/min per square metre. We also regularly adjust revolutions per minute (rpm) speed to achieve adequate flow, LV decompression, peripheral perfusion, and end organ function.

Our multivariate analysis demonstrated that preoperative liver dysfunction, and postoperative VDRF, tracheostomy, and RV failure requiring RVAD support were significant predictors of post LVAD mortality. These variables have previously been reported as potential risk factors for early post LVAD death in several published series[8-10]. High preoperative LFTs are an indication of poor end organ perfusion and RV dysfunction, which are certainly expected to increase postoperative mortality. These patients are coagulopathic, which cause postoperative bleeding, tamponade, and makes fluid management more challenging, especially with RV dysfunction which frequently co-exists with abnormal liver function. VDRF and trachesotomy both indicate critical illness and prolonged ICU support which are also expected to be predictors of poor outcome.

Several major centers around the world have also reported excellent survival outcomes, analogous to those reported in our study. A multi-institutional analysis from the United Kingdom and Germany[11] published survival rates of 89% at 30 d, 76% at 1 year and 66% at 2 years, from 139 CF LVAD implantations over a 6-year period. The average duration of support in this study was 514 d. No differences were identified between HeartMate II and HeartWare devices in terms of survival, although there was a trend towards more transfusions in the HeartMate group. These findings match our results when comparing the two types of devices. John *et al*[12] from the University of Minnesota published their single institutional experience with 130 CF LVADs. Overall, 30 d, 6 mo, and 1 year survival was 95.1%, 83.5%, and 78.8%, respectively. Driveline infections (25%), GIB 18% and stroke were the most common adverse events.

Possibly the most common and hazardous adverse events of the old generation pulsatile flow LVADs were resistant pocket/driveline infections and pump thrombosis[4,13,14]. Both these complications resulted in frequent device exchanges. Newer generation devices are more reliable and durable and fortunately these events are less frequent with CF LVADs[15-17], as clearly demonstrated in our study. Device exchange was performed in 6% of our patients, of which 77% (10/13) were for pump thrombosis and 13% (3/13)

for severe driveline and pocket infections. Our overall pump thrombosis rate was 6% (12/200), with 10/12 (83%) of these incidence occurring between 2006-2012 and only two cases of pump thrombosis over the past 3 years. Based on initial reports that suggested that anticoagulation could be less aggressive for Heartmate II devices, we followed a less aggressive anticoagulation policy, which may explain the higher frequency of pump thrombosis during the first six years of our CF-LVAD program. Since 2012, all patients receiving CF LVADs are postoperatively started on Asprin 81 mg and Warfarin with an INR target of 2.0-2.5. In addition we have recently been creating a larger sized pump pockets which reduces the effect of diaphragmatic excursion on the angle of the inflow cannula, thus reducing the incidence of pump thrombosis. Our driveline infection rate was only 7% which is significantly lower than the reported incidence of 20%[15]. It has been over 3 years since we have had a driveline infection. We feel that our success in preventing this challenging complication is linked with a new antibiotic and dressing protocol which was initiated at the end of 2011. The night before surgery patients are given 1.5 g of IV vancomycin, 2 g of IV cefepime, 400 mg IV Fluconazole and 600 mg of IV Rifampin. In penicillin or cephalosporin allergic patients, cefepime is substituted with 2 g of IV Aztreonam. Postoperatively, 4 doses of IV Vancomycin (15 mg/kg) every 12 h, 4 doses of IV Cefepime (2 g) every 12 h (or 2 daily doses of IV Aztreonam) and 2 daily doses of IV Fluconazole (400 mg) and IV Rifampin (600 mg) are administered. In the operating room, the drivelines are covered with Acticoat 3 Flex, which is a silver coated antimicrobial barrier that lasts for 3 d, followed by application of a tegaderm. Chlorhexidine and sterile water is used every 3 d to clean the driveline area, after which a new acticoat dressing is applied[5].

In our series, GIB was the most common adverse event (43/200, 21%), followed by RV failure (38/200, 19%) and stroke (31/200, 15%) rates which are similar to previously published data[1,17-22]. The occurrence of GI bleeding makes postoperative LVAD management more challenging, as temporary discontinuation of anticoagulation is required, which may increase the risk of pump thrombosis and stroke. GI bleeding is also a common cause for early postoperative readmission[23]. The frequent association of GIB with CF LAVDs is presumed to be from the lack of pulsatility which causes AV malformations and angiodysplasia. A similar mechanism, known as Heyde's syndrome[24], has been described in severe aortic stenosis, which also causes AV malformations and GIB. In addition, acquired von Willebrand syndrome has been reported as a potential cause for the development of GIB[25]. This frequent complication can be minimized through close INR monitoring, although recent studies have suggested that prophylactic administration of Octreotide may reduce the incidence of GIB[26,27]. RV failure is also a common LVAD related complication with a complex underlying mechanism. LV decompression causes a leftward shift of the interventricular septum, which reduces its contractility thus impairing RV function[28]. It is also challenging for the RV to keep up with the sudden increase in LV output which further decreases RV function. In addition, subtle changes in the pulmonary microcirculation before and after LVAD implantation also add to RV dysfunction[19,23]. We have previously published the patients who develop post LVAD RV failure and only require inotropic support with Milrinone, have equivalent outcomes to patients without RV failure[29]. It is only when RVAD support is required, does the morbidity and mortality increase, which is clearly demonstrated in our current study. Although certain risk factors predicting RV failure and RVAD support after LVAD implantation have been described, such as renal and liver failure, leucocytosis, high CVP/PCWP ratio, high CVP and decreased right ventricular stroke work index, predicting severe RV failure still remains a challenge[29-31].

Two types of LVADs have been implanted at our institution, HMII and HVAD. Of the 200 LVADs, 179 were HMII and 21 were HVADs. These devices have similarities and divergences. The HMII is an axial flow pump with an electromagnetically suspended rotor. The larger HMII device requires an additional pump pocket formation in the upper abdominal preperitoneal space[32]. The HVAD is a centrifugal flow pump, characterized by a smaller size, which allows for its placement within the pericardial cavity[33]. Although the number of HVADs we implanted was insufficient to generate results for meaningful conclusions, so far we haven't identified a significant difference in the overall mortality rate (32% for HMII *vs* 23% for HVADs, $P = 0.301$) or other complications. There only appeared to be a higher rate of blood transfusions with the HMII (20% *vs* 9%), which possibly corresponds to the need to form a pump pocket. Nevertheless, the higher transfusion rate did not correspond with higher incidences of re-exploration for bleeding and had no significant impact on survival.

An area of controversy and discussion amongst LVAD centers is patient's age as exclusion criteria for LVAD implantation. Several studies[8,10] have shown worse outcomes in older LVAD patients, although this was not observed in our analysis. In our 200 patient cohort, 14 patients were above the age of 70. Our oldest patient was 81 years. Survival at 2 years for patients above 70 was 62%. In addition, age was not found to be an independent predictor of survival. Other reports agree with our findings[34,35]. We feel that in appropriately selected patients, age should not be a contraindication to implantation[36].

Our study was not without limitations. Considering that this was not a prospective, randomize trial, it was subject to limitations inherent to any retrospective analysis. In addition statistical power was limited. Selection bias may also be present, since this is a single institution study. Finally, data on functional status and quality of life were not collected, which is an important target of LVAD therapy.

In conclusion, our single institutional analysis demonstrates superb short and long term outcomes, up to 4 year, with CF LAVDs. Compared to old generation devices, major adverse events such as pump thrombosis and driveline infections and frequent device exchanges, are now less frequent. Nevertheless, certain LVAD-related complications, such as GIB, stroke and RV failure do continue to occur. In addition to identifying new means of power transmission, new LVAD technology aims at reducing these adverse events. Preoperative hepatic and RV dysfunction appear to be predictors of post LVAD survival, which should certainly be taken into account in the patient selection process, whereas other significant variables, such as age, sex, etiology of heart failure, other comorbidities and reoperative cardiac surgery, do not appear to influence short and long term survival.

COMMENTS

Background

As the availability of left ventricular assist device (LVAD) therapy expands, there are still concerns regarding the relatively frequent occurrence of postoperative LVAD complications. Improvements are still required if LVADs are to become a plausible alternative to heart transplantation or a therapeutic option for patients in earlier stages of heart failure. The aim of this study was to review the authors' institutional experience over 8 years with 200 continuous-flow (CF) LVADs.

Research frontiers

To our knowledge this is the largest single institutional CF LVAD report.

Innovations and breakthroughs

Their single institutional analysis demonstrates excellent short and long term outcomes, up to 4 years, with CF LAVDs. Compared to old generation devices, major adverse events such as pump thrombosis and driveline infections and frequent device exchanges, are now less frequent. Nevertheless, certain LVAD-related complications, such as gastrointestinal bleeding, stroke and right ventricular failure do continue to occur.

Applications

Post-operative management of LVADs and appropriate patient selection.

Terminology

CF LVADs: Continuous-flow left ventricular assist device. They are centrifugal or axial flow pumps that replace the function of the failing heart.

Peer-review

The authors present an interesting review of experience with LVAD and analytical results about postoperative prognosis.

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ORIGINAL ARTICLE

Prospective Study

Comparison of echocardiography and device based algorithm for atrio-ventricular delay optimization in heart block patients

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Abstract

AIM: To compare the atrio-ventricular (AV/PV) delay optimization by echocardiography and intra-cardiac electrocardiogram (IEGM) based QuickOpt algorithm in complete heart block (CHB) patients, implanted with a dual chamber pacemaker.

METHODS: We prospectively enrolled 20 patients (age 59.45 ± 18.1 years; male: 65%) with CHB, who were implanted with a dual chamber pacemaker. The left ventricular outflow tract velocity time-integral was measured after AV/PV delay optimization by both echocardiography and QuickOpt algorithm method. Bland-Altman analysis was used for agreement between the two techniques.

RESULTS: The optimal AV and PV delay determined by echocardiography was 155.5 ± 14.68 ms and 122.5 ± 17.73 ms ($P < 0.0001$), respectively and by QuickOpt method was 167.5 ± 16.73 and 117.5 ms ± 9.10 ms ($P < 0.0001$), respectively. A good agreement was observed between optimal AV and PV delay as measured by two methods. However, the correlation of the optimal AV ($r = 0.0689$, $P = 0.77$) and PV ($r = 0.2689$, $P = 0.25$) intervals measured by the two

techniques was poor. The time required for AV/PV optimization was 45.26 ± 1.73 min by echocardiography and 0.44 ± 0.08 min by QuickOpt method ($P < 0.0001$).

CONCLUSION: The programmer based IEGM method is an automated, quick, easier and reliable alternative to echocardiography for the optimization of AV/PV delay in CHB patients, implanted with a dual chamber pacemaker.

Key words: Atrio-ventricular delay optimization; Complete heart block; Doppler echocardiography; Dual chamber pacemaker; Hemodynamics; QuickOpt algorithm

Core tip: Optimization of sensed and paced atrio-ventricular (AV/PV) delay is required for better hemodynamics in patients with complete heart block (CHB). Aim of the present study was to compare the AV/PV delay optimization by echocardiography and intra-cardiac electrocardiogram (IEGM) based QuickOpt algorithm in patients with CHB. We prospectively enrolled 20 patients of CHB who were implanted with a dual chamber pacemaker. A velocity time-integral of left ventricular outflow tract was measured following AV/PV delay optimization by both echocardiography and QuickOpt algorithm method. An agreement between the two techniques was assessed by Bland-Altman analysis. Optimal AV and PV delay as assessed by echocardiography was 155.5 ± 14.68 ms and 122.5 ± 17.73 ms ($P < 0.0001$), respectively and by QuickOpt method was 167.5 ± 16.73 ms and 117.5 ± 9.10 ms ($P < 0.0001$), respectively. The time required for AV/PV optimization was 45.26 ± 1.73 min by echocardiography and 0.44 ± 0.08 min by QuickOpt method ($P < 0.0001$). In conclusion, automated programmer based IEGM method is a quick, easy and reliable alternative to echocardiography for optimization of AV/PV delay in CHB patients.

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INTRODUCTION

Pacemaker therapy provides a better hemodynamics in addition to pacing support in patients with bradycardia and complete heart block (CHB). A programmed atrio-ventricular (AV) interval is crucial for adequate diastolic filling, optimal cardiac output and prevention of diastolic mitral regurgitation[1]. Optimal AV delay can be assessed by Doppler diastolic flow measurement across mitral valve, or by an invasive left ventricular pressure measurement. An automated intra-cardiac electrogram (IEGM) algorithm known as "QuickOpt" from St Jude Medical, St Paul, MN, United States has the capability to assess the optimal AV delay in implanted patient[2-4]. QuickOpt algorithm is a good alternative to the standard echocardiographic method for optimal AV delay assessment. A good correlation have been demonstrated between optimal AV/PV and inter-ventricular (VV) intervals in patients with cardiac resynchronization therapy (CRT) as assessed by echocardiography and QuickOpt method[3,4]. We have compared echocardiography and QuickOpt method for AV/PV delay optimization in patients of CHB, who were implanted with dual chamber pacemaker.

MATERIALS AND METHODS

It was a prospective, single center, non-randomized, open-label, pilot study. The institute's ethics committee approval was taken prior to initiation of the study. Twenty consecutive patients of CHB who underwent pacemaker implantation (DDDR Mode, Zephyr XL DR 5826, St Jude Medical, United States) from July 2010 to December 2011 were enrolled in the study. Patients with low intrinsic atrial rate of < 40 bpm, NYHA functional class Ⅳ heart failure, permanent or persistent atrial flutter or atrial fibrillation, significant valvular heart disease, pregnancy, age < 18 years, and those enrolled in another study were excluded. All patients underwent optimization of the AV/PV delay by echocardiography and QuickOpt algorithm after at least 8 wk of pacemaker implantation. The order of measurement of the two tests was randomized with the help of a computer generated random number table. A stopwatch was used for time interval measurements for both the optimization methods.

AV delay optimization methods

Echocardiography method: AV delay optimization was performed using two-dimensional Doppler echocardiography on iE33 ultrasound system (Philips Medical Systems, WA, United States). A sweep speed of 100 mm/s was used. Doppler measurements were taken at a delay of 30 s after programming new AV and PV intervals. Optimal AV interval was determined in DVI pacing mode, while optimal PV interval was measured in VDD pacing mode. To pace atria in DVI mode, the atrial rate was increased by 10 beats per minute over the baseline atrial rate. Mitral inflow velocity was measured in the apical four-chamber view. First measurement of mitral inflow duration (both diastolic E and A wave duration) was taken at a long AV delay of > 200 ms. Thereafter, AV interval was decreased by 10 ms each time and simultaneously EA duration was measured, during end expiration. An average of three consecutive beats during expiration was taken for EA measurement. The optimal AV/PV interval by

Table 1 Optimal atrio-ventricular delay (ms) by echocardiography and QuickOpt

	Optimal AV delay (in ms)	Optimal PV delay (in ms)	*P* value
Echocardiography	155.5 ± 14.68 (130-180)	122.5 ± 17.73 (90-150)	< 0.0001
QuickOpt	167.5 ± 16.73 (150-190)	117.5 ± 9.10 (100-140)	< 0.0001

Values are in mean ± 1 SD (range).

echocardiography was the AV/PV interval at which the maximum transmitral inflow duration was documented without the interruption of A wave[5,6]. A velocity time-integral (VTI) of left ventricular outflow tract (LVOT) was measured in an apical five chamber view and an average of three beats was taken. It was measured for each of a programmed AV/PV delay. Measurement by a single echocardiographer (author - Gupta A) ruled out inter-observer bias. Taking measurements of pulsed wave Doppler at fixed points (mitral valve leaflet tips for transmitral inflow duration and 1 cm below the aortic valve for VTI of LVOT) minimized intra-observer bias.

Intra-cardiac electrocardiogram method

Optimal AV interval was measured by intra-cardiac electrocardiogram (IEGM) method using a St Jude Medical programmer (QuickOpt algorithm in Merlin™ Patient Care System Programmer, St Jude Medical, CA, United States)[2,4]. This algorithm calculates the optimal PV delay by measuring the width of atrial IEGM and adding 30 ms if intrinsic atrial depolarization of ⩾ 100 ms and adding 60 ms if intrinsic atrial depolarization is < 100 ms. The off-set factor enables delivery of ventricular pacing after atrial electrical activation and mechanical contraction are completed. An optimal AV delay was calculated as the sum of optimal PV delay and the pacing latency (50 ms). At each AV interval, LVOT VTI was assessed as per the method described above.

Statistical analysis

Continuous variables are expressed as mean and standard deviation, and categorical variables are expressed as counts. Bland-Altman plots was used for agreement between the two optimization techniques[7-9]. These plots depict the mean difference and 95%CI of the differences (mean difference ± 2 SD of difference). A difference of > 20 ms in the AV or PV interval assessed by two optimization techniques was interpreted as poor agreement[2]. For LVOT VTI, a difference of > 2 cm was considered significant for a poor agreement[2]. Correlation between the two techniques was evaluated using linear regression analysis and Pearson's correlation coefficient. A *P* value of < 0.05 was regarded significant. Comparison of LVOT VTI was done using the paired-sample Student's *t* test. Statistical analysis was carried out by Statistical Analysis System SAS 17 and Medcalc Medical Calculator.

Table 2 Left ventricular outflow tract-velocity time integral (left ventricular outflow track velocity time-integral) at optimal AV and PV delay and time required for AV/PV delay optimization

	Echocardiography	QuickOpt	*P* value
LVOT VTI at optimal AV delay (cm)	18.86 ± 4.11 (11.6-27.7)	17.82 ± 4.02 (11.46-27.7)	0.0099
LVOT VTI at optimal PV delay (cm)	19.26 ± 3.01 (13.7-23.9)	18.5 ± 2.92 (13.8-23.9)	0.07
Time required for optimization (min)	45.26 ± 1.73 (41.5-48.1)	0.44 ± 0.08 (0.31-0.57)	< 0.0001

Values are in mean ± 1 SD (range).

RESULTS

Thirty CHB patients had dual chamber pacemaker (Zephyr XL DR 5826 model of St Jude Medical, United States) implantation from July 2010 to December 2011. Twenty eligible patients were included in the study. Ten excluded patients had permanent/persistent atrial flutter or atrial fibrillation (n = 6), slower intrinsic atrial activity of less than 40 bpm (n = 2) or NYHA Class Ⅳ heart failure (n = 2). The mean age of 20 enrolled patients was 59.45 ± 18.1 years; 13 were males and 7 were females. Seventeen patients had degenerative CHB and 3 had congenital CHB. Presenting complaint of syncope or pre-syncope was present in 17 patients. The mean left ventricular ejection fraction was 59.25% ± 7.8%.

Comparison of optimal AV/PV delay measured by echocardiography and QuickOpt algorithm

The optimal AV and PV delay determined by echocardiography was 155.5 ± 14.68 ms and 122.5 ± 17.73 ms, respectively and by QuickOpt was 167.5 ± 16.73 ms and 117.5 ± 9.10 ms, respectively (Table 1). The optimal PV delay was significantly shorter than optimal AV delay by both echocardiography and QuickOpt algorithm (P < 0.0001). Mean time required for optimisation for AV/PV delay was 45.26 ± 1.73 min by echocardiography and 0.44 ± 0.08 min by QuickOpt algorithm, P < 0.0001 (Table 2). There was a good agreement between optimal AV delays as assessed by the two techniques. Only 4-patients had > 20 ms difference in optimal AV interval (Figure 1). However, correlation of the optimal AV intervals assessed by two techniques was poor (Figure 2; r = 0.0689, P = 0.77). There was a good agreement of optimal PV delay with just 4-patients having > 20 ms difference in the optimal PV interval (Figure 3) and a poor correlation between the two techniques (Figure 4; r = 0.2689, P = 0.25).

Comparison of the LVOT VTI achieved at optimal AV/PV delays using the echocardiography and QuickOpt algorithm

Mean LVOT VTI at optimal AV delay was 18.86 ± 4.11 cm by echocardiography and 17.82 ± 4.02 cm by

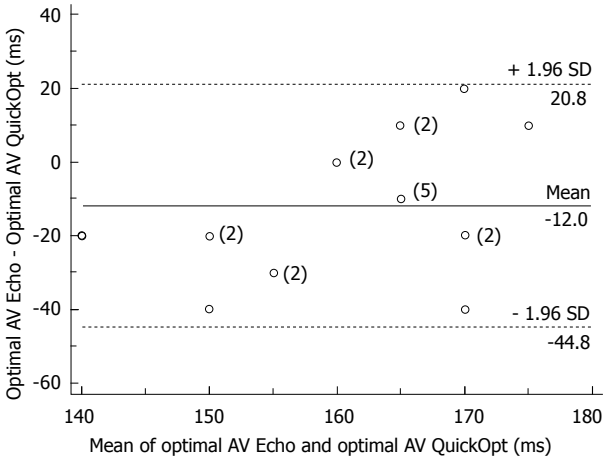

Figure 1 Bland - Altman plot of differences in optimal AV interval measured by echocardiography and QuickOpt.

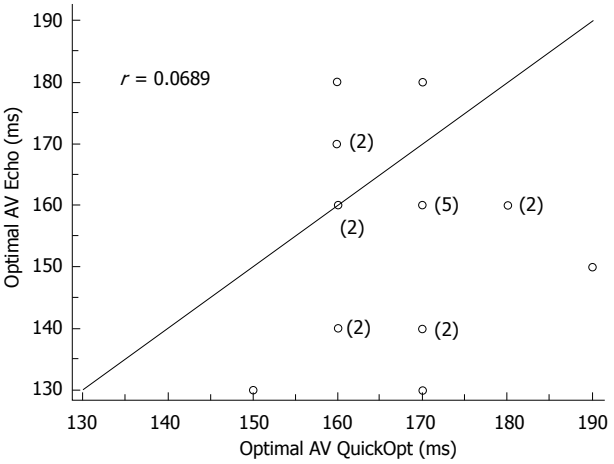

Figure 2 Correlation of optimal AV intervals measured by echocardiography and QuickOpt.

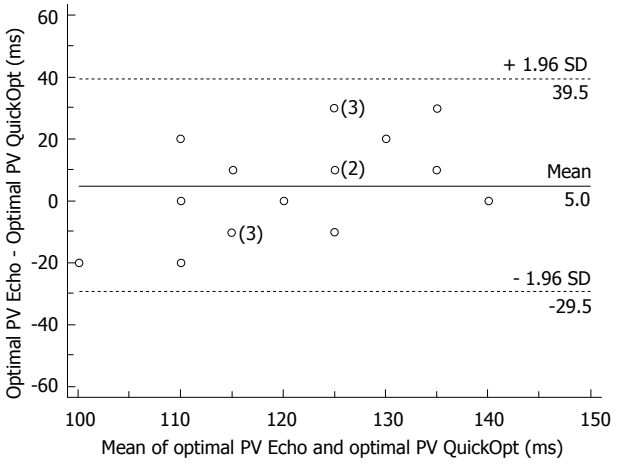

Figure 3 Bland - Altman plot of differences in optimal PV interval measured by echocardiography and QuickOpt.

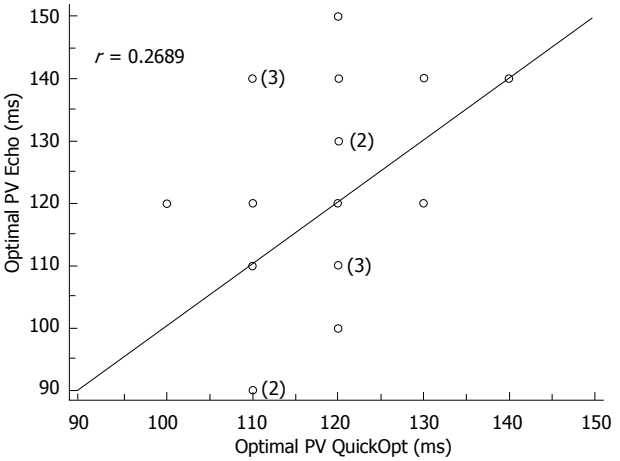

Figure 4 Correlation of optimal PV intervals measured by echocardiography and QuickOpt.

QuickOpt algorithm (P = 0.0099, Table 2), suggesting a better hemodynamic response by echocardiography. Similarly, mean LVOT VTI at optimal PV delay was 19.26 ± 3.01 cm by echocardiography and 18.5 ± 2.92 cm by QuickOpt algorithm (P = 0.07), suggesting a trend towards better hemodynamic response by echocardiography (Table 2). There was a good clinical agreement between LVOT VTI at optimal AV delay assessed by these two techniques, with 4-patients having > 2 cm difference in the LVOT VTI (Figure 5). Also, the correlation of LVOT VTI measured at optimal AV delay was good by two techniques (r = 0.9216, P < 0.0001). Similarly, there was a good agreement between LVOT VTI at optimal PV delay determined by these two techniques, with just 4 of 20 patients having more than 2 cm difference in the LVOT VTI (Figure 6) and a good correlation of LVOT VTI as assessed at optimal PV delay by two techniques (r = 0.8218, P < 0.0001).

DISCUSSION

The present study has demonstrated a good agreement and poor correlation between optimal AV and PV delay as assessed by echocardiography and QuickOpt algorithm in patients with CHB. There was also a good agreement and good correlation of LVOT VTI as determined at optimal AV and PV delay by two techniques. Various studies had shown a good correlation between LVOT VTI as determined by echocardiography and QuickOpt algorithm in patients with heart failure on CRT[3,4]. Gold *et al*[10], demonstrated an excellent correlation between the IEGM method and the maximum achievable invasive LV dP/dt measurement during CRT implantation in both AV and PV modes. Baker *et al*[3], studied AV/PV and VV delay optimization in heart failure patients implanted with a CRT-D or dual chamber ICD. They measured maximum LVOT VTI guided by echocardiography and QuickOpt algorithm. The concordance correlation coefficient between echocardiography and QuickOpt method for AV, PV and VV delays was 97.5%, 96.1%, and 96.6%, respectively (P < 0.05). Kamdar *et al*[2], studied AV and VV delay optimization in CRT patients by echocardiography and QuickOpt method. There was a good correlation of two methods for LVOT VTI

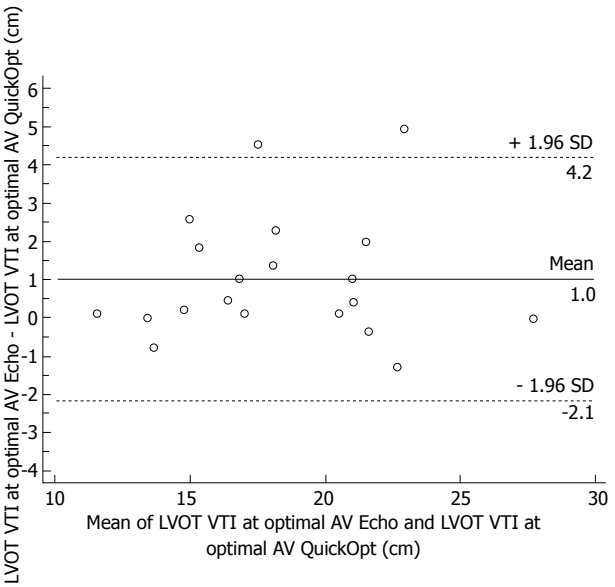

Figure 5 Bland - Altman plot of differences in left ventricular outflow tract velocity time-integral at optimal AV delay measured by echocardiography and QuickOpt. LVOT: Left ventricular outflow tract; VTI: Velocity time-integral.

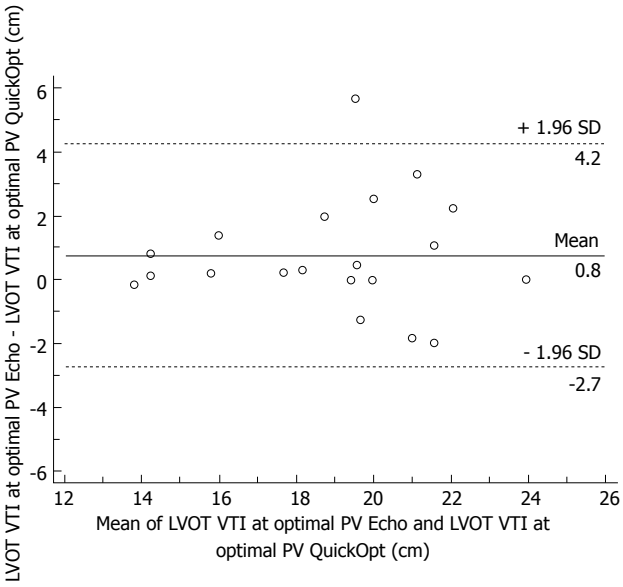

Figure 6 Bland - Altman plot of differences in left ventricular outflow tract velocity time-integral at optimal PV delay measured by echocardiography and QuickOpt. LVOT: Left ventricular outflow tract; VTI: Velocity time-integral.

optimization (R^2 = 0.77, P < 0.001), though it was significantly better ($P \leqslant 0.001$) with echocardiography compared to QuickOpt method. However, an agreement between the two methods was poor, with 15 out of 26 patients had > 20 ms difference in optimal AV interval and 10 out of 26 patients had > 20 ms difference in the optimal VV interval. The frequent optimization study using the QuickOpt method (FREEDOM trial) studied benefits of frequent AV/PV and VV delay optimisation using the QuickOpt algorithm *vs* standard of care (physician guided programming or upto 1 non IEGM based optimization like echocardiography within first 4 wk) in 1647 patients implanted with CRT[4]. This trial observed that QuickOpt optimization was as good as the standard of care programming methods which includes either physician guided or non-IEGM optimization methods by echocardiography. In the present study, there was a poor correlation between optimal AV and PV delay but good correlation between LVOT VTI at optimal AV/PV delay as assessed by echocardiography and QuickOpt algorithm. This is attributed to small changes in hemodynamics (measured by LVOT VTI) caused by large variations in AV/PV intervals.

AV synchrony provides hemodynamic benefit in addition to pacing support in CHB patients. An appropriately timed atrial systole prevents rise in mean atrial pressure, facilitates venous return, coordinates AV valve closure; thus reduces diastolic mitral regurgitation and reduces pulmonary capillary wedge pressure[11,12]. The fact that optimal AV delay results in maximum cardiac output, and small deviations can decrease cardiac output has been demonstrated in various previous studies[1,13]. Various echocardiography studies have reported optimal AV delay of 125-200 ms, and an optimal PV delay of 30-50 ms shorter to optimal AV delay[1,5,6]. Similar mean AV/PV delay was observed in present study. Janosik *et al*[1] studied Doppler derived cardiac output in 24 patients implanted with dual chamber pacemaker. The optimal delay interval during DVI and VDD pacing was 176 ± 44 and 144 ± 48 ms (P < 0.002), respectively. They demonstrated 8% increment in resting cardiac output with optimal AV delay; while same delay with paced P wave (PV delay) did not show maximum cardiac output. Kindermann *et al*[5] documented optimal AV and PV delay in 53 high degree AV block patients as 136 ± 34 ms and 76 ± 40 ms, respectively. They also reported that AV delay optimization results in 19% increase in stroke volume, compared to fixed AV delay. Similarly, Ritter *et al*[6] reported an optimal AV and PV delay of 179 ± 25 ms and 124 ± 18 ms, respectively in 19 CHB patients with dual chamber pacemaker. Ovsyshcher *et al*[14] demonstrated that optimal AV delay is associated with about 30% more cardiac output during DDD pacing, in comparison to VVI pacing.

The present study also documented a good agreement and correlation between LVOT VTI at optimal AV/PV delay by both echocardiography and QuickOpt algorithm. The hemodynamic outcome in term of LVOT VTI was significantly better with echocardiography, in comparison to QuickOpt algorithm. This is possibly because of IEGM based electrical optimization may not be equal to the best mechanical and hemodynamic performance, as achieved by echocardiography. The time required for AV/PV delay optimization in present study was 45.26 ± 1.73 min by echocardiography and 0.44 ± 0.08 min by QuickOpt (P < 0.0001). To best of our knowledge, there is no available published literature about similar comparison between two methods for AV/PV delay optimization in CHB patients. The average time required for VV delay optimization in CRT patients as reported by Hansalia *et al*[15] was significantly lower with

QuickOpt method in comparison to echocardiography (1.5 ± 0.87 min *vs* 41 ± 8.3 min, P = 0.006). Thus, QuickOpt is a cheap, fast, simple, automatic and more practical method of AV delay optimization in "real world" practice which can be performed within a minute during regular clinical follow-up using the device programmer.

The present study has few limitations such as use of non-invasive echocardiography method for hemodynamic assessment, which have inherent bias. A single echocardiographic method of transmitral inflow duration was used for AV delay optimization, instead of using other methods such as impedance cardiography[5], peak endocardial acceleration[6], left ventricular invasive pressure measurement (LV dP/dt_{max})[16], *etc.* A study with larger number of patients is required to validate the results, as the present study was of small sample size. The effects of upright position and exercise on optimal AV delay were not assessed. We only measured hemodynamic response and not the clinical benefit in enrolled patients.

In conclusion, the present study demonstrated that an automated programmer-based IEGM method is quick, easy and reliable alternative to time consuming echocardiography method for AV delay optimization in patients of CHB, implanted with dual chamber pacemaker.

COMMENTS

Background

Optimization of atrio-ventricular (AV/PV) delay is required for better hemodynamics in patients with complete heart block (CHB).

Research frontiers

The present study compared the AV/PV delay optimization by echocardiography and intra-cardiac electrocardiogram based QuickOpt algorithm in CHB patients, subjected to dual chamber pacemaker.

Innovations and breakthroughs

The authors found that QuickOpt algorithm is an automated, easy, quick and reliable alternative to echocardiography for AV/PV delay optimization in CHB patients.

Applications

In a real world practice, AV/PV delay optimization can be performed within a minute using QuickOpt algorithm.

Terminology

IEGM: Intra-cardiac electrogram; CHB: Complete heart block; LVOT VTI: Left ventricular outflow tract velocity time integral; AV/PV: Paced atrio-ventricular/sensed artio-ventricular; CRT: Cardiac resynchronisation therapy.

Peer-review

The authors stated that optimization of sensed and paced atrio-ventricular (AV/PV) delay is required for better hemodynamics in patients with CHB. They studied the AV/PV delay optimization using echocardiography and intra-cardiac electrocardiogram (IEGM) based QuickOpt algorithm in 20 CHB patients. The results revealed a good agreement between optimal AV and PV delay determined by the two methods. Authors concluded that the automated programmer based IEGM method is a quick, easier and reliable alternative to echocardiography for the optimization of AV/PV delay in CHB patients subjected for dual chamber pacemaker. The tables and figures are presented appropriately.

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SYSTEMATIC REVIEWS

Digoxin: A systematic review in atrial fibrillation, congestive heart failure and post myocardial infarction

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Author contributions: Virgadamo S and Elayi CS designed the research; Virgadamo S performed the research; Charnigo R, Darrat Y and Morales G reviewed the data; Virgadamo S wrote the paper; Charnigo R edited the paper; Darrat Y, Morales G and Elayi CS supervised the paper; all authors approved the final manuscript.

Conflict-of-interest statement: All authors declare that they have no competing interests.

Data sharing statement: The table is available from the corresponding author at samy-claude.elayi@uky.edu.

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Abstract

AIM: To review digoxin use in systolic congestive heart failure, atrial fibrillation, and after myocardial infarction.

METHODS: A comprehensive PubMed search was performed using the key words "digoxin and congestive heart failure", "digoxin and atrial fibrillation", "digoxin, atrial fibrillation and systolic congestive heart failure", and "digoxin and myocardial infarction". Only articles written in English were included in this study. We retained studies originating from randomized controlled trials, registries and included at least 500 patients. The studies included patients with atrial fibrillation or heart failure or myocardial infarction and had a significant proportion of patients (at least 5%) on digoxin. A table reviewing the different hazard ratios was developed based on the articles selected. Our primary endpoint was the overall mortality in the patients on digoxin *vs* those without digoxin, among patients with atrial fibrillation and also among patients with atrial fibrillation and systolic heart failure. We reviewed the most recent international guidelines to discuss current recommendations.

RESULTS: A total of 18 studies were found that evaluated digoxin and overall mortality in different clinical settings including systolic congestive heart failure and normal sinus rhythm (n = 5), atrial fibrillation with and without systolic congestive heart failure (n = 9), and myocardial infarction (n = 4). Overall, patients with systolic congestive heart failure with normal sinus rhythm, digoxin appears to have a neutral effect on mortality especially if close digoxin level monitoring is employed. However, most of the observational studies evaluating digoxin use in atrial fibrillation without systolic congestive heart failure showed an increase in overall mortality when taking digoxin. In the studies evaluated in this systematic review, the data among patients with atrial fibrillation and systolic congestive heart failure, as well as post myocardial infarction were more controversial. The extent to which discrepancies among studies are based on statistical methods is currently unclear, as these studies' findings are generated by retrospective analyses that employed

different techniques to address confounding.

CONCLUSION: Based on the potential risks and benefits, as well as the presence of alternative drugs, there is a limited role for digoxin in the management of patients with normal sinus rhythm and congestive heart failure. Based on the retrospective studies reviewed there is a growing volume of data showing increased mortality in those with only atrial fibrillation. The proper role of digoxin is, however, less certain in other subgroups of patients, such as those with both atrial fibrillation and systolic congestive heart failure or after a myocardial infarction. Further studies may provide helpful information for such subgroups of patients.

Key words: Digoxin; Atrial fibrillation; Heart failure; Myocardial infarction; Mortality

Core tip: This systematic review evaluates mortality with the use of digoxin in congestive heart failure (CHF) with sinus rhythm, atrial fibrillation with and without CHF, and post myocardial infarction. In patients with CHF with sinus rhythm, there continues to be a niche for digoxin use as an adjunctive therapy for symptomatic control with the understanding that there is no effect on mortality. The role for digoxin among patients who only have atrial fibrillation seems very limited; however, those with atrial fibrillation and systolic congestive heart failure or post myocardial infarction need further assessment as many questions remain regarding the benefit of digoxin in this population.

Virgadamo S, Charnigo R, Darrat Y, Morales G, Elayi CS. Digoxin: A systematic review in atrial fibrillation, congestive heart failure and post myocardial infarction. *World J Cardiol* 2015; 7(11): 808-816 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i11/808.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i11.808

INTRODUCTION

Digoxin is one of the oldest drugs used today in cardiovascular medicine in the United States and around the globe. It is used frequently to treat heart failure symptoms and to decrease the ventricular rate in atrial fibrillation (AF). Digoxin was one of the first treatments for heart failure management and was shown to decrease hospitalizations without decreasing mortality in patients with sinus rhythm and left ventricular ejection fraction (LVEF) of less than 45%[1]. Nowadays digoxin remains indicated for patients with persistent symptoms despite optimal medical therapy even with the advent of several new classes of cardiovascular medications with proven benefit on symptoms and survival [including beta blockers, angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers and mineralocorticoid antagonists]. In the setting of AF, digoxin is not mentioned in the 2014 guidelines anymore as an option for rate control, except among patients with AF and heart failure; however, concerns arose regarding its safety even in this subgroup of patients[2-4]. No randomized controlled clinical trials have been performed to date to assess the efficacy and safety of digoxin in patients with AF. Most of the current data regarding the safety and efficacy of digoxin are based on observational studies which have had conflicting results. We review the data available regarding the use of digoxin in congestive heart failure (CHF), AF, and after myocardial infarction, as well as the current guidelines indications for digoxin use from the American College of Cardiology/American Heart Association (ACC/AHA) and European Society of Cardiology (ESC).

Digoxin mechanisms of action

Digoxin's primary mechanism of action is through inhibition of sodium-potassium adenosine triphosphatase (ATPase). Its role in heart failure patients is based on its inotropic properties, due to inhibition of sodium-potassium ATPase which leads to increased intracellular calcium concentrations through the sodium-calcium exchanger[5-8]. This causes the cardiac action potential to lengthen which causes lower heart rates as well as increases myocardial contractility due to the increased calcium for sarcomeric excitation-contraction coupling[8]. Digoxin also has neurohormonal effects and causes improved baroreceptor sensitivity, decreases norepinephrine concentration, and decreases activation of the renin-angiotensin system[5,6,9].

From the electrophysiologic standpoint, digoxin has a parasympathetic effect on the sinoatrial node, by decreasing the automaticity as well as on the atrioventricular conduction system by decreasing conduction and increasing the effective refractory periods[6].

MATERIALS AND METHODS

Literature search

A comprehensive PubMed search was performed using the key words "digoxin and congestive heart failure", "digoxin and AF", "digoxin, AF and systolic congestive heart failure", and "digoxin and myocardial infarction". Only articles written in English were included in this study. We retained studies originating from randomized controlled trials, registries and included at least 500 patients. The studies included patients with AF or heart failure or myocardial infarction and had a significant proportion of patients (at least 5%) on digoxin. A table reviewing the different hazard ratios was developed based on the articles selected. Our primary endpoint was the overall mortality in the patients on digoxin *vs* those without digoxin, among patients with AF and also among patients with AF and systolic heart failure. We reviewed the most recent international guidelines to

discuss current recommendations.

RESULTS

Literature review

A total of 18 studies were found that evaluated digoxin and overall mortality in the different clinical settings including systolic heart failure and sinus rhythm (*n* = 5), AF with and without heart failure (*n* = 9), and myocardial infarction (*n* = 4).

Congestive heart failure with sinus rhythm

For over 200 years, digoxin has been used to treat patients with systolic heart failure in normal sinus rhythm, but over the past several decades digoxin has been scrutinized regarding its therapeutic benefit and risk. As studies began to show the benefits of ACEI in reducing mortality, clinicians began to question the role of digoxin. This led physicians to inquire whether discontinuing digoxin from patients' medical regimens had any effect, especially if patients were also taking ACEI, since no long term benefit had been shown with digoxin.

The Randomized Assessment of Digoxin on Inhibitors of Angiotensin-Converting Enzyme (RADIANCE) study randomized 178 patients with New York Heart Association (NYHA) Class Ⅱ-Ⅲ heart failure, LVEF of < 35%, and normal sinus rhythm to evaluate whether removing digoxin had any clinical significance. This study found that stable patients on digoxin, ACEI, and diuretics had an increased risk of clinical decline when digoxin was removed from their medication regimen with a 5.9 estimated relative risk (95%CI: 2.1-17.2) of worsening heart failure compared to those continuing digoxin. In addition, patients' no longer taking digoxin had lower quality-of-life scores, decreased ejection fraction and increased heart rate and body weight[10]. The RADIANCE study established the short term benefit of digoxin in preventing worsening functional decline, exercise capacity and LV ejection fraction in patients with heart failure and normal sinus rhythm[10]. Furthermore, the Prospective Randomized Study of Ventricular Failure and the Efficacy of Digoxin (PROVED) trial also demonstrated the efficacy of digoxin in patients with mild to moderate systolic heart failure on diuretic therapy[11]. Both studies however had a short term follow up (12 wk for RADIANCE and 20 wk for PROVED)[10,11]. It remained unknown whether the results would be similar with longer follow-up. This led the Digitalis Investigation Group to perform a randomized, double-blinded placebo-controlled trial to evaluate the effects of digoxin on mortality and hospitalizations in patients with heart failure and normal sinus rhythm[1].

The DIG study enrolled 6800 patients with LVEF of 45% or less and they were randomized to receive digoxin (3397 patients) or placebo (3403 patients) in addition to diuretics and ACEI. The DIG study failed to demonstrate a beneficial effect of digoxin on overall mortality with 1181 deaths in the digoxin group (34.8%) and 1194 deaths in the placebo group (35.1%) giving an estimated risk ratio (RR) of 0.99 (95%CI: 0.91-1.07, P = 0.80)[1]. Also, no difference was seen in cardiovascular deaths with 1016 in the digoxin group (29.9 %) *vs* 1004 in the placebo group (29.5%) with RR = 1.01 (95%CI: 0.93-1.10, P = 0.78)[1].

However, there was a trend towards a lower risk of mortality secondary to heart failure with 394 deaths in the digoxin group compared to 449 in the placebo group with a RR of 0.88 (95%CI: 0.77-1.01, P = 0.06). Overall, the number of hospitalizations attributed to worsening heart failure was lower in the digoxin group compared to placebo with a RR of 0.72 (95%CI: 0.66-0.79, P < 0.001)[1]. When combining death from any cause or hospitalization due worsening heart failure, the digoxin group had fewer events (RR = 0.85; 95%CI: 0.79-0.91, P < 0.001). This was also seen when combining heart failure deaths or hospitalizations due to worsening heart failure (1041 *vs* 1291, RR = 0.75; 95%CI: 0.69-0.82, P < 0.001). In addition, a subgroup analysis of the prior outcome, digoxin appeared to have the greatest beneficial effect among those at highest risk, especially those with lower ejection fraction, enlarged hearts, and those in NYHA functional class Ⅲ or Ⅳ[1].

A post-hoc analysis evaluated men in the DIG study according to serum digoxin concentrations to assess if drug concentration had an association with mortality and hospitalizations. In this analysis, there was a reduction in all-cause mortality in patients with lower serum digoxin levels (0.5-0.8 ng/mL) with a 6.3% (95%CI: 2.1%-10.5%, P = 0.005) absolute lower mortality rate compared with patients receiving placebo. As the serum digoxin concentration increased, the absolute risk in mortality increased to the point that those with levels greater than 1.2 ng/mL had an 11.8% (95%CI: 5.7%-18.0%, P < 0.001) higher absolute mortality rate than patients receiving placebo. Similar conclusions persisted even with multivariable adjustments[12].

Finally, a recent meta-analysis by Hood *et al*[13] reviewed 13 randomized controlled trials where patients were randomized to digoxin and focused on mortality, hospitalization, and clinical status. This meta-analysis showed that digoxin had no effect on mortality which was mostly driven by the data from the DIG study. This meta-analysis also found that in the four studies that provided data on hospitalizations for worsening heart failure, digoxin had significantly fewer hospitalizations due to worsening heart failure with an overall relative risk reduction of 23.4% and number needed to treat ranging from 13-17[13].

Current guidelines

The most current ACC/AHA and ESC guidelines recommendation on the use of digoxin in heart failure with reduced ejection fraction and normal sinus rhythm are based on the prior studies. The ACC/AHA guidelines in 2013 (class Ⅱa, level of evidence B) as well as the

ESC guidelines in 2012 (class IIb, level of evidence B) recommended digoxin for symptomatic improvement and improved quality of life as well as to decrease hospitalizations for heart failure exacerbations[14,15]. The guidelines emphasize the importance of initiating goal-directed medical therapy as the primary treatment for heart failure due to its known mortality benefit. However, the guidelines continue to allow physicians discretion regarding digoxin and emphasize the importance of close monitoring for digoxin toxicity[14,15].

Atrial fibrillation with and without congestive heart failure

In the general population, AF is the most common sustained cardiac arrhythmia. For many years, the primary approach to treatment was to maintain normal sinus rhythm with anti-arrhythmic medications and cardioversion, as a rhythm control strategy was thought to decrease morbidity and mortality compared to a rate control strategy. However, after the Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) trial as well as other randomized clinical trials, there was a shift in practice towards maintaining rate control in asymptomatic patients, as these trials exposed no significant improvement in mortality with rhythm control[16-22]. Digoxin was one of the four rate control medications used in the AFFIRM trial and remains an option for rate control.

Over the past two decades, controversy regarding the use of digoxin in patients with AF has arisen due to the potential for adverse effects. An initial retrospective analysis of AFFIRM trial data found that digoxin was associated with lower survival[23]. Yet, these findings were attributed to the patients' comorbid conditions which placed them at increased risk of death, rather than to an adverse effect of the medication. This observation was confirmed in another retrospective analysis of AFFIRM trial[24].

Subsequently, a Swedish study evaluated one year mortality among patients admitted to the coronary care unit with AF, CHF, or both in relation to digoxin. This study found that long term use of digoxin was associated with lower survival in patients with AF without CHF, with an adjusted estimated hazard ratio (HR) of 1.42 (95%CI: 1.29-1.56)[25]. However, no significant increase in mortality risk was seen in patients with CHF alone or in combination with AF.

Two retrospective studies re-evaluated the safety of digoxin use from the AFFIRM database by correcting for potential confounders, but they used different methodologies and found apparently conflicting results[4,26]. The first retrospective analysis regarded digoxin as a time dependent covariate in a propensity-adjusted Cox model and found that digoxin was associated with increased all-cause mortality, with a HR of 1.41 (95%CI: 1.19-1.67, $P < 0.001$) as well as increased cardiovascular and arrhythmic mortality[4]. The increased all-cause mortality was also seen in patients with (HR = 1.41, 95%CI: 1.09-1.84, $P = 0.010$) and without (HR = 1.37, 95%CI: 1.05-1.79, $P = 0.019$) heart failure[4].

Shortly after, a second retrospective analysis was published using propensity matching to evaluate digoxin use at baseline. This analysis found no significant difference in all-cause mortality (HR = 1.06, 95%CI: 0.83-1.37, $P = 0.640$) or hospitalizations[26]. The differences in results between the two retrospective analyses appear to arise from the different statistical methods used, with each analysis carrying some potential bias[27]. The study by Whitbeck and colleagues had an indication bias that the authors mitigated using adjustment for covariates and propensity scores[4]. Meanwhile, the second study suffered from crossover bias and a depleted sample size associated with matching[26]. Although the authors' stated conclusions were not in agreement, it is worth noting that there was some overlap in their 95%CI for all-cause mortality and that the overlapping portion (1.19-1.37) is consistent with a clinically significant, deleterious effect of digoxin in this patient population.

The aforementioned analyses of AFFIRM data have been followed by many other studies. In an observational study using the National Health Insurance Research Database in Taiwan, 4781 patients with AF were studied. In this analysis, digoxin was associated with an increased risk of all-cause mortality, with an adjusted HR of 1.21 (95%CI: 1.01-1.44, $P = 0.037$)[28]. During subgroup analysis, digoxin portended worse survival among patients without heart failure but not among those with heart failure[28].

In one of the largest retrospective analyses evaluating newly diagnosed AF, the The Retrospective Evaluation and Assessment of Therapies in Atrial Fibrillation study evaluated 122465 patients in the Veterans Affairs health care system. The study found digoxin to be associated with increased mortality after multivariate adjustments (HR = 1.26, 95%CI: 1.23-1.29, $P < 0.001$) and propensity matching (HR = 1.21, 95%CI: 1.17-1.25, $P < 0.001$)[3]. This conclusion persisted even after accounting for kidney function and history of documented heart failure, heightening the concern that digoxin reduces survival. However, data regarding the degree of left ventricular dysfunction or the NYHA class were not available; it is unknown how accounting for the severity of heart failure would impact this study's findings.

Another large retrospective analysis of the Anticoagulation and Risk Factors In Atrial Fibrillation Cardiovascular Research Network trial evaluated digoxin in patients with new onset AF and no history of CHF. This observational study used patients belonging to the Kaiser Permanente database and living mainly on the west coast of the United States. In this study, digoxin was shown to be associated with a higher risk of death with HR = 1.71 (95%CI: 1.52-1.93, $P < 0.001$)[29]. This conclusion was robust in distinctions between intention-to-treat and as-treated analyses.

A post-hoc analysis of the Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin

K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF) evaluating digoxin use and its association with cardiovascular events was performed. The trial enrolled 14171 patients of which 5239 patients were taking digoxin at baseline. In this analysis, baseline digoxin use was associated with an increased all-cause mortality with an adjusted HR of 1.17 (95%CI: 1.04-1.32, $P = 0.009$)[30]. Similar findings persisted when accounting for covariates using a regression model as well as with a time-dependent model. In subgroup analysis, the all-cause increased mortality was observed among patients with and without heart failure, as judged by left ventricular function or NYHA status[30].

In a population based retrospective analysis evaluating digoxin in patients 65 years or older with and without heart failure, an increased risk of all-cause mortality was detected in analyses based on propensity matching and multivariable Cox regression modeling. In this study, the heart failure group had a 14% greater hazard of all-cause mortality with digoxin (adjusted HR = 1.14, 95%CI: 1.10-1.17, $P < 0.001$), similar to the non-heart failure group which had a 17% greater hazard of all-cause mortality with digoxin (adjusted HR = 1.17, 95%CI: 1.14-1.19, $P < 0.001$)[2].

Hazard ratios for total mortality are reported in Table 1 for the main AF studies with digoxin, as well as for patients with or without CHF.

Current guidelines

The 2014 AHA/ACC/Heart Rhythm Society (HRS) guidelines (Class IIa, level of evidence B) and 2010 ESC guidelines (Class IIa, level of evidence C) do not consider digoxin as a first line therapy for rate control in AF; however, digoxin can be considered in combination with a beta blocker and/or nondihydropyridine calcium channel blocker when the ventricular rate is poorly controlled in patients with underlying left ventricular dysfunction[36,37]. Due to controversy and concern regarding increased mortality in many post-hoc analyses, the guidelines continue to stress caution when administering medication and to periodically check digoxin levels, in an attempt to reduce adverse effects especially in the long term setting[36,37].

Digoxin use in myocardial infarction

The AHA/ACC and ESC guidelines agree that in certain clinical situations digoxin use in patients presenting with ST elevation myocardial infarction is effective; moreover, digoxin use has been deemed appropriate for AF rate control in patients presenting with CHF and ongoing ischemia[38,39]. With increased attention toward the risk/benefit tradeoff of digoxin therapy, a recent retrospective analysis evaluated whether patients chronically taking digoxin had increased in-hospital mortality when admitted for acute coronary syndrome. The analysis considered 20331 patients of which 244 were taking digoxin upon admission to the hospital, using multivariate modeling as well as propensity score matching. Neither statistical method showed significantly increased in-hospital mortality[40]. On the other hand, several studies performed in the 1990's evaluated outcomes among patients surviving a myocardial infarction (remotely after the index event) and found increased mortality with digoxin[40-43]. For instance, the retrospective study by Køber *et al*[41] found post-MI patients being treated with digoxin at one year and five years to have 38% and 74% mortality respectively *vs* much lower rates among those not receiving digoxin (8% at one year and 26% at five years), both differences being statistically significant[41]. However, many patients in these older studies were not on current standard therapies including beta blockers.

Current guidelines

Both the AHA/ACC and ESC guidelines address the use of digoxin in the acute management of patients who present with acute ST elevation myocardial infarction. Both sets of guidelines agree that, in patients who present with acute heart failure symptoms due to severe LV dysfunction and AF with rapid ventricular rates and ongoing ischemia, digoxin may be given intravenously to improve rate control without undue concern for negative inotropic effects from beta blockers and calcium channel blockers[38,39]. A potential downside of digoxin in this clinical setting though may be an increase oxygen consumption.

DISCUSSION

This review of the current literature regarding the use of digoxin in CHF with sinus rhythm, AF with and without CHF, and post myocardial infarction highlights the concern regarding mortality risk when using digoxin. In patients with CHF with sinus rhythm, there continues to be a niche for digoxin use as an adjunctive therapy for symptomatic control once goal directed therapy has been optimized, with the understanding that there is no effect on mortality as seen in the DIG study and with a close monitoring of digoxin level.

However, no randomized controlled trial has evaluated the role of digoxin in conjunction with the current mainstay treatment strategy for CHF. It is unknown whether findings from the DIG study or prior studies can be applied to the modern strategy for heart failure[13,44]. Recent observational studies have conflicting findings regarding digoxin when evaluating patients on current optimal heart failure therapy. Although the conflicts might be resolved by a contemporary randomized trial, such a trial may not take place[45-47]. Furthermore, as novel agents like Ivabradin and the new angiotensin receptor neprilysin inhibitor become more prevalent along with left ventricular assist devices, digoxin may become less relevant in this patient population especially that these new therapies have shown to improve survival[48,49].

Questions remain regarding digoxin as a rate control strategy for those with and without heart failure. A

Table 1 Hazard ratio estimates from studies describing the effects of digoxin on total mortality in patients with atrial fibrillation

Study	Subjects	Hazard ratio estimates[1]		
		Overall mortality	AF without CHF	AF with CHF
Shah *et al*[2]	140111	NA	[2]1.17 (95%CI: 1.14-1.19, $P < 0.001$)	[2]1.14 (95%CI: 1.10-1.17, $P < 0.001$)
Turakhia *et al*[3]	122465	[2]1.26 (95%CI: 1.23-1.29, $P < 0.001$)	[2]Significant, details not given	1.29 (95%CI: 1.23-1.36, $P < 0.001$)
TREAT AF		1.21 (95%CI: 1.17-1.25, $P < 0.001$)		1.28 (95%CI: 1.21-1.36, $P < 0.001$)
Hallberg *et al*[25]	38419	NA	[2]1.42 (95%CI: 1.29-1.56, $P < 0.001$)	1.00 (95%CI: 0.94-1.06)
Freeman *et al*[29]	14787	NA	[2]1.71 (95%CI: 1.52-1.93, $P < 0.001$)	NA
ATRIA-CVRN				
Washam *et al*[30]	14171	[2]1.17 (95%CI: 1.04-1.32, $P = 0.0093$)	1.18 (95%CI: 0.94-1.46)	1.24 (95%CI: 0.98-1.57)
ROCKET AF		1.14 (95%CI: 1.01-1.29, $P = 0.0402$)		
Gjesdal *et al*[31]	7329	[2]1.53 (95%CI: 1.22-1.92, $P < 0.001$)	NR	[2]Significant, details not given
SPORTIF III and V				
Chao *et al*[28]	4781	[2]1.21 (95%CI: 1.01-1.44, $P = 0.037$)	[2]1.28 (95%CI: 1.05-1.57)	0.88 (95%CI: 0.62-1.23)
Whitbeck *et al*[4]	4058	[2]1.41 (95%CI: 1.19-1.67, $P < 0.001$)	[2]1.37 (95%CI: 1.05-1.79, $P = 0.019$)	[2]1.41 (95%CI: 1.09-1.84, $P = 0.010$)
AFFIRM				
Friberg *et al*[32]	2824	1.10 (95%CI: 0.94-1.28, $P = 0.23$)	NR	NR
SCAF		1.04 (95%CI: 0.89-1.21)		
Gheorghiade *et al*[26]	1756	1.06 (95%CI: 0.83-1.37, $P = 0.640$)	1.08 (95%CI: 0.8-1.47, $P = 0.609$)	1.08 (95%CI: 0.69-1.69, $P = 0.743$)
AFFIRM				
Pastori *et al*[33]	815	[2]2.22 (95%CI: 1.42-3.48, $P < 0.001$)	NR	NR
Rodriguez-Manero *et al*[34]	777	1.42 (95%CI: 0.77-2.60, $P = 0.2$)	0.94 (95%CI: 0.20-4.41, $P = 0.9$)	1.6 (95%CI: 0.9-2.9, $P = 0.9$)
AFBAR				
Mulder *et al*[35]	608	[2]0.41 (95%CI: 0.19-0.89)	NR	NR
RACE II				

Data also subdivided to those with and without congestive heart failure when applicable. [1]May apply different statistical methods to estimate hazard ratios. [2]Statistically significant. CHF: Congestive heart failure; AF: Atrial fibrillation; NR: Not recorded; NA: Not applicable; SPORTIF: Stroke prevention using oral thrombin inhibitor in atrial fibrillation.

recent meta-analysis reviewing over 300000 patients with AF, CHF or both found that digoxin was associated with an overall 21% increased relative risk of all-cause mortality (HR = 1.21, 95%CI: 1.07-1.38, $P < 0.01$). The meta-analysis also showed increased risk of all-cause mortality during subgroup analyses of patients with AF (HR = 1.29, 95%CI: 1.21-1.39, $P < 0.01$) and CHF (HR = 1.14, 95%CI: 1.06-1.22, $P < 0.01$) even if the hazard ratio was lower than for the other subgroups included in this analysis (*i.e.,* AF without CHF)[50].

To date, no randomized trial has been performed to evaluate the use of digoxin and its associated risk in AF patients. Such a trial might provide clarity about whether digoxin should be indicated in this population but is unlikely to happen considering the generic nature of the drug, the development of new drugs[48,49] and the burden of current healthcare costs. There are also some ethical concerns in enrolling subjects in a trial on a drug where previous studies have shown at best a neutral effect on mortality while many others raise some serious concern on safety. Therefore our understanding of digoxin's adverse effects will likely continue to be driven by retrospective analyses, which have their inherent biases and limitations in trying to evaluate associations corrected for confounders. In many retrospective studies, it is unclear what digoxin dose and/or serum levels patients had during the trials. This may be the driving force for many of the noted adverse outcomes, as prior studies evaluating digoxin in heart failure patients found that those with higher digoxin levels experienced worse outcomes[12]. In the absence of more definitive data from a prospective randomized controlled trial, the widespread adoption of a rate control strategy for AF favoring digoxin as a single first line agent has been appropriately removed from the 2014 ACC/HRS/AHA guidelines; indeed, reasonably safe and inexpensive alternatives such as beta blockers or calcium channel blockers are readily available. The subgroup of patients for which digoxin remains most controversial, in our opinion, consists of those patients with AF and CHF, for whom a benefit for digoxin could potentially extend beyond rate control (*i.e.*, inotropic effect); these patients often have low blood pressure and may be very sensitive to negative inotropic drugs[51]. Another potential clinical situation that may warrant the careful use of digoxin is AF with very low blood pressure when beta blockers and calcium channel blockers cannot be utilized.

Finally, digoxin use in patients following a myocardial infarction requires further investigation, especially immediately post MI. In this particular situation, negative inotropic drugs such as beta blockers and calcium blockers can have a deleterious effect by precipitating or worsening CHF, and digoxin may be used to control AF with rapid ventricular response since it lacks negative inotropic properties. Overall, it seems unreasonable at this point of time with the available data to recommend discontinuing patients that are stable on digoxin or to start new patients on digoxin in some indications, provided that digoxin is used cautiously.

The worrisome signal linking digoxin to increased mortality has been identified by various studies employing different designs and/or statistical methods, even though this signal has not been clearly confirmed

by prospective randomized controlled trial data. It is possible that the increased mortality is due to dosages that were inappropriately high for some patients, but this remains impossible to ascertain from existing data. Based on the potential risks and benefits, as well as the presence of alternative drugs, there is little role for digoxin among patients who only have AF. The proper role of digoxin is, however, less certain in other subgroups of patients, such as those with AF and systolic CHF or at the acute phase of a myocardial infarction. Further studies may provide helpful information for such subgroups of patients.

COMMENTS

Background

Digoxin is one of the oldest drugs used today in cardiovascular medicine around the world, and was one of the first treatments for heart failure management. Currently, this drug is frequently used to treat heart failure symptoms and to decrease the ventricular rate in atrial fibrillation (AF). In regards to heart failure management, digoxin remains indicated for patients with persistent symptoms despite optimal medical therapy. In the setting of AF, digoxin is no longer mentioned in the 2014 guidelines as an option for rate control, except among patients with AF and heart failure; however, concerns arose regarding its safety even in this subgroup of patients. Current data regarding the safety and efficacy of digoxin is based on observational studies with conflicting results as no randomized controlled clinical trials have been performed. Authors' aim is to review the data available regarding the use of digoxin in congestive heart failure (CHF), AF, or after myocardial infarction, as well as the current guidelines for digoxin use from the American College of Cardiology/American Heart Association (ACC/AHA) and European Society of Cardiology (ESC).

Research frontiers

The concern linking digoxin to increased mortality has been shown by various studies; however, this has not been confirmed by prospective randomized controlled trials. If digoxin's role in patients with only AF is limited, its role and safety in certain subgroups of patients such as those with systolic CHF and AF or during the acute phase of a myocardial infarction remain unclear. Further studies may provide helpful.

Innovations and breakthroughs

Digoxin has been used for centuries to treat systolic congestive heart failure, AF and after myocardial infarctions. Authors' goal was to review manuscripts concerning digoxin and mortality in these populations. The authors discussed the current data available and concisely displayed the data in tabular form to summarize the findings. The authors also reviewed the current recommended guidelines from the ACC/AHA and ESC regarding each subgroup when available.

Applications

Given the potential risks and benefits of digoxin, as well as the presence of alternative drugs, there is little role for digoxin among patients who only have AF. The proper role of digoxin is, however, less certain in other subgroups of patients, such as those with AF and systolic CHF or at the acute phase of a myocardial infarction. Further studies may provide helpful information for such subgroups of patients.

Peer-review

In this systematic review, the authors have provided a thorough and critical analysis of the use of digoxin in multiple clinical settings including patients with systolic congestive heart failure, AF or after myocardial infarction.

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CASE REPORT

Catheter-based intervention for symptomatic patient with severe mitral regurgitation and very poor left ventricular systolic function - Safe but no room for complacency

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Institutional review board statement: This case series is approved by the Institutional Review Board of Rigshospitalet University Hospital (Denmark), Hull and East Yorkshire Hospitals NHS Trust (United Kingdom) and the Royal Brompton Hospital (United Kingdom) where the patients were treated.

Informed consent statement: All patients gave informed consent.

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Abstract

Many patients with left ventricular systolic dysfunction have concomitant mitral regurgitation (MR). Their symptoms and prognosis worsen with increasing

severity of MR. Percutaneous MitraClip® can be used safely to reduce the severity of MR even in patients with advanced heart failure and is associated with improved symptoms, quality of life and exercise tolerance. However, a few patients with very poor left ventricular systolic function may experience significant haemodynamic disturbance in the peri-procedural period. We present three such patients, highlighting some of the potential problems encountered and discuss their possible pathophysiological mechanisms and safety measures.

Key words: Mitral regurgitation; Mitral valve; Left ventricular systolic dysfunction; Chronic heart failure; MitraClip; Percutaneous

Core tip: We described three patients with severe mitral regurgitation and very poor left ventricular systolic function undergoing percutaneous MitraClip treatment. These patients experienced haemodynamic instability peri-procedurally immediately upon reduction of their mitral regurgitation. These patients shared a few features such as right ventricular dysfunction and pulmonary hypertension which may help to identify those who may develop such peri-procedural haemodynamic compromise. Our cases highlight that although MitraClip is generally a safe procedure, there should not be complacency especially when treating patients with very poor left ventricular function. Extreme caution and vigilance should be exercised when treating such patients.

Loh PH, Bourantas CV, Chan PH, Ihlemann N, Gustafsson F, Clark AL, Price S, Di Mario C, Moat N, Alamgir F, Estevez-Loureiro R, Søndergaard L, Franzen O. Catheter-based intervention for symptomatic patient with severe mitral regurgitation and very poor left ventricular systolic function - Safe but no room for complacency. *World J Cardiol* 2015; 7(11): 817-821 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i11/817.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i11.817

INTRODUCTION

Approximately 35%-50% of patients with left ventricular systolic dysfunction (LVSD) develop significant mitral regurgitation (MR)[1]. As the severity of MR increases, their symptom and prognosis worsen with a 5-year mortality up to 60% in those with severe MR[2]. Whether treating MR in these patients alters the prognosis or progression of LVSD is not known. Percutaneous intervention for mitral regurgitation is now possible and can reduce the severity of MR even in patients with advanced heart failure with associated improvement in symptoms, quality of life and exercise tolerance[3,4]. However, special consideration should be given to patients with very poor left ventricular (LV) systolic function who may have very fragile hemodynamics that can be easily disturbed. We present a series of cases which outline some potential concerns.

CASE REPORT

Case 1

A 72-year-old man presented with NYHA class Ⅲ symptoms of heart failure and was found to have a dilated LV with severe LVSD and moderate-to-severe MR due to ischemic heart disease (IHD) (Table 1). He had previously undergone coronary artery bypass grafting, and had a history of cerebrovascular accident. Echocardiography demonstrated significant right ventricular (RV) impairment and moderately raised pulmonary arterial systolic pressure (PASP) (Table 1).

A centrally placed MitraClip reduced the severity of MR to mild. Although there was no change in blood pressure (BP) which was maintained in the region of 96/50 mmHg, trans-esophageal echocardiogram (TEE) showed worsening of LV systolic function (LV ejection fraction < 10%) and spontaneous echo contrast in the LV. After reversal of general anaesthesia, he had an episode of ventricular fibrillation that was successfully defibrillated with a single biphasic DC shock. Adrenaline and dobutamine support had to be administered for 48 h. At discharge, echocardiography demonstrated mild residual MR with LV ejection fraction (LVEF) returned to 20%. His symptoms improved to NYHA Ⅱ.

Case 2

A 78-year-old lady with severe MR, a dilated LV and severe LVSD secondary to IHD had persistent NYHA class Ⅲ symptoms despite optimal medication and cardiac resynchronisation therapy (CRT) (Table 1). She had moderate aortic stenosis with a valve area of 1.4 cm^2, moderate RV impairment and raised PASP (Table 1).

A centrally placed MitraClip successfully reduced the severity of her MR to mild. However, there was acute deterioration in her LV systolic function on TEE and a reduction in her cardiac output from 3.5 to 3.1 L/min. Her BP dropped from 104/60 to 88/54 mmHg. The MitraClip was re-positioned and deployed more medially which left her with moderate MR but her LV function, cardiac output and BP recovered. At discharge, echocardiography showed residual moderate MR and LVEF 15%. Her symptoms improved to NYHA class Ⅱ which was sustained at 1-mo follow-up.

Case 3

A 68-year-old man with severe LVSD due to idiopathic dilated cardiomyopathy had severe MR and persistent NYHA Ⅲ-Ⅳ breathlessness despite optimal medication and CRT. He had RV dilation with mild RV systolic impairment and raised PASP (Table 1).

Initial placement of the MitraClip reduced the severity of his MR to trivial. However, there was worsening of

Table 1 Echocardiographic characteristics of the patients

	LVEF (%)	LVEDD (mm)	Severity of MR	Cause of MR	Mid RV diameter (mm)	RV systolic dysfunction	PASP (mmHg)
Case 1	20	64	Moderate-to-severe	Secondary	51	Severe	50
Case 2	15	68	Severe	Secondary	42	Moderate	65
Case 3	20	70	Severe	Secondary	45	Mild	65

LVEDD: Left ventricular end-diastolic diameter; LVEF: Left ventricular ejection fraction; MR: Mitral regurgitation; PASP: Pulmonary arterial systolic pressure; RV: Right ventricle.

his LV systolic function on TEE with reduction in cardiac output from 4.9 to 4.3 L/min (Figure 1) and BP from 116/55 to 97/42 mmHg. These gradually improved to baseline after a period of observation and the clip was deployed. Invasive hemodynamic study showed an immediate reduction in his pulmonary arterial systolic pressure from 57 to 46 mmHg and mean left atrial pressure from 32 to 24 mmHg. At discharge, his symptom improved to NYHA II with trivial MR and LVEF of 20% on TTE.

DISCUSSION

These cases suggest that although percutaneous intervention for MR can be safely performed in patients with poor LV systolic function[3,4], significant clinical deterioration can occur in those with very poor LV function. The exact mechanism is unclear but likely to be due to a complex interaction of various cardiac mechanics and haemodynamic factors. In clinical setting, the haemodynamic effects following acute reduction of MR has been poorly understood as most were extrapolated from surgical patients and can be confounded by factors such as cardioplegia and cardiopulmonary bypass. Siegel *et al*[5] evaluated the acute haemodynamic effects of MitraClip therapy in 107 patients and found that successful MitraClip treatment was associated with an increase in cardiac output, cardiac index and forward stroke volume with a reduction in LV end diastolic pressure and calculated peripheral vascular resistance. These haemodynamic changes were thought to reduce the risk of low cardiac output state following the reduction of MR. However, the mean LVEF in their study was approximately 60% and patients with significant LVSD was not represented in their study[5]. In a predominantly secondary MR cohort of 50 patients with overall lower normal range of cardiac index (CI) and mean LVEF approximately 50%, Gaemperli *et al*[6] reported an improvement in CI following MitraClip. However, recent report suggests that afterload mismatch can manifest as early transient worsening of LV function following Mitraclip in patients with poorer LV function (mean LVEF 26%)[7].

It is likely that complex haemodynamic factors may be involved in patients with severe MR and significant LVSD, especially in those with very low

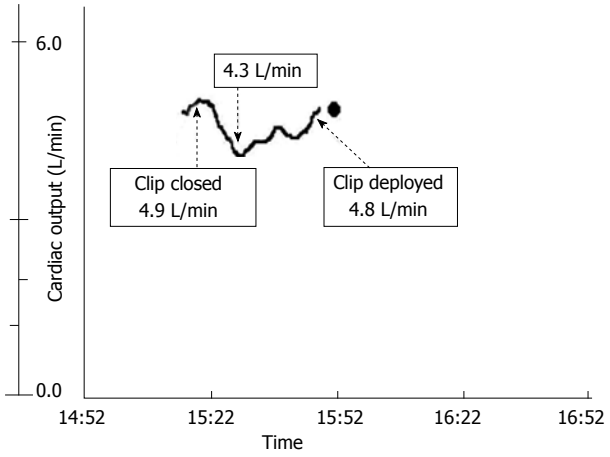

Figure 1 Cardiac output was measured by a Swan-Ganz thermodilution catheter inserted *via* the left femoral vein[8].

LVEF[8,9]. The LV contractility is regulated by the Frank-Starling mechanism, force-frequency relationship and neurohormonal control. The effectiveness of the later two mechanisms is impaired in failing human heart but the Frank-Starling mechanism can be preserved even in end-stage heart failure[10]. In the failing ventricles, the maximum LV performance is achieved at much larger LV volumes than that of the normal ventricles. This shows the importance of the Frank-Starling mechanism as a vital compensatory mechanism to maintain LV systolic contraction in the presence of significant LVSD[11]. In an experimental model using dogs with chronic heart failure, a reduction of LV end-diastolic diameter to normal level by abrupt inferior vena caval occlusion led to further reduction in LV systolic function indicating that the increase in LV preload is crucial to maintain LV systolic function[12]. Therefore, an acute and significant reduction in MR may lead to a decrease in LV end-diastolic volume (preload)[5], compromising the Frank-Starling compensatory mechanism and causes further deterioration in the LV systolic performance. In a way, this may be similar to that observed in the Fontan circulation where preload is known to be the most important determinant of cardiac output especially in the presence of a dilated or impaired ventricle[13].

Another potential factor is the poorly understood "pop-off valve" concept[14]. It was suggested that in patients with poor LV function, there is a need for the ventricle to offload into the low-impedance left atrium. However, the improvement in the outcome of mitral valve surgery in patients with poor LV function and understanding in the importance of chordal preservation has disputed this concept[15-17]. Nevertheless, it may be relevant in patients with very poor LV systolic function experiencing acute and significant reduction in MR. In experimental canine model where an external LV-to-left atrial shunt was created to mimic the effect of MR, acute shunt closure led to an increase in the LV mean systolic pressure and wall stress (afterload)[18,19]. Closure of the shunt was associated with an improvement in the haemodynamic state with reduction in LV end-

diastolic pressure (LVEDP) and increased forward cardiac output. However, in the presence of severe LV systolic dysfunction, LVEDP increased (preload) despite a reduction in LV diastolic filling and the forward flow decreased[18]. The increase in LV systolic and diastolic wall stress leads to an increase in myocardial oxygen consumption; whilst increased LVEDP may reduce coronary blood flow leading to relative subendocardial ischaemia[20]. This represents an experimental model which is not confounded by the effect of cardioplegia and cardiopulmonary bypass and may partly explain the adverse events in our patients. Since percutaneous MitraClip can be performed without cardiopulmonary bypass, the real-time beat-to-beat hemodynamic variables, LV function and MR severity can be assessed. This may help understand the pathophysiology of haemodynamic disturbance following acute MR reduction in patients with significant LVSD.

Our patients shared some common features[7] which may have contributed to the acute adverse events. They had dilated LV with advanced LVSD (LVEF $\leqslant$ 20%), pulmonary hypertension, RV systolic impairment and significant MR with substantial reduction following intervention. A cautious approach in managing the patients with very poor LV systolic function is obligatory, especially in the light of the surgical experience[1].

Every patient with significant LVSD should have individualised management within a multi-disciplinary team approach involving the interventional cardiologists, cardiac surgeons, cardiac anaesthetists and intensive care physicians, heart failure and imaging specialists and other paramedical members. Treatment strategies and options in case of procedural failure should be discussed. The patients should be pre-assessed thoroughly whilst receiving optimal heart failure therapy. TEE is mandatory to determine the anatomical suitability for the procedure and cardiac catheterisation can provide accurate haemodynamic evaluation. The assessment of LV contractile reserve and RV function may be helpful[16].

Pre-procedurally, some patients may need stabilisation of their clinical state with intravenous diuretic and/or nitrate infusion. In patients at risk of significant haemodynamic disturbance during the procedure, pre-procedural infusion of inodilators or insertion of intra-aortic balloon pump (IABP) may be considered with close liaison with all who are involved in the care of the patient including the heart failure and intensive care teams.

During the procedure, LVEF may decrease immediately after the reduction of MR in some patients. Since LVEF represents a combination of forward stroke volume (SV) and regurgitant volume (RVol) of MR, reduction in the RVol may lead to a net increase in SV even if the LVEF reduces[5,9]. Without any significant changes in the heart rate, an increase in cardiac output and systolic blood pressure are signs of positive acute haemodynamic response. Conversely, a reduction in cardiac output suggests serious haemodynamic compromise. Therefore, cardiac output monitoring is important during the peri-procedural period. Surrogate findings such as lower central venous saturation, reduction in systolic blood pressure and the appearance of spontaneous echo contrast in the LV may indicate a decrease in cardiac output. Other information, such as mitral valve opening area, left atrial or pulmonary capillary wedge pressure, left ventricular pressure[9] and if available, contractility derived from a conductance catheter[21] might help assess the situation. Continuous observation of the haemodynamic response following provisional clip deployment but before final release allows the situation to be assessed and as in patient 2, the clip to be repositioned if there is a persistent unfavourable hemodynamic response.

Adverse events may still develop some time after the procedure and so the patients should be monitored continuously until they have fully recovered from the acute effect of the procedure and general anaesthesia.

Patients with poor LV function and significant MR can benefit from percutaneous MitraClip treatment. However, some of them may experience significant acute hemodynamic disturbance peri-procedurally and caution is necessary when treating these patients.

COMMENTS

Case characteristics

Peri-procedural haemodynamic instability during percutaneous MitraClip in three patients.

Clinical diagnosis

Decreased cardiac output and blood pressure on invasive haemodynamic study.

Differential diagnosis

Afterload mismatch, access site bleeding, cardiac tamponde.

Laboratory diagnosis

Invasive hemodynamics and echocardiographic are the main diagnostic tools.

Imaging diagnosis

Trans-esophageal echocardiogram confirmed deteriorating left ventricular (LV) function and often with appearance of spontaneous echo contrast in the LV.

Treatment

Cautious and intensive haemodynamic support and monitoring before MitraClip deployment is effective in most cases; but rare cases may require repositioning of MitraClip to avoid substantial acute reduction of mitral regurgitation.

Related reports

Prior hemodynamic studies mainly limited to patients with better left ventricular ejection fraction. However, similar to this case series, Melisurgo *et al* reported a small group of patients with poor LV function experiencing afterload mismatch peri-procedurally.

Term explanation

"Pop-off valve" concept proposed that mitral regurgitation (MR) allows a degree of afterload reduction in the LV with poor systolic function and this is abolished upon acute reduction in the regurgitant volume. The resultant increase in LV afterload casues afterload mismatch leading to worsening LV function and

haemodynamic instability.

Experiences and lessons

Although MitraClip is beneficial, a minority of patients with very poor LV function and significant MR may experience significant acute hemodynamic disturbance peri-procedurally, especially in those with co-existing right ventricular dysfunction and pulmonary hypertension. Hence caution is necessary when treating these patients.

Peer-review

The article describes a group of patients with very poor LV function and severe secondary MR who may experience significant haemodynamic disturbance during percutaneous MitraClip treatment. Although it is a relatively safe procedure, there is no room for complacency and extreme caution needs to be taken when treating those patients with very poor LV function.

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LETTER TO THE EDITOR

Imaging of pannus formation in patients with mechanical heart valves

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Abstract

Patient-prosthesis mismatch (PPM) should be recognized in patients with elevated transprosthetic gradients but without leaflet immobility, since the treatment strategy may differ in either etiology. However, thrombus and/or pannus formation should be excluded before a diagnosis of PPM is made. Particularly, pannus formation may not be diagnosed with 2-dimensional transesophageal echocardiography. Electrocardiographically gated 64-section multidetector computed tomography (MDCT) may be a promising tool in diagnosing or excluding pannus formation. Our report underlines the utility of MDCT in this regard and also emphasizes the importance of recognition of PPM as a differential diagnosis in such patients.

Key words: Multidetector computed tomography; Pannus formation; Patient prosthesis mismatch; Prosthetic heart valves; Transesophageal echocardiography

Core tip: Elevated transprosthetic gradients may be caused by pannus and/or thrombus formation or patient prosthesis mismatch (PPM). The differentiation between these three diagnoses is essential since the treatment strategy may differ in either etiology. Our report emphasizes the usefulness of cardiac multidetector computerized tomography in cases with suspected pannus formation which may not be diagnosed without surgical confirmation. Moreover, we underline the importance of recognizing PPM which may easily be overlooked in patients with elevated transprosthetic gradients. Indeed, pannus, trombus or any other masses as the cause of prosthetic dysfunction should be ruled out for a diagnosis of PPM.

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TO THE EDITOR

We would like to comment on the recent article by Soumoulou *et al*[1] which reports a case of obstructed

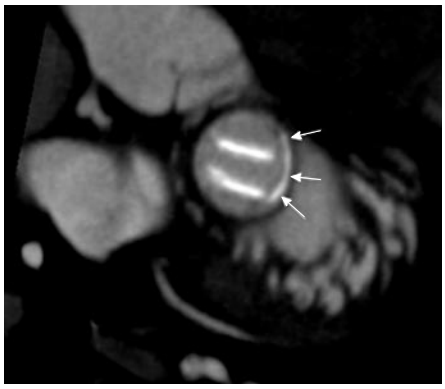

Figure 1 Pannus formation visualized as a high-attenuated periprosthetic mass.

prosthetic aortic valve caused by pannus formation, in which a preoperative definitive diagnosis could not be made by multiple imaging methods. We appreciate the authors since they emphasize the importance of clinical suspicion along with using multimodality imaging in recognizing this infrequent but serious complication of valve replacement surgery. However, two major issues remain to be addressed.

The differential diagnosis of a patient with elevated transprosthetic gradients should include not only pannus formation and thrombosis, but also patient prosthesis mismatch (PPM), since the treatment strategy may differ in either etiology. In the reported case, since there was no identifiable mass on transesophageal echocardiography (TEE) and no limited excursion of prosthetic leaflets, PPM had to be recognized before the decision of re-operation. Because, if the patient had had PPM, improvement of transprosthetic gradients after re-operation woud have been unlikely. Although the prosthetic valve size (#23 St. Jude) is not small and there is no information regarding the patient's body surface area, PPM can not be excluded unless the presence of a periprosthetic mass (pannus or thrombus) is precisely excluded. Real-time 3-dimensional TEE may be a promising tool as previously reported[2]. Although thrombus can be excluded by TEE, pannus may not be diagnosed in most of the cases. We have previously demonstrated that pannus formation may be visualized as a high-attenuated periprosthetic mass (Figure 1) and thrombus can be demonstrated as a low attenuated periprosthetic mass on electrocardiographically gated 64-section multidetector cardiac computed tomography (MDCT)[3-6]. Although the authors mention the use of cardiac computed tomography pre-operatively, there is no information regarding the slice number of the MDCT, use of intravenous contrast agent, electrocardiographic gating during the scan. Hence, without appropriate use of cardiac MDCT, pannus or thrombus may not be visualized. Fortunately, pannus formation was diagnosed peri-operatively in the current case, and the patient was succesfully re-replaced with another mechanical prosthesis.

Clinicians should be cognizant of PPM, when evaluating a patient with elevated transprosthetic gradients but without leaflet blockade. Thrombus can readliy be excluded with TEE but, pannus visualization may require more sophisticated imaging with MDCT in addition to TEE.

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