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WJC 6th Anniversary Special Issues (1): Hypertension

Pulmonary hypertension and metabolic syndrome: Possible connection, PPARγ and Caveolin-1

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Abstract

A number of disparate diseases can lead to pulmonary hypertension (PH), a serious disorder with a high morbidity and mortality rate. Recent studies suggest that the associated metabolic dysregulation may be an important factor adversely impacting the prognosis of PH. Furthermore, metabolic syndrome is associated with vascular diseases including PH. Inflammation plays a significant role both in PH and metabolic syndrome. Adipose tissue modulates lipid and glucose metabolism, and also produces pro- and anti-inflammatory adipokines that modulate vascular function and angiogenesis, suggesting a close functional relationship between the adipose tissue and the vasculature. Both caveolin-1, a cell membrane scaffolding protein and peroxisome proliferator-activated receptor (PPAR) γ, a ligand-activated transcription factor are abundantly expressed in the endothelial cells and adipocytes. Both caveolin-1 and PPARγ modulate proliferative and anti-apoptotic pathways, cell migration, inflammation, vascular homeostasis, and participate in lipid transport, triacylglyceride synthesis and glucose metabolism. Caveolin-1 and PPARγ regulate the production of adipokines and in turn are modulated by them. This review article summarizes the roles and inter-relationships of caveolin-1, PPARγ and adipokines in PH and metabolic syndrome.

Key words: Adiponectin; Caveolin-1; Leptin; Metabolic Syndrome; Pulmonary hypertension; Peroxisome proliferator-activated receptor

Core tip: Pulmonary hypertension (PH) is a devastating disease with a high morbidity and mortality rate. Recent studies indicate that the metabolic alterations that occur during the course of PH have a negative effect. Importantly, PH has been observed in patients with metabolic syndrome. Caveolin-1, a membrane protein and peroxisome proliferator-activated receptor γ, a ligand activated transcription factor are abundantly expressed in vascular cells and adipocytes. They play a significant role in maintaining vascular health, and participate in glucose and lipid metabolism. Furthermore, the proximity of vasculature and adipose tissue facilitates reciprocal influence during health and disease.

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INTRODUCTION

Chronic inflammation plays a significant role in metabolic syndrome and vascular diseases including pulmonary hypertension (PH). Adipose tissue not only functions as an energy store, but also as an endocrine system producing bioactive substances that influence metabolic and vascular homeostasis. Adipocytes play an important role in regulating inflammatory response. Obesity is associated with chronic inflammation, activation of proinflammatory

cytokines, and with the infiltration of adipose tissue with macrophages and lymphocytes[1,2]. Interestingly, increased plasma and lung levels of pro-inflammatory cytokines[3,4] and perivascular infiltration of inflammatory cells and neo-lymphogenesis in peri-bronchial areas[5-7] have been reported in human and experimental forms of PH. Both caveolin-1, a plasma membrane protein and peroxisome proliferator-activated receptor (PPAR) γ, a ligand-activated transcription factor belonging to the nuclear hormone receptor family are expressed abundantly in adipose and vascular tissues. They modulate inflammation, vascular contractility, cell proliferation, cell cycle progression, and play a significant role in maintaining vascular health, and participate in glucose and lipid metabolism[8-11]. Furthermore, perivascular adipose tissue (PVAT) has been shown to modulate vascular function. Under normal circumstances, it produces relaxing factors including nitric oxide (NO), and participates in anti-contractile function[12].

PULMONARY HYPERTENSION

A mean pulmonary artery pressure ⩾ 25 mmHg constitutes PH. A number of disparate conditions are known to give rise to PH. PH is classified into 5 major clinical groups, that has recently been updated[13]. Group 1 labeled as pulmonary arterial hypertension (PAH) includes idiopathic, heritable PAH and PAH associated with bone morphogenic protein receptor Ⅱ mutation, congenital heart defect, connective tissue diseases, portal hypertension, infection and drug toxicity. Included in this group are pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis as subcategory 1', and recently, persistent pulmonary hypertension of the newborn was assigned the subcategory 1". The next 4 groups are labeled as PH; Group 2: PH associated with pulmonary venous hypertension secondary to left ventricular diseases, Group 3: chronic lung diseases and accompanying hypoxia leading to PH, Group 4: chronic thrombo-embolic PH and Group 5 includes miscellaneous diseases such as myeloproliferative diseases, thyroid, hematological and renal diseases. Irrespective of the underlying disease, the main features of PH are impaired vascular reactivity and remodeling, elevated pulmonary artery pressure and right ventricular hypertrophy, leading to right ventricular failure and premature death. Clinical and experimental studies suggest that the endothelial dysfunction/disruption may be an important underlying factor in the pathogenesis of PH. Importantly, endothelial dysfunction and molecular changes in pulmonary vasculature are reported to occur before the onset of PH[14,15].

Endothelial cells (EC) are heterogeneous; they play a specialized role in the context of a specific organ. EC modulate Ca^{2+} entry, produce vascular relaxants such as NO, prostacyclin and endothelium-derived hyperpolarizing factor and maintain vascular tone, and participate in barrier function. Inflammation plays an important role in the pathogenesis of PH. EC bear the major brunt of injuries such as increased pulmonary blood flow and shear stress, inflammation, chemical/drug toxicity, ventilation-induced injury and hypoxia resulting in endothelial dysfunction. In response to injury, EC become activated and secrete several cytokines and adhesion molecules that can affect coagulation, barrier function, and facilitate cellular adhesion and transmigration of leukocytes leading to EC dysfunction. Endothelial dysfunction leads to impaired vascular relaxation response, and the activation of proliferative and anti-apoptotic pathways, inflammatory response, and thrombogenic state leading to progressive vascular remodeling, elevated pressure and right ventricular hypertrophy[16].

Caveolin-1 and pulmonary hypertension

In the 1950s, Palade and Yamada independently described caveolae, 50-100 nm flask shaped invaginations rich in cholesterol and sphingolipids. Caveolae are a subset of lipid rafts found on the plasmalemmal membranes of a variety of cells including endothelial, smooth muscle, epithelial cells, fibroblasts and adipocytes. Caveolae serve as a platform and compartmentalize the signaling molecules that reside in or are recruited to caveolae. Caveolae are also involved in transcytosis, endocytosis, potocytosis, and in the regulation of cell proliferation, differentiation and apoptosis *via* a number of diverse signaling pathways. Three isoforms of caveolin gene family have been identified. Caveolin-3 is muscle specific, found primarily in skeletal and cardiac myocytes. Caveolin-2 co-localizes with caveolin-1 and requires caveolin-1 for its membrane localization. Caveolin-1 (22 kD) is the major constitutive protein of caveolae[17]. Polymerase 1 and transcript receptor factor (PTRF/cavin), a caveolar coat protein, however, is required for caveolar formation and sequestration of caveolin-1 into caveolae[18]. Caveolin-1 is expressed in terminally differentiated cells including adipocytes, EC, epithelial cells, fibroblasts and myocytes. Caveolin-1 interacts and negatively regulates proteins such as Src family of kinases, G-proteins and G-protein-coupled receptors, eNOS, integrins and several growth factor receptors; and these interactions occur through caveolin-1-scaffolding domain (CSD, residue 82-101 in caveolin-1). For optimal activation, eNOS is targeted to caveolae, and caveolin-1 inhibits eNOS through its interaction. Heat shock protein (HSP) 90 binds to eNOS in a Ca^{2+}-calmodulin-depedent manner, reducing the inhibitory influence of caveolin-1, and increasing eNOS activity. However, caveolin-1 is essential for proper eNOS activation. Caveolin-1 regulates Ca^{2+} entry into EC, which is important for eNOS activation as well as the activation of other vasodilators, prostacyclin and endothelium-derived hyperpolarizing factor[19]. In addition, caveolin-1 regulates not only eNOS-derived NO but also eNOS-derived superoxide. It is involved in the sequestration of uncoupled eNOS; it prevents eNOS oxidase activity, and inhibits superoxide formation[20]. Caveolin-1 keeps smooth muscle cells (SMC) in quiescence; and it modulates Ca^{2+} regulatory molecules, increases Ca^{2+} mobilization and facilitates contractile response to agonists. Disruption of caveolin-1 has been shown to reduce myogenic tone and impair contractile responses to several agonists[21,22]. The dynamic interrelationship

between caveolin-1 and eNOS is critical for vascular homeostasis.

In several experimental models, the loss of endothelial caveolin-1 and the reciprocal activation of proliferative and antiapoptotic pathways such as PY-STAT3, cyclin D1 and Bcl-xL have been shown to occur before the onset of PH. The rescue of caveolin-1 inhibits the proliferative pathways and attenuates PH[15,23,24]. Besides, the mutation of caveolin-1 gene in humans is reported to be associated with PH[25]. Studies with caveolin-1 knockout mice have further highlighted the importance of caveolin-1 in pulmonary vasculature. The re-expression of endothelial caveolin-1 has been shown to attenuate PH, vascular dysfunction and cardiomyopathy in these mice[26]. Increased expression of PDGF-R β, the activation of PY-STAT3 and its downstream signaling pathways, cyclin D1 and Bcl-xL have been reported in pulmonary arteries from patients with PH as well as in the MCT and hypoxia models of PH[24,27-29]. The activation of PY-STAT3 is essential for PDGF-induced cell proliferation; and the inhibition of the PDGF receptor suppresses cell proliferation *via* the inactivation of STAT3 signaling[30,31]. Importantly, caveolin-1 acts as a suppressor of cytokine signaling, and inhibits PY- STAT3 activation and modulates proinflammatory cytokines[32] and it inhibits other proliferative pathways including PDGF-R β, cyclin D1, Bcl-xL. It promotes cell cycle arrest *via* a $p53/p21^{waf1/cip1}$-dependent mechanism and regulates apoptosis by inhibiting survivin[33,34].

In the monocrotaline (MCT) model of PH, at 2 wk post-MCT, there is a significant loss of endothelial caveolin-1 associated with the activation of proliferative and anti-apoptotic pathways, PH and right ventricular hypertrophy. As the pulmonary vascular disease progresses, by 4 wk, extensive endothelial caveolin-1 loss and EC damage occur, followed by an enhanced expression of caveolin-1 in vascular SMC. This is associated with a significantly increased expression and the activity of matrix metalloproteinase (MMP) 2 that is known to participate in cell proliferation and cell migration. Normally, MMP2 is inhibited by caveolin-1; the activation of MMP2 in the presence of enhanced expression of caveolin-1 in SMC suggests that this caveolin-1 may have lost its inhibitory function[15]. Enhanced expression of caveolin-1 in SMC has been reported in patients with idiopathic PAH, PAH associated with congenital heart defect and drug-toxicity[35-37]. Pulmonary arterial SMC from idiopathic PAH revealed not only enhanced expression of caveolin-1, but also Ca^{2+} dysregulation and increased DNA synthesis which could be blocked by silencing caveolin-1[35]. This caveolin-1 in SMC becomes pro-proliferative, and facilitates cell proliferation and migration. The about face of caveolin-1 function in PH is not unlike what has been reported in cancer[17]. The effect of caveolin-1, thus, may depend on its location, conformation, state of the disease and cell context.

PPARγ and pulmonary hypertension

PPARs constitute a subfamily of nuclear receptors, the master transcriptional regulators of nutrient metabolism and energy homeostasis. Three isoforms of PPAR have been identified (α, β/δ and γ). PPARα is thought to regulate fatty acid oxidation and glucose homeostasis, and is predominantly found in liver, muscle and kidneys. Recent studies have shown that PPAR β/δ agonists relax pulmonary and mesenteric arteries independent of cGMP and cAMP mechanisms. PPARγ is expressed in several types of tissue, including adipocytes, EC and SMC. It is an important regulator of genes involved in cell differentiation, cell growth, inflammation and angiogenesis. It forms an obligatory heterodimer with another nuclear receptor, retinoid-X-receptor which binds to peroxisome proliferator response elements that is located in the regulatory domains of genes[38,39]. PPARγ inhibits the production of chemokines in EC and the activation of NFκB[40]. In addition, it inhibits inter cellular adhesion molecules (ICAM) and vascular cellular adhesion molecules (VCAM)[41]. Furthermore, PPARγ increases NO production from EC and regulates superoxide generation at the EC membrane[42,43]. PPARγ has also been shown to reduce vascular SMC proliferation and migration[44]. In an arterial injury model, PPARγ was shown to have attenuated neointimal hyperplasia by modulating protein kinase G[45]. Reduction in the expression of PPARγ has been reported in human PAH and several experimental forms of PH such as vascular endothelial growth factor (VEGF) receptor blocker + hypoxia[46] and a shunt model[47] Endogenous ligand 15-deoxy-Δ(12,14) prostaglandin J2 and thiozolidinedione (TZD) compounds used in the treatment of diabetes activate PPARγ. Interestingly, TZD compound has been reported to attenuate the hypoxia-induced PH in mice[48]. However, PPARγ has also been shown to increase plasminogen activator inhibitor type-1 expression in EC which can affect vascular disease adversely[49]. PPARγ within the atheromatous lesion has a propensity to facilitate angiogenesis[50]. Furthermore, PPARγ not only upregulates caveolin-1 expression but also promotes some forms of cancer[51,52]. PPARγ does play an important role in vasculature but its effects may depend on the state of disease and the cellular context; and the activation of PPARγ may not be effective in all forms of PH.

Pulmonary hypertension and associated metabolic alterations

Metabolic alterations that occur in PH negatively impact the disease. In PH, mitochondrial metabolic shift from oxidative phosphorylation to glycolytic pathway has been shown to occur in pulmonary vasculature as well as in the right ventricle. When this shift occurs in aerobic conditions, it is termed "Warburg effect" which leads to the down regulation of mitochondrial glucose oxidation. It is accompanied by fragmented, hyperpolarized mitochondrial reticulum, decreased superoxide dismutase2, metabolic shift, increased hypoxia inducible factor (HIF)-1α, and the activation of pyruvate dehydrogenase kinase[53]. Glycolytic pathway is associated with resistance to apoptosis; an important feature of PH. EC isolated from idiopathic PAH pulmonary arteries exhibit increased glyco-

lytic rate, decreased mitochondrial DNA levels and fewer mitochondrial numbers per cell. In addition, increased glycolytic rate has also been shown to occur in the lungs of patients with idiopathic PAH[54]. Hyperpolarization of the mitochondrial membrane is thought to be a feature of Warburg phenotype, and apoptosis is induced by the activation of voltage-gated K^+ channel (Kv) and depolarization of mitochondrial membrane[55]. Mitochondrial hyperpolarization is thought to be the underlying cause of the metabolic switch observed in PH. Importantly, the loss of caveolin-1 has been shown to lead to mitochondrial dysfunction, membrane hyperpolarization, and the mitochondrial production of oxidant species. Interestingly, the glycolysis inhibition abolishes the increase in oxidant species in caveolin-1 knock-down vascular EC[56], indicating that caveolin-1 may have a key role in the regulation of oxidative stress and metabolic switch. Recent studies have shown decreased expression of mitochondrial uncoupling protein2 and increased mitochondrial potential in pulmonary arterial SMC from patients with idiopathic PAH and from experimental models of PH. Interestingly, reactive oxygen species inhibitors decrease cell proliferation in pulmonary arterial SMC with absent mitochondrial uncoupling protein2 expression[57]. In addition, treatment with dichloroacetate that increases the mitochondrial oxidative phosphorylation has been shown not only to prevent but also to reverse MCT-induced PH[58]. Thus, controlling metabolic dysfunction in PH may be a valuable therapeutic measure to prevent the progression of the disease or possibly to reverse it.

ADIPOSE TISSUE AND VASCULATURE

Adipose tissue produces a number of bioactive substances including leptin, adeponetin, and inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)-α and visfatin, and proteins such as apolipoprotein E (ApoE), plasminogen activator inhibitor 1 and apelin[59,60]. These substances influence adipose tissue and vasculature in health and disease.

PVAT surrounds blood vessels to provide support and to maintain vascular homeostasis. Close anatomical relationship between PVAT and blood vessels allows crosstalk which is essential for both vascular and metabolic homeostasis. Anti-contractile activity of PVAT is thought to be due to the release of adipose-derived relaxing factor[61]. In addition to the adipose-derived relaxing factor, PVAT releases other vaso-active factors including adiponectin, leptin, angiotensin (1-7) and NO. Under normal conditions these factors maintain vascular function and resistance[12]. PVAT shares common features with brown fat tissue, which is important for thermogenesis and plays a protective role[62].

Adiponectin was initially recognized as an insulin-sensitizing factor, now it has been found to have a role in vascular homeostasis and inflammation. Adiponectin is an anti-inflammatory adipokine; its levels are reduced in obesity. Adiponectin plays a central role in the development of metabolic syndrome and atherosclerosis; both have a low grade inflammation. Adiponectin knockout mice show an exaggerated inflammatory response and produce increased lipopolysaccharides-induced expression of VCAM-1 and ICAM-1. Treatment with adiponectin results in a dose-dependent inhibition of TNF-α-induced monocytes adhesion to EC and the expression of VCAM-1[63]. Interestingly, adiponectin is present in vascular EC at steady state, and it has been shown to have a significant role in vascular relaxation by activating eNOS[64], and PGI_2 synthase[65]. High molecular weight adiponectin stimulates eNOS phosphorylation accompanied by eNOS-HSP90-Akt complex formation and increases NO production in a dose-dependent manner; and it also inhibits caspase3 activity and promotes endothelial survival[66,67].

Adiponectin produced in perivascular tissue is highly regulated by PPARγ. Furthermore, PVAT regulates insulin-mediated vasorelaxation in adiponectin-dependent pathway. It increases eNOS activation as well as inhibits superoxide generation. Local expression of adiponectin gene and protein is increased in the presence of oxidative stress. Under oxidative stress and in the presence of low tetrahydrobiopterin, eNOS is uncoupled and generates superoxide. Under these circumstances adiponectin may increase superoxide generation by increasing eNOS activation[68,69].

Removal of PVAT has been shown to enhance neointima formation; and the local but not the systemic administration of adiponectin reduces neointima formation[70]. Obesity-induced inflammation causes increased production of pro-inflammatory adipokines and reduction in anti-inflammatory adiponectin, which contribute to pathological vascular remodeling in response to injury. Deletion of adeponectin in mice leads to PH, perivascular inflammatory infiltrates and the upregulation of E selectin[71]. Recent studies have shown increased plasma levels of adiponectin associated with endothelial dysfunction in diabetic nephropathy[72]. This suggests that the adiponectin levels increase in response to endothelial dysfunction and that the endothelial integrity may be necessary for normal adiponectin function.

Leptin, primarily expressed by adipocytes is involved in energy expenditure and plays a key role in inhibiting food intake and improving insulin sensitivity. In obese patients, the circulating leptin levels are high but they exhibit resistance to the effects of leptin. Congenital leptin deficiency is associated with marked obesity and hypogonadism[73]. Increased risk of cardiovascular diseases has been reported in obese patients with elevated levels of leptin. Leptin is considered a link between metabolic disorders and immune responses. Usually, leptin increases during the course of acute infection and inflammation. Leptin has been shown to have a direct effect on T lymphocyte type 1 helper response, and leptin alters T regulatory (Treg) response. Defective leptin receptor signaling in Treg cells reduces the development of atherosclerosis[74]. Leptin negatively affects the generation and the proliferation of the Treg cells[75], and it promotes chronic autoimmune disorders by regulating Treg cells and their

function[76].

Leptin receptors are expressed in EC, SMC and macrophages. Leptin induces vasoconstriction *via* the stimulation of sympathetic activity; and depending on intact and functional EC, it has a direct vasodilatory effect *via* NO release. In systemic hypertensive rats, a reduction in leptin levels is accompanied by a loss of perivascular anticontractile function secondary to the impaired activation of eNOS[77]. In contrast, obesity-induced increased expression of leptin enhances neointima formation. Even in the absence of obesity and increased circulating levels of leptin, overexpression of leptin in PVAT facilitates neointima formation[78]. In cell culture studies, leptin has been shown to induce vascular SMC proliferation and migration[79]. Furthermore, pulmonary arterial EC from patients with PAH, and PAH associated with scleroderma secrete leptin. In addition, Treg cells from these patients exhibit increased expression of leptin receptor (ObR) on the membrane[80], indicating that leptin may have a significant role in the pathogenesis and progression of PH.

ApoE is primarily produced in liver, but other cells such as adipocytes and macrophages also produce it, but not the preadipocytes[60]. Circulating ApoE plays an important role in the metabolism of lipoproteins. Adipocytes from ApoE knockout mice are smaller. Systemic deficiency of ApoE results in impaired clearance of triglycerides and resistance to obesity[81]. Diet-induced or leptin-deficient obesity produces a significant reduction in ApoE expression in adipocytes. Inflammatory cytokines such as TNF-α and reactive oxygen species suppress ApoE expression, whereas systemic administration of PPARγ increases ApoE expression. Interestingly, ApoE colocalizes with caveolin-1 in adipocytes, and the loss of ApoE results in the alterations in caveolar lipid composition and a significant reduction in caveolin-1 mRNA expression. Endogenous expression of ApoE preserves caveolar composition in adipocytes[82,83]. ApoE is not produced in EC, but macrophage-related ApoE is internalized by EC. ApoE increases the endothelial NO production by modulating caveolin-1/eNOS interaction and it suppresses endothelial activation, and inhibits VCAM-1 expression *via* eNOS stimulation and NO production. Interestingly, ApoE has been shown to co-precipitate with caveolin-1 but not with eNOS. Deficiency of ApoE is associated with hypercholesterolemia, and the loss of its effect on eNOS activation leads to endothelial dysfunction[84,85]. Ablation of caveolin-1 in ApoE knockout mice has shown to be protective against atherosclerosis[86]. However, PPARγ-induced increase in caveolin-1 expression in ApoE knockout mice confers protection against atherosclerosis[87]. The opposing effects of caveolin-1 may be dependent on its location and conformation. Interestingly, male ApoE knockout mice on high fat diet and associated insulin resistance have been shown to develop PH, which can be reversed by PPARγ activation[88].

Other bioactive substances produced by adipose tissue are visfatin and apelin. Visfatin has been shown to stimulate SMC growth and angiogenesis. Apelin causes NO-dependent vascular relaxation, but it is a potent vascoconstrictor in endothelium-denuded vessels[59]. The foregoing observations indicate that adipose tissue, especially PVAT possesses direct vascular protective effects which are reduced or lost in obesity, resulting in an increased incidence of vascular diseases. Even in the absence of obesity, but in the presence of alterations in the balance of bioactive substances produced by PVAT can significantly influence the state of the vasculature.

METABOLIC SYNDROME

Adipose tissue has a critical role in energy balance and insulin sensitivity. A complex network of transcripton factors is involved in adipogenesis. White adipose tissue is the predominant type in adults and it functions as a storage depot for energy; whereas the brown adipose tissue generates heat through mitochondrial uncoupling of lipid peroxidation. Adipose tissue consists of adipocytes, preadipocytes, leukocytes, macrophages and EC. Adipocytes are an active metabolic organ that secretes a number of adipokines including leptin, adiponectin and resistin, and are involved in glucose and lipid metabolism, energy homeostasis; and it modulates inflammation and vascular reactivity. In addition, adipose tissue secretes proinflammatory cytokines such as IL-6, IL-1, TNF-α and CC-chemokine ligand 2[89-92].

Inflammation plays a significant role in metabolic syndrome, and the adipocytes are considered the primary site of inflammation. Metabolic syndrome includes a number of alterations such as increased waist circumference, systemic hypertension, increased levels of glucose, and impaired cholesterol and triglyceride metabolism. The major categories included in the metabolic syndrome are obesity, disorders of adipose tissue and insulin resistance. There is a positive correlation between cardiovascular diseases and the components of metabolic syndrome such as abdominal obesity, atherogenic dyslipidemia, insulin resistance with or without glucose intolerance, and the presence of pro-inflammatory and pro-thrombogenic factors[93].

Recent studies show that EC play a key role in metabolic homeostasis. VEGF-B interacts with endothelial VEGF receptor1 also known as FLT1, and regulates endothelial transport of fatty acids into cardiac and skeletal muscle. Over expression of VEGF-B can lead to mitochondrial dysfunction, altered cardiac lipid metabolism and hypertrophy, and insulin resistance. Mice lacking VEGF-B have been shown to display decreased fatty acid uptake and lipid deposition in muscle cells. Furthermore, VEGF-B inhibition improves insulin sensitivity[94-96]. In addition to VEGF-B, PPARγ and apelin also have a role in fatty acid uptake by EC and coordinate it with the energy demand and to accommodate energy needs during fasting[97].

Caveolin-1 and metabolic syndrome

Caveolin-1 in adipocytes plays an important role in glucose and lipid metabolism. Insulin receptor (IR) colocalizes with caveolin-1, and caveolin-1 stabilizes IR-β sub-

unit at the cell membrane. It stimulates IR signaling and linking insulin action to glucose uptake. Insulin recruits glucose transporter (GLUT) 4 for glucose uptake and caveolin-1 is required for its internalization after insulin removal[98-101]. Thus, caveolin-1 plays an important role in the control of insulin signaling and facilitates GLUT4-mediated glucose uptake.

Leptin has been shown to increase the expression of caveolin-1 in adipocytes and EC, and in contrast, caveolin-1 impairs leptin signaling which in part may be responsible for inducing leptin resistance and endothelial dysfunction[102,103]. Interestingly, patients with obesity and obesity-associated type 2 diabetes, exhibit increased expression of caveolin-1 mRNA. This increase in caveolin-1 mRNA is associated with an increased expression of inflammatory markers such as leptin, C-reactive protein, MCP-1 and TNF-α[104]. In diabetic mice, increased expression of caveolin-1 mRNA and protein has been shown to be associated with impaired endothelium-dependent relaxation response despite normal eNOS expression[105]. It is likely that caveolin-1 forms a tight complex with eNOS inhibiting its activation, not unlike what is seen in the hypoxia-induced PH. In the hypoxia model of PH, the disruption of cholesterol results in the separation of caveolin-1 and eNOS resulting in increased NO production[106].

Caveolae are also the site of fatty acid entry. The enzymes involved in *de novo* synthesis of triacylglycerol from fatty acids, and glycerol-3 phosphatase are localized in the subclass of caveolae in the plasma membrane of primary adipocytes[107]. Caveolin-1 regulates triglycerides, lipoprotein metabolism and cholesterol homeostasis, and participates in lipid storage *via* transcytosis and also in its breakdown. In addition, it targets the lipid droplet accumulation in the cells. In atherosclerosis, caveolin-1 has been shown to promote cholesterol accumulation *via* transcytosis across EC, thus, negatively impacting the disease. Loss of caveolin-1 leads to decreased lipid accumulation resulting in progressive white adipose tissue atrophy[108-110]. Recent studies have shown caveolin-1 gene mutations to be associated with the atypical and severe forms of lipodystrophy and hypertriglyceridemia[111,112]. Furthermore, mutation of PTRF associated with a reduction in caveolin has been reported in patients with generalized lipodystrophy and muscular dystrophy[113]. Loss of caveolin-1 causes significant metabolic alterations, increased glucose production in the liver and metabolic inflexibility. Metabolic flexibility is the function of adjusting the changing nutrient availability. Adiponectin has been thought to provide the metabolic flexibility. Interestingly, caveolin-1 knockout mice exhibit low circulating adiponectin despite increased mRNA and intracellular adiponectin[114].

Studies with caveolin-1 knockout mice have revealed the importance of caveolin-1 in maintaining vascular and metabolic homeostasis. Caveolin-1 knockout mice exhibit PH and cellular hyperplasia in the lungs, cardiomyopathy, and metabolic deregulation. These mice are found to be resistant to diet-induced obesity, but have hypertriglyceridemia and develop insulin resistance on normal diet[98]. In addition, they exhibit increased macrophage infiltration, increased capacity for IL-6 production and an increased collagen deposition leading to increased fibrosis. Adipose tissue from these mice show increased lipolysis[115]. Re-expression of endothelial-specific caveolin-1 ameliorates cardiopulmonary changes, but has no effect on the lack of caveolin-1 in adipocytes that accounts for lipoatrophy. The endothelial-specific caveolin-1 expression, however, limits the macrophage extravasations into adipose tissue[116], indicating a significant role of endothelial caveolin-1 in modulating adipocytes-driven inflammatory response.

PPARγ and metabolic syndrome

Adipose tissue especially the white adipose tissue is the major site for PPARγ expression. PPARγ is required for adipocytes differentiation. Activation of PPARγ in fibroblastic cells leads to cell differentiation and lipid accumulation; and in addition, these cells acquire genes characteristic of fat cells[117]. PPARγ is expressed to a lesser degree in insulin target tissues such as liver and skeletal muscle. Muscle-specific PPARγ is critical for maintaining the whole body response to insulin. The loss of muscle-specific PPARγ leads to obesity and insulin resistance[118]. In addition, targeted EC deletion of PPARγ plays an important role in insulin resistance and hyperlipedemia-mediated hypertension[119].

Impaired PPARγ function is implicated in a number of metabolic disorders such as type2 diabetes, obesity and lipodystrophy. In humans, mutation of PPARγ leads to obesity and severe insulin resistance. Overexpression of this mutant gene in murine fibroblasts leads to accelerated differentiation into adipocytes and increased cellular accumulation of triglycerides[120]. PPARγ mutation is reported to be associated with insulin resistance, diabetes and hypertension[121], and also in cases of lipodystrophy associated with activated renin-angiotensin system and ensuing oxidative stress and hypertension[122]. Defect in PPARγ expression plays a significant role in PH as well as in the pathogenesis of fibrosis; importantly, scleroderma exhibits both these features[123]. The anti-fibrotic activity of PPARγ is thought to be mediated by hepatocyte growth factor and adiponectin. Adiponectin, an anti-inflammatory adipokine and a fat-specific target of PPARγ prevents hepatic fibrosis in mice[124] and hypoxia-induced PH[125]. The administration of leptin, a proinflammatory adipokine has been found to reduce the expression and activity of PPARγ in human lung fibroblasts and to augment TGF β-mediated fibro-proliferative response. Furthermore, the loss of leptin prevents bleomycin-induced lung fibrosis in mice[126].

PPARγ inhibits the production of adipokine/cytokines such as resistin, IL-6 and TNF-α, all known to promote insulin resistance. PPARγ agonist-induced adiponectin levels are reported to be low in type 2 diabetes[127]. Adiponectin increases fatty acid oxidation in liver and skeletal muscle, resulting in improved insulin sensitivity in skeletal muscle, and decreased glucose production in the liver, thus, leading to the reduction in circulating glucose,

free fatty acid and triglycerides[128]. These results suggest a protective role of PPARγ, and the crosstalk between PPARγ and adipokines determines the progression of a given metabolic/vascular disease process. PPARγ activators, TZD group of drugs have been used clinically to treat type 2 diabetes. TZDs increase the expression of proteins required for insulin signaling, and also reduce the circulating levels of low density lipoproteins and triglycerides. Furthermore, they attenuate the production of inflammatory mediators[129,130]. However, TZDs are also reported to have side effects such as increased fluid retention, increased risk of congestive heart failure, decrease in bone mineral density and fractures. Selective PPARγ modulator in experimental studies has been shown not only to increase insulin sensitivity but also to improve bone density[131,132]. Selective PPARγ modulation, thus, may significantly reduce the side effects of TZD.

Metabolic syndrome and pulmonary hypertension

Obesity is reported to be associated with PH, but the prevalence of PH in obesity is not known. The echocardiographic studies in 3790 normal subjects revealed higher pulmonary artery pressure to correlate with age, body mass index and gender; the incidence being higher in males[133]. Importantly, higher frequency of obesity, diabetes and hyperlipidemia was found in patients with precapillary PH[134]. Furthermore, obesity is a risk factor in patients with elevated pulmonary venous pressure and preserved left ventricular ejection fraction[135].

Diabetes is reported to be associated with PH independent of coronary artery disease and congestive heart failure[136], and insulin resistance is more prevalent in female patients[137]. Recent REVEAL registry analysis showed a high incidence of obesity (M:F, 31%:34%) among patients with PAH; and associated comorbidities such as diabetes and chronic obstructive pulmonary disease had a negative impact on prognosis[138,139]. In experimental studies, diabetes associated with moderate hypoxia is reported to exhibit significant endothelial dysfunction, elevated pulmonary artery pressure and RVH. It was diabetes and not the moderate hypoxia that was found to be responsible for endothelial dysfunction[140]. These observations suggest that obesity and insulin resistance negatively impact PH.

HYPOXIA, PULMONARY HYPERTENSION AND METABOLIC SYNDROME

HIF-1α, an O_2 sensor is a subunit of a family of HIF transcription factors. HIF-1α regulates numerous genes involved in adaptive responses to hypoxia and modulates metabolism, growth and angiogenesis; and promotes adaptation and cell survival under hypoxic condition. VEGF, critical for angiogenesis, is one of the target genes of HIF-1α[141]. Under normoxic conditions HIF-1α is degraded. Evidence is accumulating to suggest that reactive oxygen species (ROS) generated by mitochondrial complex Ⅲ is required for HIF-1α activation and stabilization; and in turn HIF-1α activation prevents increased production of ROS in hypoxic cells[142]. Under hypoxic conditions, cells depend on glycolysis for ATP production; and HIF-1α is necessary for metabolic switch during hypoxia[143]. Destabilization of HIF-1α has a negative impact on cell and tissue adaptation to hypoxia.

HIF-1α has been implicated in the pathogenesis of PH. HIF-1α plays a role in cell proliferation, angiogenesis, and participates in vascular remodeling. In plexiform lesions, the proliferating EC have been shown to express HIF-1α, its target gene VEGF and VEGF receptor 2[144]. Recent studies have shown that the deletion of HIF-1α in SMC attenuates hypoxia-induced PH and vascular remodeling[145]. In some types of cancer cells, HIF-1α under hypoxia conditions upregulates caveolin-1 and promotes ligand-independent activation of epidermal growth factor receptor, and increases cell proliferation and cell migration[146]. Interestingly, HIF-1α has also been shown to maintain pulmonary vascular tone during hypoxia and normoxia by decreasing myosin light chain phosphorylation; and the lack of HIF-1α increases pulmonary vascular tone[147]. In addition, the loss of HIF-1α in SMC from systemic vessels causes systemic hypertension and an exaggerated response to angiotensin Ⅱ. HIF-1α is reported to decrease the expression of angiotensin Ⅱ receptor type 1. Importantly, the HIF-1α-induced decrease in the expression of angiotensin II receptor type 1 is mediated by PPARγ[148]. In addition, HIF-1α has been shown to play a protective role in the adaptation of the heart and aorta to pressure overload by regulating TGF-β signaling in EC[149].

HIF-1α is an important regulator of glucose transport by altering GLUT1 expression in EC. Absence of HIF-1α is associated with significant defect in glucose uptake. Reduced glucose uptake in HIF-1α-deficient EC can be rescued by increased expression of GLUT1 DNA, underscoring the critical the role played by HIF-1α in glucose metabolism[150], and that the vascular dysfunction may contribute to abnormal glucose handling. Hyperglycemia has been shown to impair hypoxia-dependent stabilization of HIF-1α[151]. Both hyperglycemia and hypoxia are known to occur in diabetes. Hyperglycemia-induced destabilization of HIF-1α negatively affects the tissue adaptation to hypoxia, resulting in complications such as diabetic retinopathy, cardiovascular and renal diseases[152]. In addition, deficiency of HIF-1α has been shown to block stromal derived factor1 and impair mobilization of bone marrow-derived angiogenic cells, thus adversely affecting wound healing[153]. Interestingly, hypoxia has been shown to cause insulin resistance and the inhibition of HIF-1α in adipose tissue improves insulin resistance[154]. Thus, both in PH and metabolic syndrome, the role of HIF-1α may depend on the cells, disease state and the interaction of HIF-1α with other factors including caveolin-1 and PPARγ.

CONCLUSION

Caveolin-1 and PPARγ are abundantly expressed in EC and adipocytes. Under normal conditions, caveolin-1 and

PPARγ interact with adipokines (pro- and anti-inflammatory) and form a complex network to maintain metabolic and vascular homeostasis. Genetic mutations of caveolin-1 and PPARγ lead to vascular and metabolic diseases. PVAT has a direct role in maintaining vascular reactivity. Disruption of PVAT results in the loss of anti-inflammatory and anti-contractile factors leading to endothelial dysfunction. The initial loss of endothelial caveolin-1 results in the activation of proliferative pathways leading to vascular remodeling and PH. As the disease progresses, SMC develop enhanced expression of caveolin-1. This caveolin-1 becomes pro-proliferative and participates in cell proliferation and cell migration. In adipose tissue, the loss of caveolin-1 is associated with dysregulation of insulin and lipid metabolism. However, increased levels of caveolin-1 in diabetes and hypercholesterolemia result in eNOS dysfunction. Loss of PPARγ leads to vascular and metabolic diseases. Interestingly, PPARγ within the atheromatous lesion facilitates angiogenesis. Adiponectin, regulated by PPARγ increases insulin sensitivity, inhibits inflammation and facilitates NO production, thus, plays an important role in maintaining vascular and metabolic homeostasis. Leptin, a proinflammatory adipokine has an important role in food intake and energy conservation. Under normal conditions, leptin has a vasodilatory effect. However, obesity-induced increased levels of leptin cause endothelial dysfunction. It increases caveolin-1 expression which in turns inhibits leptin.

Vasculature and adipose tissue owing to their proximity share the complex network of transcription factors, and influence each other in health and disease. The network of these factors is rather complex and delicate, which can be deregulated by injury and/or inflammatory process leading to a stage where the cytoprotective factors become cytotoxic depending on the state of the cell/organ. Rudolf Virchow (1821-1902) a German physician is reported to have said "The body is a Cell State in which every cell is a citizen. Disease is merely the conflict of citizens of the State brought about by the action of an external force". It is not difficult to imagine that this conflict can easily spill into the neighboring organs/systems.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Transcatheter therapies for resistant hypertension: Clinical review

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Abstract

Resistant hypertension (RHTN) is a commonly encountered clinical problem and its management remains a challenging task for healthcare providers. The prevalence of true RHTN has been difficult to assess due to pseudoresistance and secondary hypertension. Atherosclerotic renal artery stenosis (RAS) has been associated as a secondary cause of RHTN. Initial studies had shown that angioplasty and stenting for RAS were a promising therapeutic option when added to optimal medical management. However, recent randomized controlled trials in larger populations have failed to show any such benefit. Sympathetic autonomic nervous system dysfunction is commonly noted in individuals with resistant hypertension. Surgical sympathectomy was the treatment of choice for malignant hypertension and it significantly improved mortality. However, post-surgical complications and the advent of antihypertensive drugs made this approach less desirable and it was eventually abandoned. Increasing prevalence of RHTN in recent decades has led to the emergence of minimally invasive interventions such as transcatheter renal denervation for better control of blood pressure. It is a minimally invasive procedure which uses radiofrequency energy for selective ablation of renal sympathetic nerves located in the adventitia of the renal artery. It is a quick procedure and has a short recovery time. Early studies in small population showed significant reduction in blood pressure. The most recent Symplicity HTN-3 study, which is the largest randomized control trial and the only one to use a sham procedure in controls, failed to show significant BP reduction at 6 mo.

Key words: Resistant hypertension; Renal denervation; Renal artery stenosis; Renal artery stenting; Transcatheter therapy; Sympathetic autonomic nervous system

Core tip: The aim of this paper is to review resistant hypertension (RHTN), including primary and secondary causes. Renal artery stenosis is one of the secondary cause of RHTN but angioplasty and stenting of renal artery for management of RHTN has failed to show any benefit. Sympathetic nervous system dysfunction is commonly noted in individuals with resistant hypertension. Renal sympathetic nerve denervation is a minimally invasive procedure which may help improve management of RHTN. However, the Symplicity HTN-3 trial failed to show a meaningful reduction in BP and has questioned this approach.

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INTRODUCTION

Resistant hypertension is defined as above goal systolic

blood pressure (SBP) despite therapy with three or more antihypertensive medications of different classes at maximum tolerable doses with one being a diuretic[1]. The definition can be extended to at goal blood pressure (BP) requiring four or more drugs of different classes[1]. The true prevalence of resistant hypertension (RHTN) is difficult to assess due to significant number of patients with poor medical compliance and/or suboptimal treatment regimen[1]. Prevalence of RHTN according National Health and Nutritional Examination Survey (NHANES) is 8.9% within the hypertension population[2]. With rising incidence of obesity, and people living longer, it is likely to become a major public health concern in the upcoming decades[1]. RHTN should be considered after excluding pseudo-hypertension and secondary causes of hypertension. It is associated with significant end organ complications including, coronary artery disease (CAD), stroke and chronic kidney disease (CKD). Prognosis is poor in individuals who have failed therapy with multiple classes of antihypertensives. The degree of reversibility of end organ damage with successful control of BP in these individuals is lacking evidence, but optimal blood pressure control in general has shown to delay onset and progression of end organ complications and it reduces the incidence of major vascular events[1]. RHTN is beginning to become a global issue, which has led to the advent of minimally invasive interventions for optimal BP control.

INITIAL DIAGNOSIS OF RHTN

RHTN is a diagnosis of exclusion. The initial step in management of poorly controlled blood pressure would be to rule out pseudo-resistance and secondary causes of HTN. Poor BP measurement technique, and use of improper cuff size can lead to falsely elevated BP readings. This can be avoided by allowing a patient to sit in a quiet room for a few minutes before checking BP, using an appropriately sized cuff and proper technique[1]. Medical noncompliance is another commonly encountered problem and has been noted in up to 40% of newly diagnosed hypertensive patients[1]. White coat hypertension is present in 20% to 30% of individuals and it should be further evaluated with ambulatory BP measurement[1]. Lifestyle factors such as obesity, excessive dietary salt intake, heavy alcohol consumption and certain medications can significantly contribute to elevation of BP, and it must be addressed before giving diagnosis of RHTN[1]. The most common secondary causes of RHTN are RAS, obstructive sleep apnea (OSA), primary hyperaldosteronism and renal parenchymal disease[1]. Fibromuscular dysplasia is a common cause of RAS in middle aged females, whereas atherosclerotic RAS is predominantly seen in the elderly. OSA is a known cause of hypertension and its severity is directly associated with difficulty in controlling BP[1]. OSA is thought to cause sympathetic dysregulation which can lead to RHTN[1]. Primary hyperaldosteronism has a prevalence of 20 percent in individuals with RHTN and its etiology can be often obscure[1]. CKD is commonly the result of long standing poorly controlled HTN and it can lead to RHTN.

RENAL ARTERY STENOSIS AS SECONDARY CAUSE OF RHTN

RAS is often noted in individuals with RHTN. Stenting or angioplasty in addition to optimal medical management for atherosclerotic RAS has failed to show any significant benefit in regards to HTN or CKD in randomized control trials (RTC)[3]. Up to 90% of renal artery stenosis in the elderly population is due to atherosclerosis[1,3,4]. A significant degree of RAS can decrease renal perfusion which leads to the over-activation of the renin-angiotensin-aldosterone axis (RAAS)[4]. RAAS overactivation leads to increase in sodium and water retention, causing elevation in systemic blood pressure[4]. The severity of stenosis required to cause over activation of RAAS is unknown, but use of ACE-inhibitor can cause acute worsening of renal function and should raise suspicion of significant RAS in these individuals[4]. There is also up-regulation of SANS which can further make it difficult to control BP[4]. Such individuals are at high risk of end organ complications including left ventricular hypertrophy, heart failure with recurrent pulmonary edema and CKD[4].

TRANSCATHETER THERAPY FOR ATHEROSCLEROTIC RENAL ARTERY STENOSIS

Theoretically, stenting of the stenotic lesion should resolve RHTN. Initial studies showed significant reduction in SBP and this led to increase in revascularization rates for renal artery stenosis[3,4]. However, recent RCT have shown such revascularization to be futile[3,4]. The "Blood pressure outcome of angioplasty in atherosclerotic renal artery stenosis trial", aka. EMMA trial, concluded that previous uncontrolled and unblended studies had overestimated the benefits of renal artery revascularization[5]. No significant difference in mean 24-h ambulatory blood pressure was noted between the control group and angioplasty group at the end of 6 mo[5]. "The Randomized comparison of percutaneous angioplasty *vs* continued medical therapy for hypertensive patients with renal artery stenosis trial" was a randomized study that enrolled patients with renal artery stenosis of 50% or greater and minimum diastolic BP of 95 on at least two antihypertensive medications[6]. Revascularization resulted in modest systolic BP improvement without any change in renal function but, there was significant post-procedural complication noted in the intervention group[6]. "The effect of balloon angioplasty on hypertension in atherosclerotic renal artery stenosis trial", also known as the "Dutch renal artery stenosis intervention cooperative (DRASTIC)" concluded that the benefit of angioplasty was "little" over medical management[7].

Table 1 Renal artery stenting/angioplasty in resistant hypertension

Ref.	Size	Follow up period	Mean SBP reduction with stenting/ angioplasty	Mean SBP reduction with medical therapy	*P* value
Cooper *et al*[3] (Coral trial)	947	43 mo	16.6 ± 21.2	15.6 ± 25.8	0.03
Van Jaarsveld *et al*[7] (DRASTIC trial)	106	12 mo	19	17	0.51
Plouin *et al*[5] (EMMA trial)	49	6 mo	12 ± 20	8 ± 16	0.46
Webster *et al*[6]	135	3-54 mo	34	8	0.018

SBP: Systolic blood pressure.

"Cardiovascular outcomes in renal atherosclerotic lesions trial (aka, CORAL)" was an NIH funded, open-label, unblinded and a multicenter randomized study[3]. It compared stenting *vs* medical therapy in atherosclerotic renal artery stenosis[3]. This trial randomized 947 individuals with elevated SBP and/or CKD with estimated GFR of 60 mL/min per 1.73 m^2 of BSA as per MDRD formula and RAS of at least 60%[3]. Patients were randomized to either only medical therapy or medical therapy plus renal artery stenting group[3]. The primary endpoint of this study was a major cardiovascular or renal event[3]. Over a 43-mo median follow up, there was no significant difference in regards to the primary endpoint between the 2 study groups[3]. SBP was noted to be reduced in the stenting plus medical therapy group by 16.6 ± 21.2 mmHg and in the medical therapy only group by 15.6 ± 25.8[3]. There was only a modest SBP lowering in stenting group compared to medical therapy group (-2.3 mmHg) with a Confidence Interval (CI) of -4.4 to -0.2 and *P* value of 0.03[3]. Once again revascularization for renal artery stenosis was proven to be futile, putting another nail into its' coffin (Table 1).

Earlier trials had a smaller sample size and the patients had clinically insignificant RAS, which question their validity. The sample size was 49 in the EMMA trial and the DRASTIC trial had 106 participants, which is too small to detect a significant difference between the study groups[5,7]. This increases the chance of a type 2 statistical error. They enrolled patients with mild RAS when a lesion of at least around 70% is deemed to be hemodynamically significant by many experts[8,9]. A crossover rate to therapy group was 44% in DRASTIC study which further obscures outcomes[7]. Earlier trials assessing effect of revascularization on RHTN used angioplasty of stenotic lesions that may not be as effective as stenting[8-10]. CORAL was one of the largest randomized trial with 947 participants comparing medical therapy *vs* endovascular stenting in addition to medical therapy for RHTN. It also included stricter criteria in regards to the degree of stenosis required to be eligible for participation, which was not seen in earlier studies. CORAL trial seems of have addressed some of the common issues with previous studies and provides the most statistically significant data.

SYMPATHETIC THEORY OF RHTN

Sympathetic autonomic nervous system (SANS) dysfunction is seen in 50% of hypertensive individuals, which makes it a promising therapeutic target[11]. The sympathetic fibers densely innervate the kidneys and are mainly located in the adventitial layer of the vascular wall of the renal arteries[12]. Activation of the afferent limb of the Renal SANS stimulates the posterior hypothalamus, the autonomic centers in the medulla oblongata and the mid brain[13,14]. All messages are integrated into the autonomic centers and are relayed back to the kidneys *via* the thoraco-lumbar paravertebral ganglia, the superior mesenteric ganglia and celiac prevertebral ganglia[13,14]. Increase in efferent sympathetic tone leads to vasoconstriction of the renal vasculature by activation of the alpha-1a receptors which leads to a decrease in blood flow to the kidneys[15]. It accelerates alpha-1b adrenergic receptor mediated tubular reabsorption of sodium and water[15]. It also causes over activation of the RAAS through the beta-1 adrenergic receptors located on the juxtaglomerular cells[15]. Sympathetic over-activity on the heart increases cardiac output and its effect on blood vessels increases peripheral vascular resistance in an effort to increase renal perfusion[13]. These pathophysiologic changes make an individual susceptible to RHTN which can lead to end organ complications over time[13,16].

THE SURGICAL APPROACH TO SYMPATHETIC DENERVATION FOR RHTN

Surgical sympathectomy was the treatment of choice for malignant hypertension before antihypertensive medications were available[11,16]. Five-year mortality from malignant hypertension was estimated to be 100%[17,18]. Thoracolumbar splanchnicectomy was first introduced in 1938[18]. Treatments ranging from radical subdiaphragmatic splanchnicectomy to less aggressive interventions such as sympathetic ganglionectomy resulted in reduced blood pressure and favorable end organ changes[11,19]. However, they were associated with undesirable adverse effects such as, orthostatic hypotension, sexual dysfunction, incontinence, anhydrosis and tachycardia[11,19]. The surgery was typically performed as a one or two step procedure and required extended hospital stay[17]. Surgical sympathectomy became a second line treatment after introduction of antihypertensives for patients whose BP was uncontrolled despite medical management[17]. Surgical sympathectomy increased sensitivity of antihypertensive drugs and had lower mortality compared to medical management alone[17]. As newer and more potent antihypertensive medications of different classes became available, this radical approach phased out due to its undesirable adverse effects. However, suboptimal control of blood pressure on maximal medical therapy, the increasing prevalence of RHTN and evidence of renal sympathetic nerve over-activity in hypertensive

individuals has sparked interest in catheter based renal sympathetic denervation as a promising therapeutic option[16,20,21].

TRANSCATHETER RENAL DENERVATION

Transcatheter renal sympathetic nerve ablation is a minimally invasive procedure. It complements the BP lowering effects of the former radical approach without its adverse effects and has a much faster post-procedural recovery time[13]. The post-operative mortality in patients treated with the surgical approach was as high as 11% compared to relatively none with RDN[18]. Contraindications to RDN mainly include GFR < 45 mL/min per 1.732 m^2, past interventions such as angioplasty or stenting, abnormal anatomy, Diabetes type 1, age less than 18 years and pregnancy[22]. One of the devices widely studied in RCT is the Symplicity Renal Denervation System by Medtronic. This device consists of a low power radio frequency generator and a disposable catheter[13]. The procedure is performed under conscious sedation through percutaneous access. The catheter tip is an opaque platinum electrode. It is hand guided into the renal artery, adjacent to the dense neural site located near the renal hilum[13]. The design of the catheter allows safe delivery of low level radio frequency energy across the arterial wall to ablate the nerves located in the adventitia of the renal artery[13]. Multiple ablations are delivered in a circumferential pattern every few millimeters within both renal arteries to ensure complete ablation. The procedure takes less than an hour and the patient is usually observed for a day after the procedure[13].

Many other catheter designs are currently being investigated. The ST. Jude's Enlig HTN Renal Denervation System uses a multi-electrode catheter which delivers the ablation in a specific circumferential pattern, eliminating the need for catheter manipulation and administering multiple ablations[22]. The EnligHTN 1 trial was a non-randomized study which evaluate the efficacy and safety of this device in 46 patients whose mean office BP was 176/96 mmHg[22]. Office BP reduced by 26/10 at 6 mo with a *P* value of < 0.0001 without any complications[22]. The Vessix V2 renal denervation system uses an over the wire balloon catheter with electrodes in a specific pattern to deliver RF energy and it is currently being evaluated in the REDUCE-HTN trial expected to complete in December 2014[23].

Catheter based ultrasound renal denervation is a newer technique which uses intravascular ultrasound for selective denervation of the renal nerves in the adventitia of the artery[24]. The device uses a catheter-based transducer, which delivers high frequency sound waves in a circumferential manner[24]. The transducer has an inflatable balloon with a water circuit that keeps the walls of the arterial lumen cool when energy is being delivered[24]. This prevents thermal damage to the vessel wall while selectively ablating the renal nerves[24]. The circumferential delivery of energy is not dependent on the position of the catheter which allows for a consistent post procedural outcome[24]. This devise is currently being evaluated in 50 patients in the ACHIEVE study, which is anticipated to be complete in February 2015[25]. Chemical renal nerve ablation is the latest technique which uses peri-adventitial dehydrated ethanol injection administered in a circumferential pattern[26]. Most of the newer devices are "energy based" and can lead to thermal injury of the vessel wall which is an advantage of chemical RDN[26]. This approach has been successful in lowering renal parenchymal norepinephrine levels at 2 wk in swine models which is a measure of reduced sympathetic activity[26]. Randomized control trial in human model is needed to evaluate its safety and efficacy.

The first reported RDN in humans was done by Schlaich and Colleagues in 2009[27]. The subject was a 59-year-old male patient with history of two TIA, untreated OSA secondary to intolerance to CPAP, and RHTN who was on seven antihypertensive medications[27]. He underwent this procedure without any complications[27]. Reductions were noted in renal norepinephrine spillover and mean office blood pressure, while the renal blood flow increased[27]. "The Catheter-based renal sympathetic denervation for resistant hypertension" was a multicenter safety and proof-of-principle cohort study, which evaluated the BP lowering effect and safety of renal denervation in 50 patients from Europe and Australia[28]. Eligible patients had an office SBP ⩾ 160, and were on three or more antihypertensive agents of which one was a diuretic with no previous ablations, stenosis, and bilateral kidneys with an anatomy that was conducive to the procedure[28]. Out of the 50 patients, 45 underwent the procedure and 5 were disqualified primarily due to dual renal artery anatomy[28]. Patients who underwent the procedure had a mean office blood pressure reduction of 27/17 at 12 mo with one complication of renal artery dissection during the procedure[28].

The Symplicity HTN-1 trial was a major open label study with a total of 153 patients enrolled at centers in the United States, Europe and Australia[29]. They were followed for 24 mo and were noted to have a mean BP reduction of 32/14[29]. Statistically, *P* value for the reduction was noted to be < 0.0001 for SBP and diastolic BP (DBP) at intervals of 1, 3, 6, 12 and 18 mo, except for *P* value of = 0.002 for DBP at 24 mo[29]. The complication rate was three percent with three patients experiencing groin access site pseudoaneurysm and one patient experiencing renal artery dissection[29]. A final 3 year report evaluated follow up data of only 88 of the 153 patients and noted a mean SBP reduction of 32 mmHg with a 95%CI of -35.7 to -28.2[30]. Complications over the three year period were one new renal artery stenosis which needed stenting and three unrelated deaths[30].

The Symplicity HTN-2 was the first multicenter, prospective RCT that evaluated the effectiveness of transcatheter renal denervation. Primary end point was change in seated SBP at the six month point[12]. A total of 106 eligible participants aged 18 to 85 years who had SBP ⩾ 160 mmHg or ⩾ 150 mmHg if patient was a type 2 diabetic despite compliance with treatment on ⩾

Table 2 Renal nerve denervation in resistant hypertension

Ref.	Sample size	Follow up duration	Mean SBP reduction in RDN group (in mmHg)	Mean SBP reduction in control group (in mmHg)	*P* value
Worthley *et al*[22] (EnilgHTN 1 trial)	46	6 mo	26	No randomized control group	0.0001
[a]Krum *et al*[28]	45	12 mo	27	No randomized control group	0.001
Simplicity HTN-1 investigators[b]	153	24 mo	32	No randomized control group	0.0001
Esler *et al*[12] (Simplicity HTN-2)	106	6 mo	32 ± 23	1	0.0001
Bhatt *et al*[32] (Symplicity HTN-3)	535	6 mo	14.13 ± 23.93	11.74 ± 25.94	< 0.001

[a]Follow up data available for *n* = 9 at 12 mo; [b]Follow up data available for *n* = 18 at 24 mo. SBP: Systolic blood pressure; RDN: Renal denervation; HTN: Hypertension.

3 antihypertensive medications were screened[12]. A total of 52 patients were randomized to renal denervation group at 24 participating centers in Australia, Europe and New Zealand[12]. BP in the intervention group was reduced by 32/12 mmHg (SD ± 23/11 mmHg) from a baseline of 178/97 mmHg (*P* value < 0.0001) compared to change of -1/0 mmHg from baseline of 178/97 mmHg in control group (*P* value = 0.77 for SBP and 0.83 for DBP) with no significant post procedural complications[12]. Thirty six month data was recently presented which showed a reduction in BP by an average of 33/14 (*P* value < 0.01) in 40 of the study participants[31].

The Symplicity HTN-3 is the largest sham controlled, single blinded trial to recruit 535 patients. Inclusion criteria were SBP ⩾ 160 mmHg on stable antihypertensive regiment with ⩾ 3 medications of different classes at full tolerated doses with one being a diuretic[32]. The primary endpoint was change in office SBP measurement at 6 mo and a secondary endpoint assessed 24 h ambulatory BP[32]. Patients were randomized in a 2:1 fashion between RDN group and control group[32]. Within the RDN group, SBP was reduced by 14.13 mmHg with a mean SD of ± 23.93 and in the control group, SBP was reduced by 11.74 mmHg with a mean SD of ± 25.94 at 6 mo (*P* value < 0.001 for change for baseline for both groups)[32]. With ambulatory BP monitoring, RDN group showed a reduction in SBP by 6.75 mmHg with mean SD of 15.11 and in the control group, SBP was reduced by 4.79 mmHg with a mean SD of 17.25[32]. The trial did meet its safety end point[33]. Compared to former studies, Symplicity HTN-3 is the largest and the only blinded RTC which included a sham procedure in the control group. It is the first trial to show that there was no significant difference between the RDN when compared to medical management alone. Symplicity HTN-4 was also a RCT which was estimated to enroll 580 patients but was suspended after release of data from the Symplicity HTN-3 trial[34]. It was similar to Simplicity HTN-3, but its eligibility criteria required participant to be on ⩾ 3 antihypertensive medications of different classes with one of them being a thiazide or a thiazide like diuretic and SBP ⩾ 140 mmHg but less than 160 mmHg[35] (Table 2).

DISCUSSION

The long term benefits of optimum BP control on end organ prognosis is beyond doubt. Newer antihypertensive agents are increasingly selective and efficacious but the prevalence of RHTN is still a public health burden. This prevalence is likely to increase with increasing incidence of obesity and longevity. RHTN is essentially a diagnosis of exclusion and should be considered in individuals after pseudoresistance and secondary causes of HTN are ruled out. Angioplasty and stenting can successfully treat RHTN in individuals with renal artery stenosis due to fibromuscular dysplasia but it has proven to be futile in atherosclerotic RAS. Renal denervation for RHTN may be an excellent therapy with low complication rates. Rare complications such as RAS requiring stenting, renal artery dissection and access site pseudoaneurysm have been noted[28,30]. The current safety profile of RDN is limited to 3 years and it appears to be fairly acceptable[36]. However, long term safety of such intervention is currently unknown[28,30]. Earlier trials presented promising results but the data from Symplicity HTN-3 trial may have brought RDN to a screeching halt for the time being. In comparison to former trials, Symplicity HTN-3 is the largest RTC, and it is the only one to include a sham group which underwent an angiography instead of denervation. Most trials used office BP reduction as primary endpoint that can vary significantly and is not as accurate as ambulatory BP monitoring. This was also addressed in Symplicity HTN-3 trial and it didn't show a meaningful SBP reduction between the two groups, thus, providing us with the most objective data on RDN. Nerve regrowth has been documented in individuals after renal transplant, questioning the durability of RDN, which is currently unknown[28]. RDN also does not completely eliminate the need for medical management and most patient still need to continue on an oral antihypertensive medications. In the meanwhile, RDN continues to be an option after failure with lifestyle and medical management in approved markets[36]. Is there a sub group of individuals with RHTN that may benefit from RDN? Future studies are need to address this question. Much has to be established about the efficacy and long term safety of RDN.

Any conclusions based on currently available data may be premature.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Exercise training in hypertension: Role of microRNAs

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Abstract

Hypertension is a complex disease that constitutes an important public health problem and demands many studies in order to understand the molecular mechanisms involving his pathophysiology. Therefore, an increasing number of studies have been conducted and new therapies are continually being discovered. In this context, exercise training has emerged as an important non-pharmacological therapy to treat hypertensive patients, minimizing the side effects of pharmacological therapies and frequently contributing to allow pharmacotherapy to be suspended. Several mechanisms have been associated with the pathogenesis of hypertension, such as hyperactivity of the sympathetic nervous system and renin-angiotensin aldosterone system, impaired endothelial nitric oxide production, increased oxygen-reactive species, vascular thickening and stiffening, cardiac hypertrophy, impaired angiogenesis, and sometimes genetic predisposition. With the advent of microRNAs (miRNAs), new insights have been added to the perspectives for the treatment of this disease, and exercise training has been shown to be able to modulate the miRNAs associated with it. Elucidation of the relationship between exercise training and miRNAs in the pathogenesis of hypertension is fundamental in order to understand how exercise modulates the cardiovascular system at genetic level. This can be promising even for the development of new drugs. This article is a review of how exercise training acts on hypertension by means of specific miRNAs in the heart, vascular system, and skeletal muscle.

Key words: Exercise training; Hypertension; MicroRNA; Heart; Vascular system; Macrocirculation; Microcirculation; Muscles; Angiogenesis

Core tip: Numerous studies have shown that exercise training exerts beneficial effects on hypertension. Thus, several important studies have established links between exercise training, hypertension and the post-transcriptional regulators known as miRNAs. It is interesting to note that exercise training helps to control hypertension through these regulators, by promoting changes in the cardiovascular system towards normality. This review summarizes the way in which exercise training acts on the cardiovascular system to control the side effects of hypertension on the heart, macro- and microcirculation, and skeletal muscles.

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INTRODUCTION

Exercise training (ET) is a well-known form of preventing or reducing cardiovascular disturbances. It is able to prevent or reduces the vascular changes that are the precursors of high blood pressure, such as diminished nitric oxide (NO) availability and increased oxidative stress. It is also able to reduce sympathetic nervous system (SNS) activity and cardiac output, improve angiogenesis and reduce peripheral vascular resistance. Therefore, ET has been used as a most successful non-pharmacological therapy for the treatment of hypertensive patients. It promotes a reduction in blood pressure and helps to reduce the medication used by these patients (in some cases, it promotes discontinuation of the medication used); thereby decreasing the side effects of pharmacotherapy and the financial cost of hypertension to public health[1]. Despite the continuous advances in options of pharmacological therapies for hypertension, it remains an important and growing public health problem worldwide, affecting more than one billion people across the planet[2]. Today, it is estimated that it kills nine million people per year[3]. It is in this context that ET has a high relevance in hypertension, contributing as an additional tool for the treatment or prevention of this disease.

Hypertension is a persistent elevation of systemic blood pressure with multifactorial causes. Its development is determined by a cluster of environmental factors associated with genetic susceptibility. The mechanisms by which hypertension is generated (such as hyperactivity of SNS, overactivation of the renin-angiotensin-aldosterone system, endothelial dysfunction, and others) are responsible for the gradual development of pathological manifestations in the form of vascular, cardiac and renal diseases, such as atherosclerosis, stroke, pathological cardiac hypertrophy, myocardial infarction, heart and kidney failure[2]. Whereas, ET is able to minimize the effects of multiple factors that induce the development of hypertension, and by extension, it also helps to prevent or reduce the development of the aforementioned pathological manifestations.

ET promotes numerous cardiovascular and muscular adjustments that are antihypertensive. These adjustments depend on the amount of ET, which is determined by the volume (training time), intensity (degree of training load) and frequency of ET (number of training sessions at any given time)[4]. In this context, aerobic exercise promotes physiological cardiac hypertrophy[5], reduction in systolic blood pressure (SBP) and heart rate (both at rest and under submaximal loads)[6,7], increases the lumen diameter of the coronary arteries[8] and cardiac blood flow[9], increases the circulating NO[10], corrects the peripheral capillary rarefaction in hypertensive animals[7], promotes revascularization[11] and reduces peripheral vascular resistance. ET also promotes important metabolic adaptations that reflect on blood pressure control, for example, reduction of plasma triglycerides and low-density lipoproteins, as well as increased insulin sensitivity in tissues[12]. In addition to aerobic exercise, physical resistance training with anaerobic characteristics is also able to induce physiological cardiac hypertrophy[13,14]. Moreover, positive effects have been shown on reducing systolic, mean, and diastolic blood pressure, and heart rate in trained when compared with untrained rats[14].

It is interesting to note that aerobic or resistance training may promote different adaptations in the cardiovascular system, but all adaptations are beneficial to regulating the blood pressure. However it is not only the type (aerobic or anaerobic) of exercise that is important, but also the modality of exercise performed (for example running, walking, cycling and swimming)[15]. In this case, Nualnim *et al*[16] has shown that swimming training was able to promote hypotensive effects and improve the vascular function in adults over 50 years of age. Cycling exercise (30 min, 5 d per week, for 3 mo) significantly decreased the resting blood pressure and increased the NO plasma concentration in older (59-69 years) normotensive women, suggesting that aerobic ET exerts the beneficial effect of increasing NO production in previously sedentary older humans[10]. Furthermore, moderate intensity walking decreased the baseline SBP of postmenopausal women with hypertension[17], and treadmill exercise improved the endothelial function and vascular stiffness in coronary and mesenteric arteries of spontaneously hypertensive rats, which may be related to decreased oxidative stress and increased endothelial-dependent NO production[15].

In view of the beneficial effects of ET on the treatment of hypertension, and the new genetic findings revealed in the last decades, several scientists have turned their attention to a new class of gene expression regulators, known as microRNAs (miRNAs), which have been shown to be important factors in the gene regulation of hypertension and possible therapeutic targets for this disease[2]. The miRNAs are small, noncoding RNAs with approximately 17-25 nucleotides in length, which act as potent posttranscriptional regulators of gene expression. They can couple with sites in 3'-untranslated (3'-UTR) in the messenger RNAs (mRNAs) of protein-coding genes and negatively regulate their expression[18-20]. The posttranscriptional regulation realized by the miRNAs in 3'-UTR is dependent on the degree of complementarity between them and the target mRNA. Thus, the miRNA does not require perfect complementarity for target recognition. Due to the fact that they have small sequences and act without the need for complete pairing[21], a single miRNA can regulate up to 200 mRNAs, and more than one miRNA can regulate a single mRNA[22].

As hypertension is developed on the basis of genetic susceptibility associated with environmental factors, many studies have shown associations between it and miRNAs; and others between it, miRNAs and ET as a way to prevent or minimize the harmful effects of environmental and/or genetic factors that promote hyperten-

sion. Based on the abovementioned data, the aim of this review is provide an overview of how ET can help to regulate blood pressure by means of specific miRNAs in the heart, vascular system, and skeletal muscle.

EFFECTS ON THE HEART

Hypertension is the major risk factor for congestive heart failure and chronically induces a chronic pressure overload on the heart. Sustained high blood pressure induces pathological cardiac hypertrophy (CH) and contractile dysfunction as compensatory mechanisms to reduce left ventricle wall stress. In addition to the increased size of cardiomyocytes, the growth of extracellular matrix is exacerbated and consequently there is interstitial fibrosis, and abnormalities occur in the systemic and coronary vasculature[23].

Whereas, ET consists of a frequent, but intermittent stimulus of hemodynamic volume overload on the heart, which induces physiological CH. In this condition, the increase in size of the cardiomyocytes predominantly occurs by expression of sarcomere proteins, and the process is concatenated with preserved or improved cardiac function[20]. Indeed, it is known that ET is able to decrease systolic and diastolic blood pressure in hypertensive humans and rats, and that the physiological CH or pathological CH triggers different signaling pathways, which in turn trigger specific transcription factors. Consequently, the pattern of gene expression is different in the two types of CH[24-26]. In addition, there is an intricate network of transcriptional and posttranscriptional mechanisms involved in the differential expression of these genes, and there is still much to clarify as regards the differentiation of physiological and pathological phenotypes of CH[26].

The miRNAs are part of the posttranscriptional mechanism, performing negative regulation of several target mRNAs involved in both physiological and pathological CH. The miRNAs are essential in different cell processes involved in the regulation of cardiovascular phenotypes, such as cardiomyocyte growth, remodeling, interstitial fibrosis, and heart failure. Several studies that postulate the relations between CH, hypertension and miRNAs have emerged. The miRNAs more frequently cited in cardiomyocytes studies are the miRNA-1, -133, -30, -21, -98, -378, -221, -22, -27, -212/132, -199 and -350 with several targets that are involved in the adaptive response of CH[27,28].

Recent studies have supported the suggestion that CH may be caused by inflammatory signaling, and that this may be mediated by miRNAs. The miRNA-155 is expressed in macrophages and is a key mediator of cardiac injury in hypertensive heart disease, by the regulation of cardiac inflammation, dysfunction and hypertrophy in pressure overload[29]. Moreover miRNA-155 directly targets endothelial nitric oxide synthase (eNOS) and the type 1 receptor of Angiotensin Ⅱ (AT1R), primordial targets that regulate the tonus of vascular smooth muscle cells (VSMC), and hence the peripheral cause of pressure overload on the heart[29-32].

With regard to ET and CH, Fernandes *et al*[5] have shown that swimming training was able to increase miRNA-27a and 27b [targeting angiotensin-converting enzyme (ACE)] and to decrease miRNA-143 [(targeting angiotensin-converting enzyme 2 (ACE2)] in the heart of rats. The CH induced by ET involves the regulation of miRNAs related to increased AT1R expression without the participation of Angiotensin Ⅱ. Parallel to this, the increase in ACE2, Angiotensin (1-7) and type 2 receptor of Angiotensin Ⅱ in the heart has also suggested that miRNAs were involved in upregulation of the non-classic renin-angiotensin aldosterone system (RAAS), counteracting the classic cardiac RAAS in physiological CH[5]. Thus, it is plausible to suggest a relationship between ET and CH through the regulation of several targets by miRNA-155, -27a, -27b, -143.

A hallmark related to hypertension and pathological CH is the reactivation of a set of fetal cardiac genes, which are repressed postnatally and replaced by the expression of adult genes. These genes include atrial natriuretic peptide/B-type natriuretic peptide, skeletal α-actin, and β-myosin heavy chain (βMHC). The causes and consequences of fetal gene expression in the adult heart have not been completely elucidated, but is known that chronic stress on the heart, such as hypertension, increases βMHC (slow ATPase activity) and decreases αMHC (fast ATPase activity), which has been implicated in impaired cardiac function[13,33,34]. It is well known that in cardiovascular disease, the expression of βMHC increase while αMHC decreases and that ET is able to reverse these abnormalities in rats[20,33,34].

The miRNAs -208a, -208b, and -499 are called "*myomiRNAs*", which regulate the expression of slow myosin, playing an important role in the control of cardiac disease progression[35-38]. The inhibition of miRNA-208a with Locked Nucleic Acid-Modified Anti-miRNA-208a (LNA-antimiRNA-208a) induced reversion in MHC switching during heart failure in hypertensive rats, reduced deleterious cardiac remodeling, and prevented the deterioration of cardiac function and lethality in rats[39]. In another study, the circulating miRNA-16, -19b, -20b, -93, -106b, -223, and -423-5p were equally reversed by both LNA-antimiRNA-208a and captopril therapy, and the results were correlated with the changes in βMHC expression in the time course of hypertension or therapy in rats[40]. With regard to ET, recent studies performed in our laboratory showed that ET decreases cardiac miRNA-208a expression in healthy Wistar and obese Zucker rats, induces upregulation of targets as THRAP-1, Purβ and Sox6, and improves the balance between the βMHC and αMHC gene expression[41,42]. Thus, miRNA-208a is another pathological miRNA naturally reversed by ET, and its downregulation is involved in the increase in several targets that constitute a gene program to improve the contractile efficiency of the heart[35]. Regarding circulating miRNAs, the miRNA-208a and -499 also reflect cardiovascular damage and a poor prognosis in patients with viral myocarditis, acute myocardial infarction, hyperten-

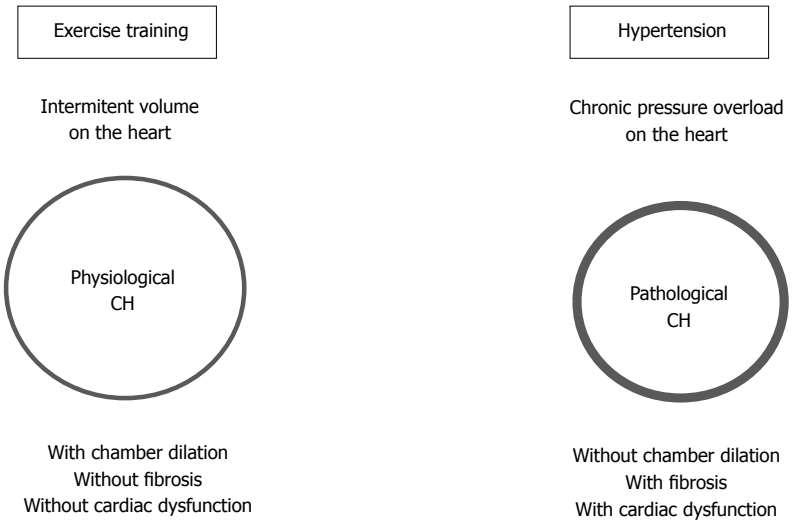

Figure 1 Effects of aerobic exercise training on the cardiac miRNAs in hypertension. CH: Cardiac hypertrophy; COL Ⅰ: Collagen 1; COL Ⅲ: Collagen 3; ACE: Angiotensin-converting enzyme; ACE2: Angiotensin-converting enzyme 2; THRAP-1: Thyroid hormone-associated protein 1; Purβ: Purine-rich element binding protein B; α-MHC: α-Myosin heavy chain; β-MHC: β-Myosin heavy chain.

Effect of exercise training	Targets	Phenotype	Effect of hypertension
↑ miRNA-29c	COL Ⅰ; COL Ⅲ	Fibrosis	miRNA-29c ↓
↑ miRNA-27a ; miRNA-27b	ACE	Vascular resistance	miRNA-27a; ↓ miRNA-27b
↓ miRNA-143	ACE 2	Vasodilation	miRNA-143 ↑
↓ miRNA-208a	THRAP-1; Purβ; Sox6	Balance between α/β-MHC expression	miRNA-208a ↑

sion or diastolic dysfunction[43].

Hypertension induces cardiac fibrosis. The aberrant expression of matrix extracellular proteins is determinant in the differentiation between pathological and physiological CH[20,44]. There are also several miRNAs involved in the fibrotic response to stress and ischemic stimulus: the miRNA-21, -24 family, -29 family, -101, -206, -132, -214, involved in fibroblast survival, growth and differentiation or dysfunction of extracellular matrix[44]. The downregulation of miRNA-29b induces upregulation of several extracellular matrix genes in the heart during pathological CH, including collagens and elastin[45]. In addition, the miRNA-29c has recently been implicated in the immunopathogenesis of atrial fibrillation, showing a relationship between cardiac arrhythmia and abnormalities in miRNA-29c expression[46]. ET is able to increases cardiac miRNA-29c expression, and a study conducted by Soci *et al*[20] has shown that swimming training increased miRNA-29c, and downregulated collagens Ⅰ and Ⅲ in the CH of rats. Thus, the future perspective related to fibrosis, immune system and cardiac automatism points to an integrative and regulatory role of miRNAS in the fibrotic response of the heart, requiring further investigations about miRNAs and its mRNA targets involved in the processes that regulate diastolic function, cardiac automatism and hypertension. Another interesting miRNA that plays an important role in the dysfunction of cardiovascular system is the miRNA-34a, which is induced in the ageing heart. The inhibition of this miRNA reduces fibrosis following the acute myocardial infarction and improves recovery of myocardial function[47]. In this way, studies linking this miRNA, cardiac fibrosis, and ET will contribute to the knowledge and treatment of cardiovascular dysfunctions in the future.

According to the studies conducted by our laboratory, ET is able to reverse or prevent pathological processes involved in hypertension[5,20,24,25,48], but further studies are needed, and will be performed to investigate the relations between ET, hypertension, and CH in the perspective of miRNAs.

The Figure 1 summarizes the effects of ET or hypertension on some cardiac miRNAs.

EFFECTS ON THE VASCULAR SYSTEM

Macro- and microcirculation interact in the vascular system, and their changes contribute to end-organ damage in hypertension[49]. However, these two vessel types must to be discussed separately because they are differently regulated[50].

Macrocirculation

Macrocirculation comprises large arteries such as the brachial, radial, femoral, aorta, epicardial arteries, and others vessels with the purpose of supplying blood from heart to peripheral tissues, and also performs the function of transforming the pulsatile flow into a steady flow necessary to supply oxygen to the tissues. In order to do this, good arterial compliance and distensibility are required, but in hypertension these properties of the arteries are affected[51].

In hypertension, the structure, mechanical behavior and function of vessels are affected, with a reduction in lumen diameter and thickening of the tunica media (structural change), increased vascular stiffness (mechanical change), and impaired NO-dependent vasodilation

(functional change)[51]. The direct relationship between hypertension and thickening of large vessels is due to an adaptive response of VSMC to increased internal arterial radius secondary to increased wall tensile stress imposed by pulsatile blood flow. Thus, VSMC become hypertrophied and change from the contractile phenotype to a proliferating phenotype. In addition, there is an increased collagen content in vessels, which contributes to increases in arterial thickness[49]. Furthermore, hypertension promotes decreased endothelial NO production, which leads to more collagen expression and VSMC growth, affecting the vascular thickening and stiffness[52]. In fact, NO is able to inhibit the expression of collagen, and lack of NO may induce excessive proliferation of VSMC[53]. Thus, ET can act positively to prevent or reverse these vascular changes induced by hypertension, such as hypertrophic remodeling that may be caused by loss of mitogenic quiescence of VSMC, resulting in their proliferation and establishment of a hypertrophied phenotype[53,54]. ET may also act on the vascular stiffness caused by high expression of collagen, which can be induced by hyperactivity of the renin-angiotensin system, and may also act on the impaired endothelium-dependent vasodilation, improving the NO availability[51].

A large body of evidence has indicated that ET exerts positive effects on preventing or reversing the structural, mechanical, and functional vascular changes in hypertension. Indeed, Moraes-Teixeira *et al*[55] have shown that the effects of treadmill ET (1 h/d, 5 d/wk, 20 wk) were able to decrease the circumferential wall tension and intima-media thickness in the aorta of exercised spontaneous hypertensive rats (SHR) compared with non-exercised animals, without significant differences in the lumen diameter among the studied groups at the end of protocol. Moreover, treadmill exercise increased the percentage of elastic fibers; percentage of eNOS density in the aortic wall, and decreased blood pressure in exercised SHR compared with their non-exercised controls. These results were interesting, because they showed that ET was able to prevent or reverse the capacity of hypertension to modulate the thickening and stiffening of large vessels in rats. Furthermore, Guimarães *et al*[56] assessing arterial stiffness by carotid-femoral pulse wave velocity in hypertensive patients has shown that interval ET (16 wk of training) decreased arterial stiffness in trained subjects. In addition, aerobic ET (30 min, 3 times/wk, 4 wk) was able to reduced arterial stiffness in young men with a family history of hypertension[57]. Jordão *et al*[58], have shown that treadmill exercise was able to reduce the mRNA expression of collagen I and Ⅲ; prevent rupture of the internal elastic lamina, and improve the orientation of VSMC in the aorta of trained SHR compared with non-trained animals. Furthermore, endurance training attenuated the oxidative stress in the aorta of SHR, showing a possible suppressive effect of the exercise on the development of arteriosclerosis[59], and was able to increases endothelium-dependent relaxation through NO pathways in the aorta of SHR[60].

Microcirculation

Microcirculation is a network of vessels that includes the smallest arteries, arterioles, capillaries and venules which, by definition, have an inherent physiological characteristic of responding to increasing pressure by a myogenic reduction in lumen diameter, rather than a definition based on the vessel diameter and structure. Microcirculation has the function of optimizing nutrient and oxygen supply within the tissue in response to variations in demand; avoiding potential fluctuations in the pressure at the level of the capillaries, and determining the overall peripheral resistance[49,61].

In hypertension, the mechanisms regulating vasomotor tone may be abnormal, leading to altered vascular function; and structural and mechanical alterations may also occur, such as an increased wall-to-lumen ratio and arterial stiffness. Furthermore, the rarefaction of arterioles and capillaries affecting the microvascular network has been observed in hypertension, and at first, it seems to be a functional change that involves the constriction of microvessels to the point of nonperfusion, and the second change is structural, when the nonperfused vessels may disappear. It is important to note that these factors will contribute differently in each vascular bed, and may vary between models of hypertension[49,50,61]. Central to these alterations there is impaired NO availability, secondary to oxidative stress, mainly due to the increased production of reactive oxygen species and reduced antioxidant capacity, as well as increased cyclooxygenase-derived contractile products[49,50,62-66].

Microvascular damage is a predictor of long-term adverse cardiovascular prognosis. Endothelial dysfunction has been considered an independent predictor of adverse cardiovascular events, providing a better predictive value of future cardiovascular events than each traditional risk factor alone identified in the Framingham study[67]. Moreover, the abnormal artery structure and the arterial stiffness of small vessels are predictors of later cardiovascular events and have prognostic implications[68-71]. As regards rarefaction, further prospective study is needed to determine whether it presents a clinically relevant predictive value, however it is important to note that microvascular rarefaction will reduce oxygen delivery resulting in ischemia, which may be responsible for much of the end-organ damage associated with hypertension[49,61].

Given the central role that all these alterations play in vascular biology, it seems attractive to consider the relevance of therapeutic improvement in function, structure and mechanical alterations in hypertension. Thus, non-pharmacological approaches, such as ET, are able to improve blood pressure control and vascular alterations in hypertension. It is well established that ET decreases blood pressure[24], and although endurance training, dynamic resistance training and combined training were associated with decreases in blood pressure, until clearer evidence emerges, it may be prudent to prescribe endurance training for the hypertensive individual[24]. In accordance with Cornelissen *et al*[72], aerobic endurance training

decreases blood pressure through a reduction in vascular resistance.

Regular ET improves endothelial function in hypertensive patients as well as in animal models of hypertension[73,74]. Although to a lesser extent, some recent studies have also confirmed the beneficial effects of continuous aerobic ET on the endothelial function of small arteries, such as in arteries of gastrocnemius muscle from rats with chronic NO synthase inhibition[75]; in mesenteric resistance arteries and small coronary arteries from SHR[15]; and in resistance arteries from young pre-hypertensive patients[76]. Emerging evidence has increasingly demonstrated that diverse beneficial effects induced by ET in hypertension are mediated, at least in part, by reversing oxidant stress[77]. In fact, in small arteries and arterioles the reduced oxidative stress significantly contributes to endothelium-dependent vasodilation that has been enhanced by ET[15,78].

In addition to the functional improvement, structural and mechanical changes are also mediated by exercise in hypertension. Recently, we demonstrated that aerobic treadmill exercise reverts the increased arterial stiffness of both mesenteric resistance and small coronary arteries, mediated by changes in the extracellular matrix[15], although it did not modify the increased wall-to-lumen ratio of these arteries in hypertension. However, the vascular remodeling induced by ET may be dependent on the vascular bed studied, and thus either improvement[79,80] or no effects[15,79,80] already have been observed in microcirculation in hypertension. In addition to structural changes, aerobic ET corrects capillary rarefaction in hypertension[7,79]. Indeed, a balance between angiogenic and apoptotic factors to prevent microvascular abnormalities in hypertension has been observed as an effect of ET[7]. In addition, the decrease in oxidative stress induced by ET in SHR seems to be associated with the normalization of the reduced number of endothelial progenitor cells in a vascular endothelial growth factor (VEGF)/eNOS-dependent pathway, thus promoting a peripheral revascularization induced by aerobic ET[11].

Although many vascular effects of ET have been established in hypertension, little is known in the literature about their beneficial effects on miRNAs of the small and large vessels. However, recently new approaches in ET have highlighted the key role of miRNAs in the modulation of hypertension.

The miRNAs are involved in all biological processes, including cellular proliferation, differentiation, cellular migration and apoptosis, and their deregulation often results in the development of cardiovascular diseases. As there is high expression of miRNAs in the vascular system, growing evidence suggests that miRNAs may be important in the development of endothelial dysfunction, vascular remodeling and reduced angiogenic capacity, features that are frequently observed in the pathogenesis of hypertension[2,81,82]. More specifically, the phenotypes of VSMC and endothelial cells, as well as the inflammatory activation of macrophages is regulated by miRNAs, which may promote the structural changes that lead to vascular remodeling[83]. VSMC maintains remarkable plasticity, able to react to various forms of vascular stress or injury by switching from the contractile phenotype to a proliferating and synthetic phenotype[2].

Studies have associated hypertension and alterations in the expression of miRNAs with the angiogenic process, endothelial dysfunction, changes in the RAAS, and in the phenotype of VSMC[2], but as regards the role of ET, there is almost nothing in the literature showing a direct connection between the modulation of miRNAs and vascular changes in hypertension. For example, nothing has yet been shown in the literature relating hypertension and the effects of exercise to the miRNAs existing in aorta. However, considering the atherosclerosis, Wu *et al*[84] investigated the effects of treadmill ET for a period of 12 wk, 5 times per week, 60 min/d, on the aorta of male ApoE null C57BL/6J mice with atherosclerosis, which were fed a high-fat diet. The authors showed that ET significantly decreased the angiotensin II and endothelin 1, and prevented the formation of plaques and foam cells in comparison with the control group, followed by decreased expression of miRNA-155, and increased expression of miRNA-146a and miRNA-126 in the aorta of the trained mice, with more pronounced changes in the groups treated with Simvastatin. The miRNA-146a interacts with the 3'-UTR of the tumor receptor-associated factor 6 (*TRAF6*) gene, negatively impacting the toll-like receptor 4 (TLR4)-TRAF6 signaling, and then reduces the inflammatory response in atherosclerosis[85]. The decrease in miRNA-155 expression is an essential factor for increasing eNOS expression and NO production, because eNOS is directly targeted by this miRNA[31,86]. In atherosclerosis, miRNA-155 is drastically upregulated[31], because inflammation factors increase miRNA-155 *via* activation of nuclear factor (NF)-κB, activator protein-1, and Rho kinase. The miRNA-155, together with yet unidentified cytosolic RNA-binding proteins, bind to the eNOS mRNA 3'-UTR and destabilize eNOS mRNA, resulting in decreased eNOS and NO production. However, anti-miRNA-155, statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) and other Rho kinase inhibitors prevent the increase in miRNA-155[86], and then maintain the eNOS and NO production, or possibly increase their production. However, in hypertension, could ET diminish miRNA-155 expression in vessels? To our knowledge, at present there are no studies to answer this question. Nevertheless it is known that exercise can modulate the miRNA-155 expression in atherosclerotic vessels.

The study of Wu *et al*[84] has also shown that ET was able to increase the miRNA-126 expression in the aorta of the studied mice. The miRNA-126 suppresses the cell adhesion molecule expression and negatively regulates the endothelial receptor of α4β1 integrin, thereby interfering with adhesion of leucocytes to the endothelium[87,88], as well as enhancing angiogenesis[87]. In agreement with Harris *et al*[87], miRNA-126 is expressed in endothelial cells, but not in VSMC, and miRNA-126 in other tissues might simply reflect the vascularity of the organ. Target deletion of miRNA-126 in mice promotes hemorrhaging, loss of

Table 1 Vascular effects of hypertension and exercise training in macro- and microcirculation

Hypertension	Exercise training	Ref.
Hypertrophy of VSMC	Decreased intima-media thickness	[49,52,55-57]
Excessive proliferation of VSMC	Improved orientation of VSMC	[49,53]
Increased collagen content	Decreased mRNA collagen expression	[49,51,58]
Decreased availability endothelial NO	Increased endothelial NO production	[49,51,55]
Increased endothelium-dependent contract factors	Increased endothelium-dependent relaxation	[59,75]
Increased ROS production	Increased antioxidant capacity	[49,50,59,62-66]
Increased overall peripheral resistance	Reduced vascular resistance	[24, 49,50]
Rarefaction of arterioles and capillaries	Increased peripheral revascularization	[7,49,50,61,79]

VSMC: Vascular smooth muscle cells; NO: Nitric oxide; ROS: Reactive oxygen species.

Table 2 Some miRNAs associated with hypertension that has potential to be regulated by exercise training

miRNAS	Targets	miRNA function	Ref.
miRNA-16	VEGF	Control of angiogenesis and vascular integrity	[7]
miRNA-21	PTEN; Bcl-2	Involved in nitric oxide production; apoptosis	[2,7,80,82]
miRNA-24	Trb-3	Mediator of contractile phenotype in VSMC	[81-83]
miRNA-92a	Integrin α5; eNOS	Involved in the regulation of endothelial function	[88]
miRNA-126	VCAM-1; PI3KR2; Spred1	Suppress cell adhesion molecule; Proangiogenic	[48,85,87,88]
miRNA-143/145	KLF4; KLF5	Involved in the plasticity of VSMC	[81]
miRNA-146a	TRAF6; KLF4	Involved in inflammatory response	[85]
miRNA-155	eNOS; AT1R	eNOS expression and NO production	[85]
miRNA-221/222	P27Kip1	Involved in the proliferation of VSMC	[81]

VEGF: Vascular endothelial growth fator; PTEN: Phosphatase and tensin homolog; Bcl-2: B-cell CLL/lymphoma 2; Trb-3: Tribbles-like protein-3; eNOS: Endothelial nitric oxide synthase; NO: Nitric oxide; VECAM1: Vascular cell adhesion molecule 1; PI3KR2: Phosphatidyl-inositol 3-kinase regulatory subunit beta; Spred1: Sprouty-related; EVH1: Domain-containing protein-1; KLF4: Krüppel-like factor 4; KLF5: Krüppel-like factor 5; TRAF6: TNF receptor-associated factor 6; AT1R: Type 1 receptor of angiotensin Ⅱ; P27Kip1: Cyclin-dependent kinase inhibitor 1B.

vascular integrity and defects in endothelial cell proliferation, migration and angiogenesis[89]. The miRNA-126 is one of the most abundant miRNA in endothelial cells, and plays an anti-atherogenic role by enhancing endothelial repair[2,82,84].

Another miRNA that deserves to be remembered is the miRNA-34a because its overexpression has been associated with senescence of endothelial cells. This miRNA targets SIRT1 (sirtuin 1) in the endothelial cells[90] and may serve to mediate the effect of aging upon the vasculature[91]. The inhibition of SIRT1 impairs the eNOS methabolism in the endothelial cells *via* SIRT1/eNOS axis[92,93], allowing us to suppose that this miRNA may be related with development or progression of atherosclerosis or hypertension. However, such as in heart, the literature has not shown studies linking the miRNA-34a, vascular cells, and ET.

In addition to the above mentioned data, other miRNAs are able to participate in the vascular changes. In this case, the miRNA-143/145 cluster is related to the phenotype plasticity of VSMC, and together with miRNA-21 and miRNA-24, they are involved in the differentiation and proliferation of these cells. Thus, several studies have shown that they are downregulated in injured vessels[2,81-83]. The miRNA-21 is involved in vascular remodeling that affects both VSMC and endothelial cells. The overexpression of miRNA-21 induces a synthetic VSMC phenotype, as observed after vascular injury, and it is also a critical miRNA for angiogenesis[2,81,82]. The miRNA-221/222 have an ambiguous response after vascular injury, enhancing the proliferation of VSMC, whereas it may be atheroprotective in endothelial cells[81,83]. The miRNA-221/222 may also be involved in the control of eNOS expression[94]. The miRNA-92a is a negative regulator of endothelial function, and its overexpression represses *eNOS* gene expression and angiogenesis[88]. The abovementioned miRNAs have been found to be related to hypertension. Bátkai *et al*[2] and Synetos *et al*[95] provided an overview of the role of miRNAs in the development and consequences of hypertension.

Recent data from our research group has shown the effects of ET on vascular miRNAs involved in hypertension. Our study reveals some of the molecular mechanisms of ET in physiological revascularization observed in hypertensive rats. Swimming ET restored the balance between injury and repair in the vascular process collaborating with the regression of hypertension, and remarkably, restoring normal expression of skeletal muscle microcirculation miRNA-16, -21, and -126[7]. This alteration occurred parallel with normalization of VEGF, eNOS, and PI3KR2 levels, as well as the proapoptotic (Bad) and antiapoptotic (Bcl-2, Bcl-x, and p-Bad$_{ser112}$:Bad ratio) mediators, indicating that balance between angiogenic and apoptotic factors may prevent microvascular abnormalities in hypertensive rats[7].

The study of miRNAs may generate hypotheses about the mechanisms by which exercise affects the pathophysiology of hypertension. ET probably causes al-

terations in many of the miRNAs that are deregulated in the vascular system. Thus, a promising tool emerges for the treatment and expansion of knowledge about hypertension.

Effects of hypertension and ET in the vascular system are summarized in Table 1, and some miRNAS, its target, and miRNA function are in the Table 2.

EFFECT ON THE SKELETAL MUSCLE

Microvascular abnormalities, such as reduction in blood flow and microvascular rarefaction, are clear evidence of disturbance of the angiogenic process related to changes in the muscle fiber profile in hypertension[61,96,97].

It is interesting to note that studies have shown that ET-induced blood pressure reduction in SHR was correlated with both normalization of arterial wall-to-lumen ratio and a great increase in capillary-to-fiber ratio in skeletal muscle. Indeed, evidences have shown that ET improves both endothelial function and muscle fiber profile, counteracts microvascular rarefaction and decreases blood pressure in hypertension[7,11,79,80,98].

It is known that the angiogenesis represents a primary adaptive response of the skeletal muscle to aerobic ET, hence contributing to the improvement in muscular aerobic capacity (oxygen transportation, provision and extraction)[79]. On the other hand, many conditions, such as cardiovascular disease (CVD) risk factors, lead to alteration in the capillary support of skeletal muscles, and may consequently, impair the offer of oxygen and nutrients, which is related to alteration in the distribution of the skeletal muscle fiber types towards an increase in type Ⅱ fibers. As yet, little is known about the origin of the transition from type Ⅰ fibers to type Ⅱ in the soleus muscle of SHR; however, studies have shown that it is related to capillary rarefaction followed by alterations in metabolic properties[96,99].

Studies have shown that when there is a transition between the types of fibers of the skeletal muscle, the different morphological properties of the muscular fiber are changed in the following manner: the capillary density and activities of the energy metabolism enzymes are altered at an early stage during the transition, and precede the change in myofibrillar ATPase activity and the contractile characteristics of the muscle[100].

In mammals, the skeletal muscle fibers are usually classified as type Ⅰ and type Ⅱ fiber, according to the different activities of the myosin ATPase after pre-incubation at different pHs, and the type Ⅱ fibers can be subclassified into ⅡA, ⅡX/D and ⅡB. The type Ⅱ fibers are characterized as being fast twitch with predominance of glycolytic metabolism, while the type Ⅰ fibers are slow twitch with predominance of oxidative metabolism[96].

Evidences in the literature have shown that the skeletal muscle of hypertensive individuals, and of SHR, contains a higher percentage of type Ⅱ fast twitch, glycolytic fibers compared with their normotensive controls[7,96,100,101]. It is interesting that the results obtained in the analysis of the composition of the fiber types of the soleus skeletal muscle (which presents an average of 90% of type Ⅰ fibers and 10% of type Ⅱ fibers), performed both by histochemical myosin ATPase reaction and SDS-PAGE gel electrophoresis for detection of MHC for each type of fiber, were positively correlated regardless of the technique applied[101]. According to Bortolotto *et al*[101] the main result obtained in their study was that in all stages of hypertension (4, 16 and 24 wk), the soleus muscle of SHR presented a higher proportion of type Ⅱ fibers than the soleus muscle of Wistar Kyoto rats (WKY), as well as hybrid fibers, those that contain two types of MHC in the same muscle fiber isolate, in the case of SHR, a higher proportion of ⅡA + ⅡX hybrid fibers. The presence of a higher proportion of hybrid fibers is an indication of the transition of muscle fiber type in the muscle under consideration.

Some studies have associated the effects of ET with pharmacological treatment. Minami *et al*[102] showed the effects of ET either associated with treatment with perindopril (ACE inhibitor) or without it, on the capillarity and fiber types in the soleus muscle of SHR. The authors observed that chronic treatment with perindopril increased the exercise capacity in untrained animals; however, this effect was not synergic to the exercise capacity acquired as a result of ET alone. Whereas, the treatment with perindopril associated with ET promoted adaptive alterations in the soleus muscle, such as increase in capillary density and percentage of type Ⅰ fibers[102]. Although no alteration in the composition of types of fiber was observed in the trained SHR and SHR treated with perindopril groups when compared with the sedentary SHR group, the authors observed higher capillarization in these groups, which may be attributed to the improvement in exercise capacity. A more recently study from the same group showed that pharmacological treatment with a calcium channel blocker (azelnidipine), or a type Ⅰ angiotensin Ⅰ receptor antagonist (olmesartan) or even the ET significantly increased capillary density and percentage of type Ⅰ fibers in the soleus muscle of SHR[103]. Although the results in the literature are still controversial with respect to the alterations in proportion of the types of fiber in response to ET, it was also not possible to observe the comparison between the profile of the types of fiber in the trained SHR group compared with its normotensive control WKY, with the aim of checking normalization with the fiber type composition.

Recently, Fernandes *et al*[7] for the first time, showed evidence that aerobic ET corrected the alteration in the composition of fiber types in the soleus muscle of SHR when compared with WKY. This result is probably linked to the increased capillarization and citrate synthase activity observed with ET, since these adaptations are related to changes in fiber type in the skeletal muscle. Altogether, these ET-induced adaptations contribute to the increase in oxygen consumption and exercise tolerance, and the decrease in BP levels observed in the trained hypertensive group.

Although studies have reported change in the profile of skeletal muscle fibers in hypertension, none of them

observed change in muscle mass in hypertensive rats up to 24 wk of age[7,96,100,101,104]. It is interesting that Carvalho *et al*[105] determined the soleus muscle changes in the expression of MHC isoforms, diameter of fiber types and muscle atrophy during the transition of ventricular hypertrophy to heart failure induced by aortic stenosis. The animals developed a myopathy in the soleus muscle, characterized by a decrease in the percentage of type I fibers and increased frequency of type Ⅱa fibers, in cardiac hypertrophy (after 18 wk) and heart failure (after 28 wk). However, atrophy of type Ⅱa fibers occurred only during heart failure.

Recently, for the first time in the literature, Damatto *et al*[106] reported changes in MHC isoforms and soleus muscle atrophy induced by heart failure in SHR. The setting of heart failure in SHR at 18 mo of age was observed and muscle disorders were associated with myogenic regulatory factors and expression of myostatin and follistatin.

Many studies have shown the beneficial effect of ET on muscle atrophy and correction of changes in fiber types in animals with heart failure due to various etiologies, such as myocardial infarction, and sympathetic hyperactivity[99,107], however no study up to now has reported the effects of ET on these changes in animals with heart failure with the etiology of hypertension.

In spite of the important role of exercise in the prevention and treatment of hypertension, the mechanisms involved in these vascular and muscle changes are not fully understood. The analysis of miRNAs has made it possible to understand the development of various types of CVD, and the elucidation of these processes regulated by miRNAs and identification of new targets of miRNA in the pathogenesis of disease is a very valuable strategy for both prevention and treatment of hypertension.

Recent studies have revealed that myogenic transcription factors involved in differentiation and muscle contraction also activate the expression of a set of miRNAs with the function of "adjusting" the output of the transcription network, resulting in precise cellular responses to signals of development, physiology and pathology. The integration of these small RNAs into the muscle transcriptional program further expands the accuracy and complexity of the regulation of genes in muscle cells, since miRNAs are capable of regulating various mRNAs, and mRNAs can be targets of many miRNAs[108-110].

The miRNAs-1, -133a-b, -206 and -208 are muscle-specific and have been studied thereby contributing to muscle development. It is interesting that these miRNAs provide up to 25% of miRNAs expressed in skeletal muscle; they are recognized by their control of the growth, differentiation and contractility of muscle[111-116]. Additional miRNAs have been described; these regulate myoblast proliferation or differentiation, and include miRNAs-24, -26a, -27b, -125b, -148a, -181, -214 and -489[116-119]. Curiously, high expression of miRNA-128a was found in skeletal muscle, and increased during myoblast differentiation, regulating target genes involved in insulin signaling, which include insulin receptor (Insr), insulin receptor substrate 1 (Irs1) and phosphatidylinositol 3-kinases regulatory 1 (Pik3r1). In fact, Motohashi *et al*[116] showed that overexpression of miRNA-128a in myoblasts inhibited cell proliferation by targeting Irs1. In contrast, inhibition of miRNA-128a induced myotube maturation and myofiber hypertrophy *in vitro* and *in vivo*.

The miRNAs-1 and -133 are expressed in cardiac and skeletal muscle and they are transcriptionally regulated by myogenic differentiation factors, such as MyoD, myogenin, Mef2 and SRF (serum response factor)[110-113]. The miRNA-1 promotes differentiation of cardiac and skeletal progenitor cells and exit from the cell cycle in mammals[109], while the miRNA-133 inhibits differentiation, and maintains cells in a proliferative state[111].

Increased expression of miRNA-1 in skeletal muscle of mice after 3 h of a single session of aerobic ET was observed by Safdar *et al*[120]. This increase was associated with a reduction in the expression of its target histone deacetylase 4 (HDAC4), a transcriptional repressor of muscle gene expression, and by the increase in myogenic differentiation factors such as MyoD, and thus would promote remodeling of the lesion caused by the training session[113,120]. Conversely, the chronic effect of exercise led to a decrease in the expression of miRNA-1 associated with muscle hypertrophy in favor of the expression of important genes in muscle growth, such as c-Met, hepatocyte growth factor and Insulin-like growth factor 1 (IGF-1). IGF-1 is a potential target of miRNA-1, which could partly explain the hypertrophic phenotype during the initial responses resulting from ET overload[121,122].

The miRNA-206 is the only miRNA specifically expressed in skeletal muscle, and its expression appears to be induced by MyoD and myogenin during myogenesis, promoting differentiation[113,114,123]. HDAC4, PAX7, MET and Notch3 are some of the target *miRNA-206* genes related to the muscle differentiation process[113,115,123].

These skeletal muscle miRNAs also appear to participate in muscle diseases including cardiac hypertrophy, heart failure and muscular dystrophy, such as Duchenne muscular dystrophy[113,115,122,124].

Studies have reported that an intron of the αMHC (*Myh6*) gene encodes a miRNA -miRNA-208a, which is necessary to increase βMHC (Myh7) in the heart of adult animals in response to stress and hypothyroidism[35]. Given that miRNA-208a and their host myosin, αMHC, are only expressed in the heart, these results raise interesting questions with respect to which other miRNAs could control the fiber type and gene program in skeletal muscle contractile proteins[38].

van Rooij *et al*[38] showed the existence of two miRNAs in MHC genes. The *βMHC* gene encoding the miRNA-208b, which has an identical sequence to the seed miRNA-208a, and differs at only three nucleotides in the 3' region. A third member of this family is miRNA-499, encoded by the gene *Myh7b*, a little studied myosin that shares extensive homology with the *βMHC* gene. These two miRNAs are expressed in skeletal muscle, are related with an oxidative profile, such as in the soleus, and have a feature of type I fibers with predominance of *βMHC*.

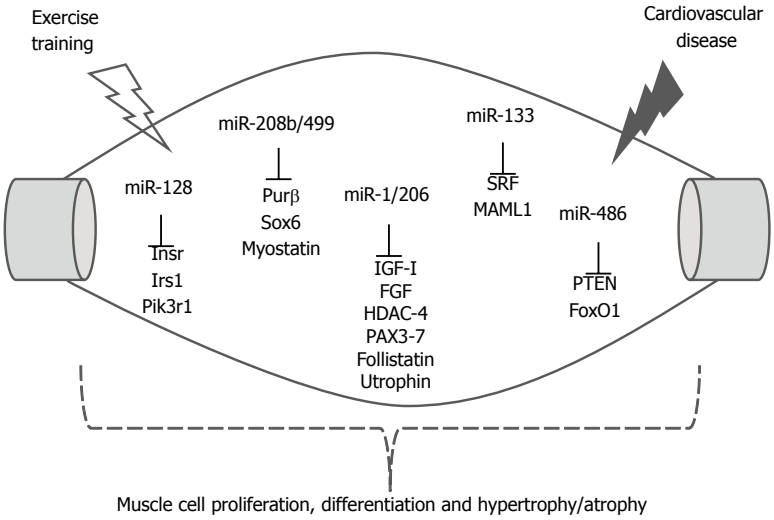

Figure 2 Skeletal muscle miRNAs and selected target genes regulating cell proliferation, differentiation and hypertrophy/ atrophy by exercise training and cardiovascular diseases. The relationship between miRNAs and the mRNAs that encode proteins is shown. Insr: Insulin receptor; Irs1: Insulin receptor substrate 1; Pik3r1: Phosphatidylinositol 3-kinases regulatory 1; Purβ: Purine-rich element binding protein B; HDAC4: Histone deacetylase 4; IGF-1: Insulin-like growth factor 1; FGF: Fibroblast growth factor; SRF: Serum response factor; MAML1: Mastermind 1; PTEN: Phosphatase and tensin homolog; FoxO1: Forkhead box protein O1.

Interestingly, deletion of miRNA-208b and miRNA-499 did not alter the expression of another miRNA in the soleus muscle, and the analysis of fiber type showed little or no difference in the number of type I muscle fibers in any of the mutant animals compared with the wild type. However, in the generation of double knockout animals (dKO) for miRNAs-208b and -499 there was a substantial loss of type I muscle fibers in the soleus muscle of dKO. The loss of slow fibers in dKO mice was also evident from the reduction in protein and gene expression of βMHC, and a concomitant increase in the expression of isoforms of myosin fast type IIa and type Ⅱb and Ⅱx[38].

Moreover, overexpression of miRNA-499 was sufficient to induce complete conversion of all fibers from soleus fast into slow fibers with a type I profile. Notably, when the animals were subjected to an exercise tolerance test on the treadmill, those with overexpression of miRNA-499 ran 50% more than the wild-type, indicating a higher aerobic endurance resulting from the reprogramming of muscle fibers with the induction of predominance of type I fibers, slow-twitch and oxidative metabolism. Moreover, the authors investigated the possible targets of these miRNAs related to the control of βMHC. The findings showed that the transcription factors Sox6 (a member of family Sox transcription factors) and Purβ are targets of miRNA-208b and -499 in skeletal muscle, and dKO animals have increased expression of both factors[38]. Other studies have also shown that Sox6 and Purβ inhibit the expression of βMHC in skeletal muscle involved in changing the profile of muscle fibers[125,126].

No studies have been conducted to evaluate the expression of these miRNAs and change in fiber type in CVD, in particular in hypertension. Knowing that in hypertension and CVD there is a change in muscle fiber profile, it would be appropriate to think that miRNAs-208b and -499 would participate in this change and that aerobic ET would be a strong candidate for the standardization of these parameters, since it is well known that ET increases the oxidative metabolism associated with a predominance of type I fibers (Figure 2).

CONCLUSION

Considering that hypertension affects over one billion people across the world, that ET plays a key role as non-pharmacological therapy for hypertensive patients, and that genic therapies from miRNAs may represent new strategies in combating the development and/or progression of hypertension, is reasonable to go more deeply into studies to acquire more knowledge about which miRNAs are induced by ET and which are related to protection of the cardiovascular system. Most important, these studies may guide scientists in future gene therapies for the treatment of hypertension with specific miRNAs. Finally, complementing the above discussion is important to comment about circulating miRNAs that results of cellular damage and that have been presented as biomarkers of cardiovascular diseases[127]. In this way, the circulating miRNA-1, -133a, and -208b were higher in patients with myocardial infarction in relation to patients who had unstable angina[128], and the miRNA-499 was increased in individuals with acute myocardial infarction compared with patients without myocardial infarction[129]. Also, patients with coronary artery disease or diabetes may presents reduced levels of circulating endothelial-enriched miRNAs, such as miRNA-126[130]. Moreover, it was reported a linkage between circulating miRNAs, human cytomegalovirus, and essential hypertension[131]. On the other hand, the literature has not presented studies linking circulating miRNAs, cardiovascular diseases, and ET. In this way, further studies need be realized. However, it is clear that circulating miRNAs will be used in the future also as biomarkers of the therapeutic efficacy of ET in the treatment of hypertension.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Prehypertension: Underlying pathology and therapeutic options

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Abstract

Prehypertension (PHTN) is a global major health risk that subjects individuals to double the risk of cardiovascular disease (CVD) independent of progression to overt hypertension. Its prevalence rate varies considerably from country to country ranging between 21.9% and 52%. Many hypotheses are proposed to explain the underlying pathophysiology of PHTN. The most notable of these implicate the renin-angiotensin system (RAS) and vascular endothelium. However, other processes that involve reactive oxygen species, the inflammatory cytokines, prostglandins and C-reactive protein as well as the autonomic and central nervous systems are also suggested. Drugs affecting RAS have been shown to produce beneficial effects in prehypertensives though such was not unequivocal. On the other hand, drugs such as β-adrenoceptor blocking agents were not shown to be useful. Leading clinical guidelines suggest using dietary and lifestyle modifications as a first line interventional strategy to curb the progress of PHTN; however, other clinically respected views call for using drugs. This review provides an overview of the potential pathophysiological processes associated with PHTN, abridges current intervention strategies and suggests investigating the value of using the "Polypill" in prehypertensive subjects to ascertain its potential in delaying (or preventing) CVD associated with raised blood pressure in the presence of other risk factors.

Key words: Prehypertension; Renin-angiotensin system; Therapeutic lifestyle changes; Polypill

Core tip: There is a current debate over the ideal means of intervening in prehypertension. Since it is the cardiovascular risk that constitute the basis for intervention in both prehypertension and hypertension, the review discusses the following points, that: (1) categorizing blood pressure levels is based on mere 20 mmHg brackets; hence this doctrine may be re-visited to include other cardiovascular risk factors to categorize patients regardless of their blood pressure level; (2) investigating the therapeutic potential of intervening in all pathophysiological processes associated with prehypertension; and (3) ascertaining the therapeutic value of the "Polypill" in prehypertensives as means of primary prevention.

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SEARCH AND ARTICLE COLLECTION METHOD

Initially, PubMed and Scopus were searched using the

key words prehypertension, epidemiology, and treatment (as such or in the form of derived terms as appropriate) alone and in combination, were used to identify a set of primary articles. Other searches using pertinent key words were conducted as required; such was particularly utilized during search for pathophysiologically-related publications. For example, treatment + prehypertension led to renin-angiotensin system, which led to angiotensin converting enzyme system, angiotensin converting enzyme inhibitors, angiotensin receptor blockers and so on. To the resulted articles we added our own collection. Reviews were used as another source to include more articles. All searches were limited to English language.

The main aim of this review was to provide an overall understanding of the current status of the medical profession opinion on prehypertension and to map the so far recommended or suggested therapeutic strategies. It also reflected on other potential therapeutic options. The selection of cited references was made to serve that aim.

CONCEPTUAL OVERVIEW

Definitions

The concept of prehypertension (PHTN) was introduced in 1939 by Robinson and Brucer who were first to draw attention to the range of blood pressure (BP) between 120-139 mmHg (systolic) and 80-89 mmHg (diastolic) as being of value in determining clinically overt hypertension (HTN)[1]. Almost three decades later, the same BP range was given the name "borderline hypertension"[2], then the name changed to "high-normal blood pressure" in 1997[3]. The name "prehypertension" was given in 2003[4]. The nomenclature went further by some authors[5] who categorized BP levels between 130-139/85-89 mmHg (the upper half of PHTN range) as "Stage 2" PHTN. Also, the upper half of the HTN range was given the name of "high normal" blood pressure by the European Society of Hypertension/the European Society of Cardiology[6].

HTN is defined as a systolic/diastolic pressure level of $\geqslant$ 140/90 mmHg)[7,8]. HTN is a major world health problem and is among the most prevalent chronic conditions with rates that reach up to 70% of adult population in some countries[9] and is on the increase[10]. HTN has been identified as the leading global risk factor for disease burden[11] and it is considered to be the most important modifiable risk factor for coronary heart disease, stroke, congestive heart failure and end-stage renal failure[12].

Salient issues pertaining to blood pressure and cardiovascular risk

Prior to start the discussion on the main topic of this review i.e prehypertension, there are a number of issues that are of significant value to this topic and they address the relationship of changes in BP and their correlation with cardiovascular (CV) morbidity and mortality. These are; the J-blood pressure curve concept; the central versus peripheral blood pressure relation with CV events; and the complex interaction of different antihypertensive drugs on blood pressure and cardiac hemodynamics. A brief account of each of these follows.

The J-blood pressure curve concept and cardiovascular risk

The "J-curve" concept describes the shape of the relationship between BP and the risk of CV morbidity or mortality[13]. Some authors consider the J-curve to be more correlated with diastolic blood pressure (DBP) than systolic blood pressure (SBP)[14] since most of coronary blood flow occurs in diastole[15]. Three pathophysiologic mechanisms have been proposed to explain the existence of a J-curve are: (1) low DBP could be an additional risk factor to coexisting or underlying poor health or chronic illness leading to increasing morbidity and mortality; (2) low DBP could be caused by an increased pulse pressure reflecting advanced vascular disease and stiffened large arteries; and (3) over-aggressive antihypertensive treatment could lead to too-low DBP and thus hypo-perfusion of the coronaries resulting in coronary events[13].

The J-curve concept is in line with the thought that BP has a continuous relationship with CV events such as myocardial infarction, stroke, sudden death, heart failure and peripheral artery disease as well as of end-stage renal disease[16-20] even at values such as 110-115 mmHg for SBP and 70-75 mmHg for DBP[21,22].

Central systolic versus peripheral systolic blood pressure and cardiovascular events

In adults, peripheral SBP (pSBP) exceeds central (aortic) SBP (cSBP) by about 10 mmHg or more[23]. This difference is greater in younger subjects, during exercise and is affected by drug therapy[23]. Because cSBP and cPP are more closely related to the load on the heart and pulsatile stress on the coronary arteries than pSBP, they are suggested to be better predictors of CV events[24,25]. Additionally, it may be highlighted that the heart, kidneys, and major arteries supplying the brain are exposed to aortic rather than peripheral pressure. Therefore, there is a rationale to believe that CV events may be more related to central rather than brachial pressure[26].

The increase in central pressure from diastolic to systolic values is determined by the compliance of the aorta as well as the ventricular stroke volume. A high central pulse pressure (PP) is considered to be a marker of increased artery stiffness and represents a well-established independent predictor of CV morbidity and mortality[27-29] in hypertensive individuals and even in those considered as having normal BP[30]. PP significantly predicts major adverse CV events including unstable angina pectoris, myocardial infarction, coronary revascularization, stroke, or death[31]. An independent correlation between aortic PP and coronary artery disease was established in men, along with age and hypercholesterolaemia[32,33]. The late decrease in DBP after the age of 60, associated with a continual rise in SBP, is consistent with increased large artery stiffness. Higher SBP, if left untreated, may accelerate large artery stiffness and thus perpetuate a vicious cycle[21].

Indeed, central pressure was found to be more (than peripheral pressure) correlated with indicators such as carotid intima-media thickness[24,34,35] and left ventricular mass[35-37]. Also, aortic pulse pressure was found to be significantly and independently correlated with angiographically determined coronary artery stenosis[38] and more related to CV events than brachial pressure[24,39-41] and responds differently to certain drugs[25,42]. For example, it was found that the β-blocker, atenolol, is inferior to other major anti-hypertensive drug classes in preventing CV events. β-blockers exert differential effects on brachial *vs* central pressure which may help to explain the adverse findings with atenolol in outcome studies and provides support for the hypothesis that drugs which lower central pressure the most will be more effective[43-48].

Antihypertensive drugs and cardiovascular events

The interaction of antihypertensive drugs on BP and coronary hemodynamics (and hence CV events) is complex. For example, not all antihypertensive drugs have similar effects on pulse pressure. Blockers of the renin-angiotensin system, calcium antagonists and diuretics improve arterial compliance and thus lower SBP more than DBP and therefore diminish pulse pressure. In contrast β-blockers, because they decrease heart rate, increase stroke volume would have a less favorable effect on pulse pressure than the other drugs. Yet, decreased heart rate may allow for more prolonged diastolic perfusion of the coronary vascular bed and *vice versa*; whereas, short-acting calcium antagonists and other arteriolar vasodilators (such as hydralazine, minoxidil) are prone to cause myocardial ischemia in susceptible patients[49].

Antihypertensive drugs that reduce left ventricular hypertrophy (LVH) and hypertensive vascular disease are more effective over the long term in improving coronary flow reserve than drugs that have little or no effect. Thus, blockers of the renin-angiotensin system, calcium antagonists as well as the diuretics, have been shown to reduce LV hypertension[50] and hypertensive vascular disease[51-53] and improve arterial compliance[54] better than β-blockers.

EPIDEMIOLOGY OF PHTN

Many studies in various countries were performed to determine the magnitude of the PHTN rate. These have revealed that PHTN prevalence is considerable and it varies widely from country to country. For example, prevalence rate averages at 21.9% in China[55], at 32.8% in the Netherlands[56], at 34% in Taiwan[57], at 37% in the United States[58], at 40% in Ghana[59], at 48.2% in Oman[60] and at 52% in Iran[61]. Men[57-59,61] and blacks[62] are more likely to be affected than women or whites; respectively.

CARDIOVASCULAR RISK OF PHTN

PHTN is not only a caveat to develop overt HTN, but it is a major health risk on its own also. Prehypertensives were repeatedly reported to be subjected to approximately double the risk of CVD independent of progression to HTN[58,63] in addition to other cardiovascular complications[64-66].

PATHOPHYSIOLOGIC CHANGES ASSOCIATED WITH RAISED BP

This part of the review is intended to discuss briefly the significant pathophysiologic changes associated with the progressive increase in BP to provide the scientific premise for currently recommended interventions or another that is recommended by this review.

INVOLVEMENT OF THE RENIN-ANGIOTENSIN SYSTEM (RAS)

Effects of RAS on cardiovascular system in general

Angiotensin Ⅱ, an active peptide of the RAS, causes increase in BP and enhances oxidation of the low-density lipoprotein *via* stimulation of its type 1 receptor (AT1)[67,68]. It appears that it acts in this respect by inhibiting NAD(P)H oxidase-mediated oxygen synthesis and enhances antioxidant superoxide dismutase activity in the cardiovascular system and decreases nitric oxide (NO) bioavailability. The latter effect may be responsible, at least in part, for the beneficial effects of drugs inhibit RAS activity such as angiotensin converting enzyme inhibitors (ACEIs) or angiotensin Ⅱ type 1 receptor blockers (ARBs) that may act, eventually, by enhancing NO availability[69-71]. However, RAS blockade provides additional protective effect on cardiovascular function that cannot be solely explained by mere reduction of BP which is the action mediated by increasing NO availability[72].

In this context, it may be added that angiotensin-converting enzyme 2 (ACE2) converts angiotensin I to angiotensin (Ang)-1-9, that can be converted by ACE to a shorter peptide, Ang-1-7, which has an intrinsic vasodilator activity[73,74]. ACE2 have been described to be a potent negative regulator of RAS, counterbalancing the multiple functions of ACE, thus, it plays a protective role in the CV system and other organs[75].

Also, chronic activation of the RAS was shown to underlie HTN, insulin resistance, cardiac and renal disease, and polycystic ovarian syndrome and it serves as a link between obesity and low-grade systematic inflammation[76-80]. In addition, it is suggested that RAS contributes to the atherosclerotic process through angiotensin Ⅱ, which acts as a proinflammatory mediator directly inducing atherosclerotic plaque development and heart remodeling and exacerbate endothelial dysfunction[81,82]. On the other hand, blockade of RAS can offer protection from RAS-related metabolic diseases including diabetes[83-88].

The statement by Demirci *et al*[72] is further enforced by the observation that the ACE gene may be a determinant of serum ACE levels, but it does not appear to confer susceptibility to essential hypertension[89], since there are many factors that influence the genetic make-up of

blood pressure[90]. In addition, other environmental factors[91] may be involved in determining BP. Therefore, the possibility of drugs interfering in the RAS to be additionally interfering with any of these other factors cannot be eliminated.

Effect of RAS on development of hypertension

The first report on the potential of early intervention to prevent HTN was in 1990[92]. The authors showed that inhibiting RAS by captopril (an ACEI) for two weeks may intervene in the progression of HTN in young "prehypertensive" spontaneously hypertensive rats (SHRs). Later, other studies have shown that transient inhibition of the renin-angiotensin system from two weeks of age in SHRs, either with ACEIs or with ARBs, diminishes the increase in BP for up to 21 wk after cessation of treatment[93]. While others reported that permanent treatment of SHRs from conception onwards with ACEIs completely prevented hypertension[94,95].

The ARBs losartan was reported to have beneficial effect in humans[96] and rats[97,98] similar to that of captopril in SHRs, specifically, as shown by another study that transient use of losartan resulted in a long-lasting improvement of arterial contractility, an effect that was linked to endothelium-dependent vasodilatation[92,98].

Paradoxically, other authors showed that decreased BP is accompanied by severe disruption of the normal vascular architecture of intrarenal arteries[99]. These authors concluded that, apparently interference with RAS during a crucial stage of development in SHRs can initiate this disturbance and may cause intrarenal vascular smooth muscle hyperplasia, suggesting the involvement of another trophic factor that is inhibited by angiotensin II under physiologic conditions. Such led other workers[72] to suggest that the efficacy of antihypertensive treatment is also influenced by age and the hypertensive stage of the investigate animals.

INVOLVEMENT OF VASCULAR ENDOTHELIUM

The association between RAS and endothelium-dependent pathways in PHTN was suggested by more than one observation. It was shown that dysfunctional NO synthesis in PHTN may be a source of oxygen free radicals or reactive oxygen species (ROS) which may be an additive factor to develop overt HTN[100]. Jameson *et al*[101] reported that, endothelium-dependent relaxation of prehypertensive SHRs mesenteric arteries was impaired. Later, interleukin-1β (which induces inducible NO Synthase) was shown to cause a lower production of NO and a reduced generation of cGMP in these animals[102]. This observation was followed by demonstrating that lower NO level correlated with increased SBP in the same species[103]. Such was related to impaired NO production alone[104] or combined with an enhanced ROS activity which may contribute to progression of PHTN to HTN[105]. All these effects indicate that endothelial vasodilator capacity is impaired in PHTN[106].

INVOLVEMENT OF REACTIVE OXYGEN SPECIES (ROS)

It is stated above that ROS may add to developing overt HTN. Therefore, it is not surprising that antioxidant deficiency has been long implicated in HTN pathogenesis[107-109]; whereas, antioxidant treatment to reduce oxidative stress was shown to prevent development of HTN in SHRs[110]. Many studies have demonstrated that enhanced production of plasma free radicals may impair the physiologic function of vascular endothelium[111-113]. An action that may lead to increase in BP. Recently, the rationale for antioxidant trials in PHTN was reviewed by Nambiar *et al*[114].

INVOLVEMENT OF THE INFLAMMATORY PROCESS: PROSTGLANDINS AND C-REACTIVE PROTEIN

Inflammation was also implicated in the development of HTN and in endothelial dysfunction either as a primary or a secondary event[115]. Inflammation, indicated by C-reactive protein (C-RP) level, was used to predict HTN among PHTN subjects[116,117]. In addition, prostaglandin E2 (an inflammatory cytokine) was particularly shown to enhance norepinephrine-pressor response in PHTN; an effect that was abolished by indomethacin (a prostaglandin synthesis inhibitor)[118].

INVOLVEMENT OF THE AUTONOMIC NERVOUS SYSTEM

Eyal *et al*[119] suggested that α-adrenoceptors of SHRs are in a basic state of excitation even prior to the onset of overt HTN, *i.e.*, in PHTN. Prior to this observation, Fujimoto *et al*[120] demonstrated that β-adrenoceptor-mediated relaxation of arteries, in the same species, was diminished before and during development of HTN. The diminished relaxation may be because of defective hyperpolarization induced by these receptors[121]. In the same rat species, both the M3 cholinoceptors- and P2y-mediated relaxation was not altered[122] ruling out involvement of any other component of the autonomic nervous system apart from the sympathetic. However, β-blockers, compared to ACEIs, did not improve resistance arteries function after two years of use in human[123,124].

The underlying mechanism for the sympathetic involvement was also indicated by the presence of subsensitive presynaptic α2-adrenceptors which may lead to exaggerated norepinephrine secretion[125], an effect that may have a causal relevance to development of HTN[126]. Similarly, the β2-adrenceptor-mediated facilitation of neurogenic pressor response was found to be enhanced in prehypertensive SHRs, which may contribute to devel-

opment of HTN[127].

INVOLVEMENT OF CENTRAL MECHANISMS

An impaired baroreceptor control of vascular resistance was implicated in SHRs[128] and such was thought to be a primary defect[129]. In humans, baroreflex was not found to be altered, but plasma norepinephrine positively correlated to BP and associated with subsensitive α- and β-adrenoceptors[130].

Another explanation of the symapthetic overactive state in PHTN/HTN was postulated by Kotchen *et al*[131]. It is based on the observation that brain NO, as a neurotransmitter, reduces sympathetic output, and systemic angiotensin Ⅱ activates NO-producing neurons. SHRs show higher gene expression of nNOS, probably, as a compensatory mechanism for increased BP. That hypothesis was supported by the finding that hypothalamic angiotensin Ⅱ-sensitive neurons activity was greatly enhanced in PHTN[132], and that the central component of the baroreflex was also impaired[133].

INVOLVEMENT OF OTHER MECHANISMS

Other than RAS pathways should be investigated since not all the beneficial effects attributed to anti-RAS drugs (in HTN and PHTN) can be solely understandable by its mere reduction in BP. For example, RAS is activated by common comorbidity including type 2 diabetes, hyperinsulinemia and excess weight as well as it can be activated by a diet rich in carbohydrates and fats. Two clinical trials[134,135] have shown that ACEIs decrease the risk of developing type 2 diabetes in patients with HYN and/or vascular disease.

THERAPEUTIC OPTIONS OF PREHYPERTENSION

Rationale for therapeutic intervention in PHTN

PHTN and HTN are associated with a number of factors such as increased age, male gender, increased C-RP level and waist circumference[117,136]. These factors are positively correlated with the development of HTN[117]. Yet and despite the clear relationship between HTN and PHTN, treating HTN is unequivocally accepted, but the debate over the use of the term PHTN itself as a clinical category[137] or what type of intervention to be used in this case has not yet been concluded. The main reasons raising the thought of therapeutically intervening in prehypertensive subjects can be summarized as follows: (1) elevated systolic BP is the most important risk factor for cardiovascular, cerebrovascular, and renal disease[4,16,138]; (2) there is a strong association of cardiovascular mortality risk with BP[16]; (3) it is expected that many individuals with PHTN will, with time, become overt hypertensive patients[139]; and (4) for normotensive population, it was calculated that SBP increases at an average rate of about 0.5 mmHg/year[137].

Current suggested intervention strategies in PHTN

In principle, intervening in PHTN is a form of primary prevention, which can be enacted in more than one way. One is by the use of proven and safe drugs; another is by inducing individual behavioral changes. The latter is an attractive option because of its inherent "natural" appeal, perceived low cost, simplicity and safety though may not be sustainable.

Primary prevention strategies that are directed towards the individual necessitate screening all individuals in order to identify those who are over a certain "threshold". That process is followed by subjecting individuals at risk to an appropriately "tailored" intervention to each of them which incurs high cost. In addition, risk prediction in primary prevention remains imprecise and may not reflect long-term risk[140].

At the community level, primary prevention may be endorsed by passing health policies, encouraging beneficial cultural attitudes and/or imposing environmental changes. This approach is more likely to have a greater impact on individual's health[141-144].

At present, it is agreed in principle, that prehypertensive subjects should be treated. However, there is a polarizing controversy on the means of intervention. Two main strategies are recommended; one is based on "Therapeutic Lifestyle Changes (TLCs)"[4], and the second is based on using antihypertensive monodrug therapy[5].

TLCS

Previous[3] and current guidelines[5] advocate specific lifestyle modifications for prehypertensives. The most recent recommendations (JNC7 report)[4] are as follows: (1) maintain body mass index between 18.5 and 24.9 kg/m^2; this is expected to reduce SBP by 5 to 20 mmHg for each 10-kg reduction in weight; (2) consume a diet rich in fruits and vegetables, as well as low-fat dairy products; this is expected to reduce SBP by 8 to 14 mmHg; (3) restrict sodium to no more than 6 g of table salt per day; this is expected to reduce SBP by 2 to 8 mmHg; (4) walk briskly at least 30 min per day or engage in other regular aerobic physical activity; this is expected to reduce SBP by 4 to 9 mmHg; and (5) reduce alcohol consumption; this reduces SBP by 2 to 4 mmHg.

Evidence for therapeutic effectiveness of lifestyle modifications

The JNC 7 lifestyle changes are focused on weight loss, dietary restriction and exercise, which were supported by abundant clinical evidence. For example, weight loss[145], and salt restriction[146] have been shown to improve PHTN. Maintaining a body mass index between 18.5 and 24.9 kg/m^2 is expected to reduce SBP by 5 to 20 mmHg for each 10-kg reduction in weight[16].

Weight loss has been shown to be the most effective lifestyle modification strategy for prevention of hypertension[147]. Reductions in BP occur even without attain-

ment of normal body mass index. In a meta-analysis of 25 randomized, controlled trials, weight loss of 1 kg was associated with approximately 1 mmHg reduction in SBP and DBP in individuals with HTN[148]. Addition of antihypertensive medication has been shown to have an effect on BP reduction that is additive to that achieved by weight loss alone[148,149]. However, it has been shown that the type of medication prescribed may decrease the ability of the patient to lose weight[147].

Dietary pattern changes, in general[150] or specifically prescribed such as the Dietary Approaches to stop hypertension (DASH) plan[151,152] which uses a diet rich in fruits, vegetables, legumes, nuts, and low-fat dietary products and low in saturated fats, induced a significant lowering of BP. Adhering to the DASH diet can reduce BP by 8-14 mmHg, an effect that was augmented even further when dietary sodium was restricted. The OmniHeart Collaborative Research Group study[153] in which the DASH diet was modified to provide more protein and unsaturated fat and less carbohydrate, impressive reductions of BP were also achieved. The TOHP trial[147], in a substudy of the DASH trial also showed that by reducing sodium intake to less than 100 mmol in your daily diet, in addition to dietary changes provided greater benefit than either approach alone[154].

Similarly, there is ample evidence that exercise, independent of weight loss, decreases BP[154-157]. A number of clinical trials demonstrated that increased physical activity can lower BP independent of any effect on body weight, although this finding is not universal[158-160]. However, two meta-analyses concluded clearly that physical activity independently lowers BP[161,162]. In one of these meta-analyses, 27 of 50 studies reported results in nonhypertensive subgroups, which presumably include a large proportion of participants with PHTN[162]. Exercise alone has been associated with a 30% reduction in cardiac risk, making it similar to statin and antihypertensive interventions[163-166]. Hence, a number of studies have been performed to examine the effects of aerobic and/or resistance exercise on BP in hypertensive, prehypertensive, and normotensive groups, and a recent review has examined the relevant findings[167].

Nevertheless, some trials have shown that TLCs to have a modest and unsustainable impact to reduce CVD events when tested in large, long-term trials[152,168]. This observation has been challenged by the PREMIER trial[169] which studied the combined effects of diet, physical activity, and weight reduction in three groups of prehypertensive and hypertensive subjects over an 18-mo period. Although all three groups demonstrated significant reductions in BP in both prehypertensive and hypertensive subjects, the amount of decrease in the group given relatively minimal counseling was both surprising and gratifying in view of the previous difficulties with obtaining long-term behavioural changes to improve the cardiovascular risk status. These findings encourage adding counseling as an important early augmenting intervention to lifestyle modifications that may sustain beneficial therapeutic effect. This view is further supported with the findings of the largest population-based experience of lifestyle modification as a strategy to reduce cardiovascular risk factors, CVD, and mortality. The study used a comprehensive community-level approach that encompassed the health and other services like voluntary organizations, local media, businesses including the Food Industry and changes to public policy. It demonstrated a reduction in mortality from coronary artery disease by 55% in men and by 68% in women over a 20-year period[170]. Furthermore, in a recent randomized clinical trial[171] it was found that subjects with increased BP who participated in an automated online self-management program resulted in improved BP among prehypertensive or hypertensive subjects. These findings emphasize the need to involve patients for a more sustainable outcome. Similar results were obtained in overt hypertensive patients, who, in a prospective cohort study received repeated nonpharmacological recommendations to follow low-salt and low-calorie diets and to do physical activities[172]. This study concluded that adherence to follow low-salt and low-calorie diets is associated with clinically relevant long-term BP reduction and better hypertension control in clinical setting.

Although the evidence on reducing alcohol intake and reduction in BP is equivocal[173,174], a meta-analysis of trials in this respect with many of the analysed trials included prehypertensives[175] suggest that reducing alcohol intake can independently lower SBP.

THERAPEUTIC INTERVENTION WITH A MONODRUG THERAPY

All concluded studies that have been attempting to treat PHTN used one drug that affects the RAS in the form of ACEIs, ARBs or renin inhibitors. The use of other monotherapies such as β-blockers, was not shown to be, compared to ACEIs, effective in improving resistance arteries function after two years of use in human[123,124]. The involvement of RAS in PHTN and HTN was discussed earlier. This part of the review summarizes the outcome of clinical trials using drugs that affect RAS in this respect.

Clinical trials with drugs affecting RAS: ongoing clinical trials

Two clinical trials are ongoing: (1) the first trial is the PREVER-Prevention trial[176], a controlled randomized, double-blind trial designed to include individuals with PHTN given chlorthalidone 12.5 mg plus amiloride 2.5 mg or placebo. The study is to investigate if early use of drugs in individuals with PHTN may prevent cardiovascular events, target-organ damage and the incidence of overt HTN. In the 2nd International Conference on Prehypertension and Cardiometabolic Syndrome (January 31 - February 3, 2013; Barcelona, Spain) the trial co-principal investigator (Fuchs FD) announced that PREVER has finished enrollment of 1053 patients. According to the study design, patients who are still prehypertensive after three months of recommended lifestyle changes are randomized to a low-dose combination of chlorthalidone

plus amiloride or to placebo. Preliminary results from the study[177] indicate that 659 (77%) of subjects remained prehypertensive and were randomized according to the study protocol; another 7.5% had abnormal lab values, and 6.2% had progressed to developing HTN, while 9% had seen their BP drop to within normal values; and (2) The second trial is the Chinese High Normal Blood Pressure (CHINOM) trial. The study has finished enrollment of 10689 patients with BP in the range of 130-139/85-89 mmHg and at least one other cardiovascular disease risk factor (but no established diabetes, renal or hepatic dysfunction, or history of stroke or CVD). The trial randomized patients to one of three parallel treatment groups: telmisartan 40 mg, indapamide 1.5 mg, or, in the third group, placebo or a combination pill of hydrochlorothiazide 12.5 mg, triamterene 12.5 mg, dihydralazine 12.5 mg, and reserpine 0.1 mg. The primary end point of the study is combined CV events (nonfatal stroke, nonfatal MI, and CVD death), while secondary end point addresses new-onset hypertension and new-onset diabetes. In the above mentioned conference, it was announced that the CHINOM trial is still awaiting the first results which may be still several years away. However, baseline characteristics of study subjects are showing that 70% of subjects enrolled actually have more than one cardiovascular risk factor with metabolic syndrome being the most common. More than three-quarters of participants are overweight or obese, 42% have high triglycerides, and over one-third have a family history of hypertension[177].

Clinical trials with drugs affecting RAS: concluded clinical trials

The first clinical trial was the TRial Of Preventing HYpertension (TROPHY)[178,179] which examined whether early treatment of PHTN justified pharmacologic intervention with the use of an ARB (candesartan 16 mg daily) in HTN. TROPHY hypothesis to examine whether ARBs may be useful to treat PHTN was based on the following: (1) PHTN is a strong independent predictor of cardiovascular events; (2) growth factors mediated by stimulation of the sympathetic nervous system[180] and excess activity of RAS[181] tend to promote vascular hypertrophy by direct as well as hemodynamic effects. Antihypertension treatment with ACIs or ARBs, but not with β-blockers, has been reported to cause regression of arteriolar hypertrophy[123,124]; and and (3) despite intensive community efforts to promote healthy lifestyle, the prevalence of PHTN[182] in the United States is increasing.

Over a period of four years of TROPHY study, it was found that stage 1 HTN developed in nearly two thirds of patients with untreated PHTN (the placebo group). Treatment of PHTN with candesartan appeared to be well tolerated and reduced the risk of incident HTN during the study period. The authors concluded that, treatment of PHTN appears to be feasible.

Although the observations in this study indicate that candesartan may ameliorate BP in prehypertensives, a comment by the authors stated that they do not advocate treatment of the 25 million people (in the United States) with prehypertension. They added that additional studies will be needed to ascertain whether this or other strategies involving early pharmacologic treatment of PHTN would positively affect clinical outcomes.

Another trial is the PHARAO study[183] which demonstrated that ramipril (an ACEI) given to prehypertensives reduced the risk of HTN by 34.4% compared to those not taking antihypertensive drugs; however, no differences were found in cardiovascular or cerebrovascular events. The study concluded that prehypertensives are more likely to progress to overt HTN than those with optimal or normal BP when treated with ACEIs.

A third trial, on the other hand, concluded that pharmacological therapy is indicated for some patients with PHTN who have specific comorbidities, including diabetes mellitus, chronic kidney disease and coronary artery disease[184], while another trial[185] did not support the use of antihypertensive drugs in "normotensive" subjects and that, ARBs might offer less protection against myocardial infarction than ACEs.

Most recently, the AQUARIUS trial[186] examined the effect of aliskiren (a renin inhibitor) on progression of coronary atherosclerosis in a double-blind, randomized, multicenter trial study. It concluded that among participants with PHTN and coronary artery disease, the use of aliskiren compared with placebo did not result in improvement or slowing of progression of coronary atherosclerosis and that their findings do not support the use of aliskiren for regression or prevention of progression of coronary atherosclerosis.

INTERVENTION WITH MULTIDRUG FORMULATIONS: SHOULD THE "POLYPILL" BE CONSIDERED IN PHTN?

What is the "Polypill"?

The "Polypill" is a multidrug formulation with modified drug combinations containing drugs such aspirin, statins, β-blockers, ACEIs and ARBs; all of which are of proven value in reducing CVD morbidity and mortality. Approximately, half of the decline in cardiovascular mortality observed in developed countries during the last two decades is attributable to medical therapy using these types of drugs[187].

The introduction of the Polypill idea was not intended for use in PHTN, rather it was for reducing the burden of CVD in economically disadvantaged individuals to reduce cost and improve adherence. It was meant to be applied to entire or large segments of the population. The reasons behind innovating the Polypill (see below) were, in effect, the same as those for intervening with PHTN, the authors suggest considering to include prehypertensives in future Polypill clinical trials to ascertain the potential benefit of using the Polypill in these subjects.

Rationale behind the polypill composition

Some of the main relevant reasons for introducing the

Polypill are summarized as follows: (1) cardiovascular disease is the major cause of death and disability globally and affects approximately half of all individuals over their lifetimes[140]. CVD has increased in developing countries, and by the year 2020, 80% of the global CVD mortality is predicted to occur in low- and middle-income countries[188]; (2) world population is threatened by increasing obesity, sedentary lifestyles, and diabetes mellitus rates[187]. If these conditions are added to increased BP, primary intervention strategies directed towards community rather than individuals become of more therapeutic value; (3) nine to ten potentially modifiable risk factors account for 90% of the attributable risk for myocardial infarction and stroke, with similar estimates in all major regions of the world[189,190]; and (4) the prevalence of low risk factor burden is on the increase. In the US it was only 4.4% during 1971-1975, 10.5% during 1988-1994, and 7.5% during 1999-2004[191].

In addition to the above reasons, it has been shown that monodrug therapeutic intervention in PHTN has yielded mixed results, with some researchers have shown benefits[184] while others have not[185]. It seems that the existence of comorbidities determines how a prehypertensive subject is likely to respond to pharmacological intervention[192]. Hence, the authors propose to consider including prehypertensives in future clinical trial of the Polypill to investigate how much benefit they may gain by multidrug therapeutic intervention.

Clinical evidence for the polypill effectiveness

Two randomized, placebo-controlled trials investigated the therapeutic effect of the polypill. The first was conducted in 2011[193]. It was an international randomised placebo-controlled trial of a four-component combination pill ("polypill") in people with raised cardiovascular risk (over 7.5%, determined by the Framingham risk) using data on age, gender, BP, total cholesterol, HDL cholesterol, diabetes status, and cigarette smoking status. It contained aspirin 75 mg, lisinopril 10 mg, hydrochlorothiazide 12.5 mg, and simvastatin 20 mg or to placebo. The drug combination was associated with a 9.9-mmHg drop in SBP and a 0.8-mmol/L reduction in LDL cholesterol over a 12-wk treatment period.

The second clinical trial[194] studied a polypill contained amlodipine 2.5 mg, losartan 25 mg, hydrochlorothiazide 12.5 mg and simvastatin 40 mg; but contained no aspirin. The pill was given for 12 wk. The treatment showed reductions mean systolic (17.9 mmHg) and DBP (9.8 mmHg) and LDL blood level was reduced by 1.4 mmol/L.

CONCLUSION

PHTN is a major health challenge that requires extra-attention. The "challenge" resides in finding the answer to "how/what" should be the intervention strategy (or strategies) that may best reduce its health impact.

In the search for a "strategy" to intervene in prehypertension, a number of considerations may be noteworthy and can be summarized as follows: (1) the rationale behind therapeutic intervention in hypertensive, and, indeed, prehypertensive subjects is to prevent (or delay progression of) cardiovascular events and mortality caused by these conditions. Yet, it is equally accepted that presence of other comorbidities such as diabetes mellitus, obesity, dyslipidemia, *etc.* in addition to ethnic, age and gender differences should also be accounted for when an intervention strategy considered; (2) the term PHTN is based on "defining" HTN itself, which is established on a 20-mmHg per brackets. Yet, BP is confounded by many factors such as circadian rhythms, food intake, stress, exercise, emotional state etc. leading to "variable BP variability"[137,195]; (3) based on the above two points, it is plausible to suggest that, categorizing and staging of subjects on basis of BP alone may need to be re-considered. It may be more clinically useful to contain factors, together, that cause BP variability to "stage" BP levels as well as to calculate cardiovascular risk factors. Such, may produce new terminologies or new definitions of PHTN and HTN. Consequently, an intervention strategy may not be "one-size-fits-all", and may necessitate more than one intervention. Different strategies (or combination thereof) may be considered. For example, males may require a different strategy from females since differences between genders have been reported in overt HTN[196,197]. Similarly, children PB progression differs from that of adults[198] and, thus may need a different intervention strategy. Furthermore, different ethnicities have shown different patterns in both progression of their BP as well as response to therapy and, hence, they may need different intervention strategies[199-202]; (4) the first-line treatment for prehypertensives should be based on adoption of a healthy lifestyle, especially if there are other associated risk factors such as obesity, dyslipidemia, pre-diabetes or diabetes, excessive alcohol intake, sedentary lifestyle and smoking[203] as well as salt-intake restriction[152]. It is desirable if TLCs would be adopted by government and NGOs and may be "enforced" as a "policy" that is directed (in a way similar to the antismoking campaign) towards changing community behavior; and (5) if pharmacologic means will be used, such should not be confined to drugs affecting RAS, other drugs may be investigated to ascertain whether it is the mere reduction in BP that is benefitting prehypertensives or other effects.

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TOPIC HIGHLIGHT

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Peroxisome proliferator-activated receptors for hypertension

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Abstract

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily, which is composed of four members encoded by distinct genes (α, β, γ, and δ). The genes undergo transactivation or transrepression under specific mechanisms that lead to the induction or repression of target gene expression. As is the case with other nuclear receptors, all four PPAR isoforms contain five or six structural regions in four functional domains; namely, A/B, C, D, and E/F. PPARs have many functions, particularly functions involving control of vascular tone, inflammation, and energy homeostasis, and are, therefore, important targets for hypertension, obesity, obesity-induced inflammation, and metabolic syndrome in general. Hence, PPARs also represent drug targets, and PPARα and PPARγ agonists are used clinically in the treatment of dyslipidemia and type 2 diabetes mellitus, respectively. Because of their pleiotropic effects, they have been identified as active in a number of diseases and are targets for the development of a broad range of therapies for a variety of diseases. It is likely that the range of PPARγ agonist therapeutic actions will result in novel approaches to lifestyle and other diseases. The combination of PPARs with reagents or with other cardiovascular drugs, such as diuretics and angiotensin Ⅱ receptor blockers, should be studied. This article provides a review of PPAR isoform characteristics, a discussion of progress in our understanding of the biological actions of PPARs, and a summary of PPAR agonist development for patient management. We also include a summary of the experimental and clinical evidence obtained from animal studies and clinical trials conducted to evaluate the usefulness and effectiveness of PPAR agonists in the treatment of lifestyle-related diseases.

Key words: Peroxisome proliferator-activated receptors; Nuclear receptor; Isoform; mRNA; Blood pressure; Hypertension; Obesity; Angiotensin Ⅱ receptor blocker; Diabetes mellitus

Core tip: Lifestyle-related diseases are major public health problem worldwide, and the prevalence of these diseases and subsequent complications has increased rapidly over the past 20 years. It has been a decade or more since the first report of the pleiotropic effects of peroxisome proliferator-activated receptors (PPARs), and numerous studies on their novel effects continue to appear every month. In addition to their effects on blood pressure, atherosclerosis, and kidney dysfunction, anti-cancer effects of PPARγ ligands have been reported recently. The effectiveness of PPAR agonists in the treatment of lifestyle-related diseases will be increasingly appreciated. This review summarizes the current literature on PPARs.

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INTRODUCTION

PPARs are ligand-activated transcription factors of the

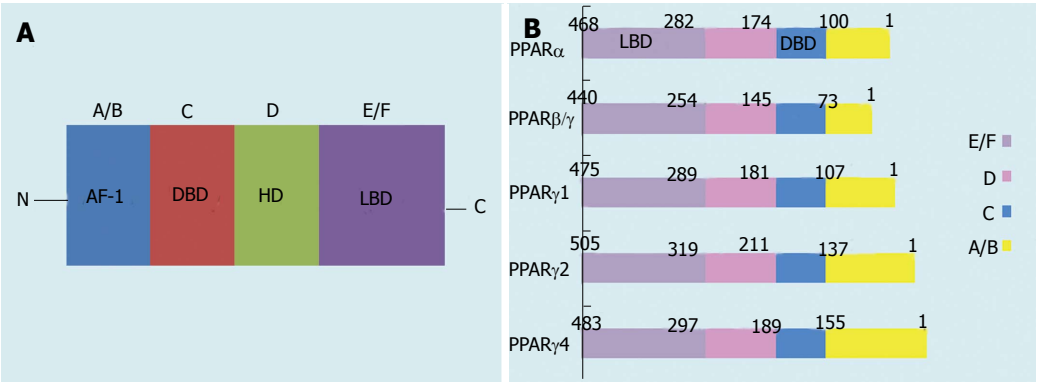

Figure 1 Schematic structure of peroxisome proliferator-activated receptor protein isoforms. A/B, C, D, and E/F indicate the N-terminal A/B domain containing a ligand-independent AF-1, the DBD, the hinge region, and the C-terminal LBD containing AF-2, respectively. AF-1 is responsible for phosphorylation, while AF-2 promotes the recruitment of co-activators for gene transcription. PPAR: Peroxisome proliferator-activated receptor; AF-1: Activation function-1; DBD: DNA-binding domain; HD: Hinge domain; LBD: Ligand-binding domain. Figure adapted from reference[8].

nuclear receptor superfamily, and they comprise four members encoded by distinct genes (α, β, γ and δ). The PPARs undergo transactivation or transrepression under distinct mechanisms that lead to the induction or repression of target gene expression[1]. PPARs bind to sequence-specific target elements in the promoter region of target genes following heterodimerization with the retinoid receptor, and in doing so, they control the majority of steps in cellular fatty acid uptake, utilization, oxidation, and storage pathways; cell growth and migration; oxidative stress; and inflammation in the cardiovascular system[1,2]. Each PPAR is primarily located in a distinct set of tissues, is stimulated by different ligands, and has different effects[2]. Certain new effects of PPARs on hypertension have been identified in recent studies, and the present mini-review focuses on the literature related to the effects that PPARs and their agonists exert in this area. Each member of the PPAR family possesses distinct functions that are determined by their ligand affinity, expression, and activity, which are dependent on the metabolic pathway and the type of tissue.

PPAR STRUCTURE

PPARs are orphan nuclear receptors belonging to the steroid, retinoid, and thyroid hormone receptor superfamily of ligand-activated transcription factors[3,4]. Three distinct receptor types have been cloned and characterized: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3)[5]. Like other nuclear receptors, these PPAR isoforms have five or six structural regions within four functional domains, termed A/B, C, D, and E/F (Figure 1A)[5]. The variable NH_2-terminal end, which is a ligand-independent transactivation domain (the A/B domain), contains activation function (AF)-1, which is a target of kinase phosphorylation[5]. The 70-amino-acid-long PPAR DNA-binding domain (the C domain) contains two highly conserved zinc finger motifs and promotes the binding of receptors to a DNA sequence in the promoter region of target genes, which is known as the peroxisome proliferator response element (PPRE)[5]. The hinge region (the D domain) acts as a docking site for cofactors. The C-terminal or ligand-binding domain (the E/F domain) is responsible for ligand specificity and the activation of PPAR binding to the PPRE, which increases target gene expression. The E/F domain uses cofactors for the transactivation *via* the ligand-dependent trans-AF-2[5]. When activated by endogenous or synthetic ligands, the PPARs, like other nuclear hormone receptors, heterodimerize with the 9-*cis*-retinoic acid receptor (retinoid × receptor)[5]. The PPAR-retinoid × receptor heterodimer undergoes conformational changes, binds to the PPRE in the promoter region of the target gene, and alters coactivator/corepressor dynamics to modulate the transcription machinery, which in turn affects the initiation of transcription (*via* upregulation or downregulation) and the abundance of messenger RNA (mRNA) in the target genes[6,7]. PPARs are also drug targets; currently, PPARα agonists (fibrates) are in clinical use for treating dyslipidemia, and PPARγ agonists (thiazolidinediones (TZDs)) are being used to treat type 2 diabetes mellitus (T2DM)[8].

PPAR EXPRESSION

The PPAR family possesses distinct functions that are determined by their ligand affinity, expression, and activity, which are dependent on the metabolic pathway and the type of tissue[1]. The characteristics of each PPAR isotype are described below.

PPARα was the first PPAR isotype to be cloned, and its name comes from its activation by peroxisome proliferator chemicals[9,10]. Its expression is greatest in tissues with a high fatty acid oxidation rates, such as heart, liver and skeletal muscle, and functions as a major regulator of fatty acid homeostasis[10-13]. PPARα expression is also significant in the adipose, adrenal and kidney tissue (particularly brown adipose tissue), and the majority of cell types, including endothelial, smooth muscle, and macrophages, in the vasculature[12-14].

PPARβ/δ (PPARδ) is expressed at relatively high levels in liver, kidney, cardiac and skeletal muscle, adipose tissue, brain, colon, and vasculature[14-17]. Unlike PPARα

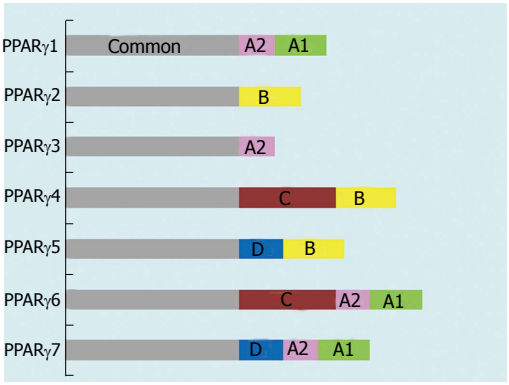

Figure 2 Domain structure of the peroxisome proliferator-activated receptor γ isoforms. PPAR: Peroxisome proliferator-activated receptor. Figure adapted from reference[8].

and PPARγ, PPARδ does not seem to be the target of available drugs[8]. The unavailability of PPARδ-targeted drugs may be due to its wide ranging expression. The physiological function of PPARδ is much less studied and understood[8].

PPARγ is highly expressed in adipose tissue and plays an indispensable role in the regulation of adipocyte differentiation, lipid storage, and glucose metabolism and in the transcriptional regulation of a number of genes involved in these metabolic processes[13,18-20]. Some key target genes of PPARγ include the fat-specific *ap2* gene, LPL, fatty acid transport, fatty acid-binding protein, FAT/CD36, acyl-CoA synthase, GLUT4, glucokinase, phosphoenolpyruvate carboxykinase, uncoupling proteins 1, 2, and 3 and LXRα[18,19,21]. PPARγ also regulates genes involved in insulin signaling and the expression of proinflammatory cytokines such as tumor necrosis factor (TNF)-α[20,21]. It also has significant anti-inflammatory effects[18,19,21] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3246744/ - R49. Most importantly, PPARγ is a well-recognized cellular target for the antidiabetic thiazolidinediones, which sensitize cells to insulin and improve insulin sensitivity and action[22-24]. To date, seven mRNA transcripts generated through different forms of initiation and the alternative splicing of five exons at the 5'-terminal region (A1, A2, B, C and D) have been identified (Figure 2)[24-26]. Each mRNA transcript is different, based on the combination of five exons. They have been designated PPARγ1, -γ2, -γ3, -γ4, -γ5, -γ6, and -γ7. PPARγ1, -γ3, -γ5, and -γ7 mRNA transcripts translate to the identical PPARγ1 protein. PPARγ2 mRNA yields PPARγ2 protein, while PPARγ4 and -γ6 mRNA transcripts produce identical PPARγ4 protein (Figure 1B)[25-27]. The PPARγ1 mRNA isoform is expressed in a range of tissues: cardiac and skeletal muscle; pancreatic β-cells; the spleen, intestines, kidneys, and adrenal gland; vascular cells such as endothelial cells (ECs) and smooth muscle cells; and monocytes/macrophages[26,28,29]. The expression of PPARγ2 mRNA is primarily restricted to adipose tissue, whereas PPARγ3 mRNA is abundant in macrophages, the large intestine (colon), and adipocytes[24,26,30]. High levels of PPARγ4, -γ5, -γ6, and -γ7 mRNA transcripts are expressed in macrophages, while PPARγ6 and -γ7 mRNAs are also detected in adipose tissue[24,25,27,30].

PPAR ACTIVATION AND REGULATORY ROLES

PPARs are found primarily within the nucleus without their ligand and localize to target gene promoters with either co-activator or co-repressor complexes[31]. To date, many ligands had been identified that activate and modulate PPAR functions[31]. Endogenous lipid metabolites from saturated or unsaturated fatty acids, for example, are able to bind to nuclear receptors and activate or repress gene expression[31]. Another group of PPAR ligands consists of lipid metabolites from essential fatty acids, such as arachidonic acid derived from lipoxygenase or cyclooxygenase activity[31]. In particular, the best-characterized endogenous ligands known to stimulate PPARα are the eicosanoids LTB4 and 8-hydroxyeicosatetraenoic acid (8(S)-HETE), while 15d-PGJ2 and 13-HODE activate PPARγ[31]. Other essential fatty acid metabolites, such as 15-HETE, have been suggested to activate PPARβ/δ[31].

The discovery of PPARs as a key regulator of metabolic pathways has provided significant insight into the mechanisms involved in this process[28,32]. PPARs act as nutritional sensors that regulate a variety of homeostatic functions, including metabolism, inflammation, and development[28,32,33]. PPARs are involved in many functions, particularly those having to do with the regulation of vascular tone, inflammation, and energy homeostasis. Therefore, they represent important targets for addressing hypertension, obesity, obesity-induced inflammation, and metabolic syndrome in general[1,32-34]. PPARs may influence the inflammatory response either directly through the transcriptional downregulation of proinflammatory genes *via* mechanisms involving transrepression or indirectly *via* their transcriptional effects on lipid metabolism[1,8]. Because of their pleiotropic effects, they are now known to be active in a number of disease conditions, and they represent potent therapeutic targets for a wide range of diseases[1,8,32,34]. PPAR agonists may be of benefit, either alone or in combination with other drugs that influence the inflammatory response, in treating hypertension, atherosclerosis, and metabolic derangements associated with obesity[34].

The endogenous ligands that bind to PPARα with the highest affinity are saturated/unsaturated fatty acids, leukotriene derivatives, and VLDL hydrolysis products[33]. Examples of synthetic ligands that bind PPARα are the fibrate class of hypolipidemic drugs, the experimental ligand Wy-14643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid) and some phthalate monoesters (monoethylhexyl phthalate), and herbicides (lactofen)[33]. PPARα is a major regulator of the mitochondrial and peroxisomal β-oxidation pathway, and as will be discussed below, it is suggested that these pathways are involved in the pathogenesis of various liver complications[33]. PPARα activation inhibits vascular smooth muscle pro-

inflammatory responses, attenuating the development of atherosclerosis[15,35]. PPARα ligands negatively regulate interleukin (IL)-6 promoter activation, and chronic treatment with fenofibrate, a PPARα agonist, suppresses IL-6-induced atherosclerosis[36]. The absence of PPARα expression is suggested to prolonged the inflammatory response, and PPARα has anti-inflammatory properties[36]. Furthermore, the PPARα ligand, fenofibrate, may repress ICAM-1 and VCAM-1 expression in endothelial cells[36]. In addition, PPARα activation has been reported to inhibit NF-κB activation and inflammatory gene expression[35].

Activation of the nuclear hormone receptor PPARβ/δ is known to both improve insulin resistance and plasma high-density lipoprotein levels and to exhibit anti-inflammatory properties in the vessel wall through the inhibition of vascular cell adhesion molecule 1 and monocyte chemoattractant protein 1 expression[37].

Although PPARγ was first to be recognized as an anti-inflammatory agent, both PPARα and PPARδ are also known to have similar effects[34]. Inflammation is a significant aspect of the damage that hypertensive disease causes[34]. PPARs are now seen as important determinants of macrophage polarization[34]. Monocyte precursors of classically and alternatively activated macrophages are being identified as important participants in the progress of metabolic syndrome-related cardiovascular disease, including hypertension, hyperlipidemia, and obesity[8,38,39]. The activation of PPARβ/δ has been shown to increase lipid catabolism in the skeletal muscle, heart, and adipose tissue and to improve the serum lipid profile and insulin sensitivity[39,40]. Further, PPARβ/δ ligands stop weight gain and reduce macrophage-derived inflammation[40]. One new approach that may prevent or regress hypertension-induced vascular, renal, and, perhaps, brain changes is the activation of nuclear receptors, which not only have metabolic effects but also exert anti-inflammatory actions through PPARα and PPARγ[41]. PPARα and PPARγ are therapeutic targets for hypertriglyceridemia and insulin resistance, respectively[42,43].

Covalent modifications include phosphorylation, ubiquitylation, O-GlcNAcylation, and SUMOylation[44]. Covalent modifications of PPARγ are key regulatory mechanisms that control both PPARγ protein stability and transcriptional activity[39,44]. PPARγ functions as a master switch in controlling adipocyte differentiation and development, and its activation has an important role in glucose metabolism by enhancing insulin sensitization[39,45]. PPARγ is a primary target for TZD-structured insulin sensitizers such as pioglitazone and rosiglitazone, which are used in the treatment of T2DM[39,45]. Additionally, PPARγ activation inhibits adhesion cascades and detrimental vascular inflammatory events[39,45]. Furthermore, although the primary action of select ARBs, which partially activate PPARγ, is to lower blood pressure, they may also be effective in treating insulin resistance and dyslipidemia absent the toxicity associated with full PPARγ agonists[39,46].

PPARγ activation is known to have an influence on the events connected with the development and progression of atherosclerotic lesions[14,15,24,34,39,47]. PPARγ and its ligands may exert direct antiatherosclerotic action[14,15,24,48-50] Consistent with the anti-inflammatory properties of PPARγ and the TZDs, aortas showed decreased accumulation of macrophages in the lesions as well as attenuated expression of proatherogenic agents. Interestingly, these changes occurred independently of improvements in dyslipidemia, glycemic control, and hypertension, which supports the assumption of a direct vascular effect[8,50,51]. Moreover, PPARγ activation plays a distinct role in regulating the physiology and expression of endothelial nitric oxide synthase (eNOS) in the endothelium, resulting in enhanced generation of vascular nitric oxide[45]. PPARγ activation-mediated vascular anti-inflammatory and direct endothelial functional regulatory actions could therefore be beneficial in improving vascular function in patients with atherosclerosis and hypertension with or without DM[45]. Unfortunately, PPARγ agonists can exert long-term effects on certain patients, including increased body weight, fluid retention, and risk of heart failure[39]. This is unfortunate, as TZDs show consistent efficacy in the treatment of T2DM[39]. More recently, there has been increased concern about the association between TZD and bone loss[39]. The association with bone loss is an especially worrisome concern because fracture is usually when it is detected[39]. The biguanide metformin is currently the first-line medication in the treatment of T2DM due to increasing concerns about the safety of TZDs[39]. While the cardiac side effect profile of rosiglitazone-like PPARγ full agonists is unfortunate, the therapeutic potential of novel pharmacological agents targeting PPARγ submaximal cannot be excluded. Interestingly, newly synthesized partial agonists of PPARγ, such as balaglitazone, MBX-102, MK-0533, PAR-1622, PAM-1616, KR-62776, and SPPARγM5, have a reduced tendency to cause the adverse effects associated with full agonists of PPARγ or may be entirely devoid of such effects[45]. Therefore, with as much as 50% of patients with ischemic stroke and transient ischemic attack also having insulin resistance, drugs capable of addressing both hypertension and insulin resistance could be of great benefit in preventing stroke[46]. In summary, PPARγ is implicated both in the maintenance of vascular homeostasis and in the pathogenesis of a number of vascular conditions such as atherosclerosis, hypertension, and restenosis[28,29,39,52]. TZDs, which are PPARγ agonists, lower blood pressure and exert protective vascular effects through largely unknown mechanisms[39,52]. In contrast, loss-of-function dominant-negative mutations in human PPARγ cause insulin resistance and severe early onset hypertension[52].

EFFECTS OF PPARS ON BLOOD PRESSURE

PPARα ligands have been reported to decrease blood pressure in various models of hypertension[36]. Several

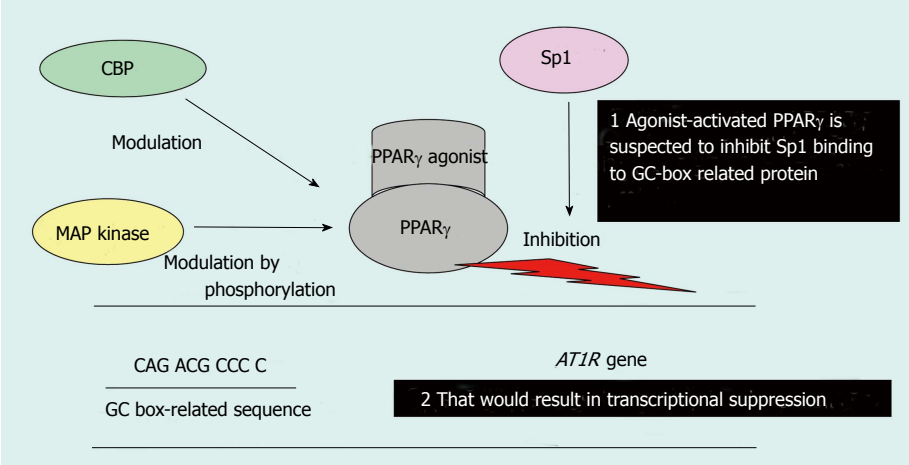

Figure 3 Possible mechanism of peroxisome proliferator-activated receptor γ-agonist-mediated transcriptional suppression of the Ang-Ⅱ type 1 receptor gene promoter. PPAR: Peroxisome proliferator-activated receptor; AT1R: Ang-Ⅱ type 1 receptor; CBP: CERB-binding protein; MAP: Mitogen-activated protein. Figure adapted from reference[71].

mechanisms have been proposed for the antihypertensive effects of PPARα agonists such as the increased excretion of Na^+ through reduced Na^+-K^+ ATPase activity in the proximal tubules, increased cytochrome P450 (CYP) 4A expression, and increased renal tubular 20-HETE production, which exerts a natriuretic effect[36,53-57]. A recent report has described a crosstalk between PPAR*a* and IL-6 in the regulation of blood pressure[36,58]. Furthermore, another report demonstrates that PPARα activation attenuates angiotensin-Ⅱ (Ang-Ⅱ)-induced hypertension through the upregulation of CYP4A and CYP2J and the attenuation of plasma IL-6, renal MCP-1 and other inflammatory markers, and the renal expression of ICAM-1 and COX-2[36].

A PPARβ/δ agonist has been reported to induce progressive systolic arterial blood pressure and heart rate reduction, and to reduce mesenteric arterial remodeling, endothelial dysfunction, and aortic vasoconstriction in response to Ang-Ⅱ[37]. These were accompanied by a significant increase in eNOS activity attributed to upregulated eNOS and downregulated caveolin 1 protein expression[37]. Moreover, the PPARβ/δ agonist also inhibited vascular superoxide production, downregulated $p22^{phox}$ and $p47^{phox}$ protein expression, decreased both basal and Ang-Ⅱ-stimulated NADPH oxidase activity, inhibited extracellular-regulated kinase 1/2 activation, and reduced the expression of proinflammatory and proatherogenic genes, including IL-1β, IL-6, and intercellular adhesion molecule 1[37]. Further, the same study showed that PPARβ/δ activation, both *in vitro* and *in vivo*, increased the expression of RGS4 and RGS5, which are regulators of G protein-coupled signaling proteins; RGS4 and RGS4, in turn, negatively modulated the vascular actions of Ang-Ⅱ[37]. PPARβ/δ activation also exerted antihypertensive effects, restored vascular structure and function, and reduced the oxidative, proinflammatory, and proatherogenic statuses[37]. Hence, PPARβ/δ was proposed as a new therapeutic target in hypertension[37].

It has been reported recently that independent of its blood glucose-lowering effects, PPARγ demonstrates pleiotropic beneficial effects on vasculature[59]. The effect may possibly be due to PPARγ-mediated inhibition of Ang-Ⅱ type 1 receptor (AT1R) expression as well as Ang-Ⅱ-mediated signaling pathways, which may result in suppression of the renin-angiotensin system (RAS) and lead to a lower blood pressure[59].

However, it has also been speculated that PPARγ-induced *AT1R* gene transcription suppression is mediated through the inhibition of Sp1 binding to DNA. This inhibition is due to the protein-protein interaction between ligand-activated PPARγ and Sp1; indeed, the PPARγ ligand-mediated suppression of AT1R expression has been demonstrated previously (Figure 3)[60-62]. Interestingly, transcription suppression was abrogated by the over-expression of the coactivator CERB-binding protein (CBP) and PPARγ phosphorylation by mitogen-activated protein (MAP) kinase, most likely because of the functional modification of PPARγ (Figure 3)[60]. Moreover, PPARγ ligands have been shown to suppress Ang-Ⅱ-induced phosphatidylinositol 3-kinase and MAP kinase and to ameliorate Ang Ⅱ-mediated inflammatory responses by interfering with the Toll-like receptor 4-dependent signaling pathway[62,63]. Therefore, PPARγ not only downregulates AT1R expression but also inhibits Ang Ⅱ-mediated signaling pathways, which may result in RAS suppression (Figure 4)[62-64]. On the other hand, transgenic mice expressing a dominant negative PPARγ P465L mutation are hypertensive, which is consistent with the phenotype of patients who have an equivalent PPARγ P467L mutation without affecting components of the RAS[59]. Thus, ligand-activated PPARγ may lower blood pressure through several different mechanisms in addition to inhibiting the RAS[59].

In terms of blood pressure, the transient administration of ARBs may prevent the development of hypertension, and high doses of ARBs may regress mild hypertension[65]. Next-generation ARBs are becoming available that are intended not only to antagonize AT1R but also to block endothelin receptors, function as nitric oxide donors, inhibit neprilysin activity, increase natriuretic peptide levels, or stimulate PPARγ[66]. It has been shown that ARBs have benefits beyond their established cardioprotective and vasculoprotective effects, including lowering risk of new-onset diabetes and its associated cardiovascular effects[67]. Furthermore, it has also been found that the drug telmisartan can selectively activate PPARγ in

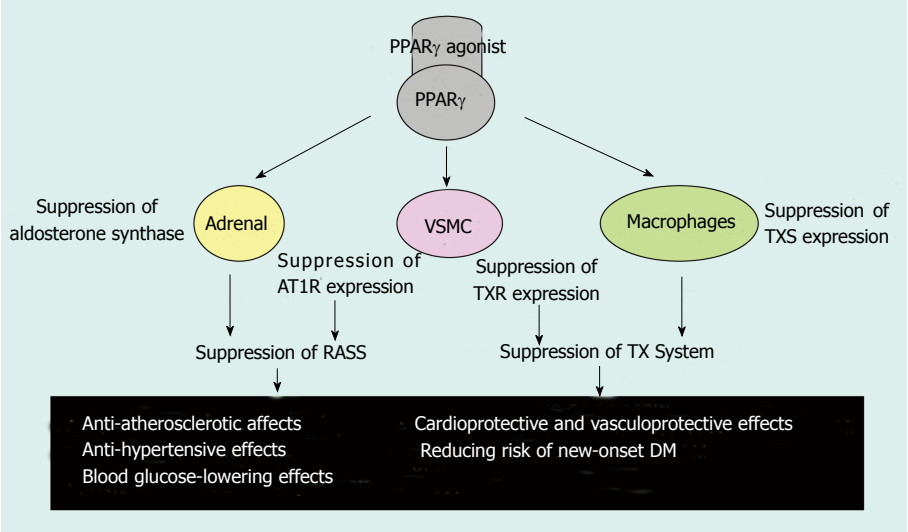

Figure 4 Possible effects of Peroxisome proliferator-activated receptor γ agonists. PPAR: Peroxisome proliferator-activated receptor; AT1R: Ang-Ⅱ type 1 receptor; RAAS: Renin-angiotensin-aldosterone system; TX: Thromboxane; TXS: TX synthase; TXR: TX receptor; VSMC: Vascular smooth muscle cells; DM: Diabetes mellitus. Table adapted from reference[71].

targeting DM, and it therefore provides an approach to the prevention and treatment of cardiovascular complications in high-risk elderly patients suffering from hypertension and new-onset DM[67]. The beneficial metabolic effects of telmisartan have been attributed to its action as an Ang-Ⅱ receptor blocker and as a partial PPARγ agonist, and it has also been found that telmisartan may have the strongest binding affinity to AT1R[43,68]. Treatment with telmisartan has been shown to significantly improve endothelial dysfunction and inhibit lipid accumulation in the liver[43]. It is possible that the favorable characteristics of telmisartan are due to its action as a partial PPARγ agonist, apart from its blood pressure-lowering effect as an Ang-Ⅱ blocker, possibly earning it the name "metabosartan"[43]. These observations suggest that because of its unique PPARγ-modulating activity, telmisartan may be one of the most promising sartans for the treatment of cardiometabolic disorders[68].

EVIDENCE FROM ANIMAL AND HUMAN STUDIES

PPARγ activation is suggested to be beneficial in inflammatory diseases, not only in humans but also in rats and pigs[69]. The question is now whether PPARγ activation mitigates immunological stress such as mastitis in livestock. In livestock species in general, however, data on the use of synthetic PPAR agonists are limited[69]. Considering the high amino acid identities ranging from 95% to 98% for the PPAR proteins in all species, one may believe that bovine and porcine PPARs could also be targeted using the existing synthetic PPAR agonists[69]. However, because only a minor overlap between the Wy-regulated genes from mouse and human primary hepatocytes was found and because PPREs are not fundamentally conserved among species, activation of the PPARs does not necessarily activate the same array of genes in one species as in another[69]. Data from the literature makes it clear that further studies on the impact of PPAR ligands in livestock are necessary as such investigations may identify unconsidered health and sanitation benefits.

It has been shown that WY14643, a potent PPARα agonist, has cardioprotective and cardiodepressive effects when used to treat encephalomyocarditis virus-induced myocarditis in diabetic mice[38]. The cardioprotective effect may be due to its anti-inflammatory properties and its ability to increase cardiac adiponectin expression, whereas the reduced cardiac efficiency may be due to its enhancement of cardiac UCP3 mRNA expression[38]. In animals, the pharmacological or genetic elevation of plasma adiponectin relieves obesity-induced endothelial dysfunction and hypertension, and prevents atherosclerosis, myocardial infarction, and diabetic cardiomyopathy[70]. These therapeutic benefits of PPARγ agonists (TZDs) are mediated by the induction of adiponectin[70]. Adiponectin protects cardiovascular health through its vasodilator, anti-apoptotic, anti-inflammatory, and anti-oxidative activities in both cardiac and vascular cells[70].

PPARγ agonists are known to lower blood pressure in humans, possibly through the suppression of the RAS, by mechanisms including the inhibition of AT1R expression, Ang-Ⅱ-mediated signaling pathways, and Ang-Ⅱ-induced adrenal aldosterone synthesis/secretion[52,71]. PPARγ agonists also inhibit the progression of atherosclerosis in humans, possibly through a pathway involving suppression of the RAS and the thromboxane system, as well as the protection of endothelial function[71]. Moreover, PPARγ-agonist-mediated renal protection, particularly the reduction of albuminuria, has been reported in diabetic nephropathy, including animal models of the disease, and in nondiabetic renal dysfunction[71]. The renal protective activities may reflect, at least in part, the ability of PPARγ agonists to lower blood pressure, protect endothelial function, and cause vasodilation of the glomerular efferent arterioles[71]. In addition, it has recently been reported that PPARγ agonists have antineoplastic effects and that they can ameliorate polycystic kidney, polycystic liver, and cardiac defects through the β-catenin, c-Myc, CFTR, MCP-1, S6, ERK, and TGF-β signaling pathways in animal models of chronic kidney disease (CKD)[71]. The multiple therapeutic actions of PPARγ agonists

leave no doubt that they will produce new approaches to lifestyle-related and other diseases[71].

However, negative (harmful) aspects of PPARs have also been reported. TZDs are insulin-sensitizing anti-diabetes agents that act through PPARγ to cause a durable improvement in glycemic control in patients with T2DM[72,73]. These benefits must be weighed against the side effects of the drug, which include weight gain, fluid retention, atypical fractures, and possibly, bladder cancer[72,73]. Despite having similar effects on glycemic control, pioglitazone and rosiglitazone appear to have different effects on cardiovascular outcomes[72,73]. Rosiglitazone has been associated with an increased risk of myocardial infarction, and its use in the United States is restricted because of cardiovascular safety concerns[72,73]. PPAR-α/γ or -γ/δ dual agonists are now under development[74,75].

As the literature has been indicating, disorders of pregnancy, such as preeclampsia and gestational diabetes, are potential targets for treatment with PPAR ligands[76]. In clinical cases, including preeclampsia, gestational diabetes, and intrauterine growth restriction, aberrant regulation of components of the PPAR system parallels the dysregulation of metabolism, inflammation, and angiogenesis[76]. These actions are the result of the roles of the PPARs in regulating human trophoblast invasion and early placental development[76]. PPARs are involved in trophoblast invasion, placental development, parturition, and pregnancy-specific diseases, particularly preeclampsia and gestational diabetes[76]. The PPAR system's involvement in pregnancy under physiologic and pathologic conditions has yet to be fully clarified due to a lack of knowledge about endogenous PPAR ligands[76]. Partially characterized inflammatory, angiogenic, and metabolic disturbances in pregnancy-related diseases suggest that these synthetic PPAR agonists may be of potential use in these conditions[76].

To date, several large clinical trials of hypertension using PPAR agonists have been conducted worldwide, including in Japan. The Losartan Intervention for Endpoint reduction in hypertension (LIFE) study compares the effects of losartan- (a PPARγ agonist) and atenolol- (a β blocker) based antihypertensive treatment on cardiovascular morbidity and mortality in a population of 9193 hypertensive patients with left ventricular hypertrophy (LVH)[77]. In the LIFE study, losartan-based treatment further reduced the primary composite end point (cardiovascular death, myocardial infarction, or stroke) by 13% [relative risk reduction (RRR) 0.87, 95%CI: 0.77-0.98, P = 0.021]. The further reduction in stroke with losartan (RRR 0.75, 95%CI: 0.63-0.89, P = 0.001) was the major contributing factor to the reduction in the primary end point[77].

The Study on Cognition and Prognosis in the Elderly (SCOPE) assessed the effect of candesartan (PPARγ agonist) on cardiovascular and cognitive outcomes in elderly patients (aged 70-89 years) with mild to moderate hypertension[78]. Patients were randomized to candesartan 8-16 mg daily (n = 2477) or placebo (n = 2460) and followed for an average of 3.7 years[78]. Other antihypertensive drugs were added if blood pressure remained greater than 160 mmHg systolic and/or 90 mmHg diastolic[78]. Due to extensive add-on therapy, particularly in patients randomized to placebo, the between-treatment difference in blood pressure was only 3.2/1.6 mmHg[78]. The main analysis showed, however, that non-fatal stroke was reduced by 28% (P = 0.04) in the candesartan group compared with the control group, and a non-significant 11% reduction in the primary endpoint of major cardiovascular events was seen (P = 0.19)[78]. In conclusion, the findings of SCOPE suggest that candesartan treatment reduces cardiovascular morbidity and mortality in old and very old patients with mild to moderate hypertension. Candesartan-based antihypertensive treatment may also have positive effects on cognitive function and quality of life[78].

The Valsartan (PPARγ agonist) Antihypertensive Long-term Use Evaluation (VALUE) trial was designed to evaluate the hypothesis that for the same blood-pressure control, valsartan would reduce cardiac morbidity and mortality more than amlodipine (a calcium channel blocker) in hypertensive patients at high cardiovascular risk[79]. Blood pressure was reduced by both treatments, but the effects of the amlodipine-based regimen were more pronounced, particularly in the early period (blood pressure 4.0/2.1 mmHg lower in the amlodipine group than the valsartan group after 1 mo; 1.5/1.3 mmHg after 1 year; P < 0.001 between groups)[79]. There was no difference between the treatment groups in the primary composite endpoint, which was the occurrence of cardiac disease[79].

The Trial of Preventing Hypertension (TROPHY) investigated whether pharmacological treatment of prehypertension prevents or postpones stage 1 hypertension[80]. Participants with repeated blood pressure measurements of 130-139 and/or 85-89 mmHg were randomly assigned to 2 years of candesartan or placebo, followed by 2 years of placebo for all[80]. The 4-year incidence of hypertension was significantly (P < 0.01) lower than that previously reported in the placebo (-11.3%) and candesartan (-11.0%) groups[80]. During the first 2 years, hypertension developed in 162 placebo and 53 candesartan participants (RRR 68%, P < 0.001)[80]. After 4 years, hypertension occurred in 197 placebo and 165 candesartan participants (RRR 18%, P < 0.009)[80]. The new definition resulted in a lower incidence of hypertension, but the outcomes were remarkably similar with both definitions and confirmed our original findings[80].

In the Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial and the Telmisartan Randomized AssessmeNt Study in ACE-I iNtolerant Subjects with Cardiovascular Disease, researchers assessed the cardioprotective and antidiabetic effects of telmisartan[67]. The collective data suggest that telmisartan is a promising drug for controlling hypertension and reducing vascular risk in high-risk elderly patients with new-onset diabetes[52]. Furthermore, several clinical studies have demonstrated the blood pressure-lowering effect of TZDs as PPARγ ligands[81]. The recent PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive Study), which included 5238 T2DM enroll-

ees, also demonstrated a significant decrease in systolic blood pressure (3 mmHg) following treatment with pioglitazone (a TZD)[82].

Disappointingly, the results from the Fenofibrate Intervention and Event Lowering in Diabetes trial failed to show a reduction in risk for the primary end-point (coronary heart disease death and nonfatal myocardial infarction) of coronary events with fenofibrate therapy[83]. There are many explanations for these results, including the use of a low cardiovascular risk diabetic population; however, more investigation is clearly needed to understand the clinical relevance of fibrates for treating CVD[83].

In a sub-analysis of the Candesartan Antihypertensive Survival Evaluation in Japan trial, researchers examined the relationship between the achieved blood pressure and cardiovascular events in hypertensive patients with T2DM, CKD, or LVH at baseline[84]. A higher achieved blood pressure was associated with an increased risk of cardiovascular events in hypertensive patients with complications (T2DM, CKD, or LVH)[84]. In patients with LVH, who achieved a systolic/diastolic blood pressure (SBP/DBP) < 130/75-79 mmHg, the risk of cardiovascular events was reduced to the same level as in those without LVH, an SBP/DBP < 130/75-79 mmHg[84]. However, the risks of cardiovascular events in patients with DM or CKD, who achieved an SBP/DBP < 130/75-79 mmHg, were still significantly higher than in those without DM or CKD[84].

CONCLUSION

Although a decade or more has passed since the pleiotropic effects of PPARγ were first reported, numerous studies on its novel effects continue to appear each month. In addition to the effects on blood pressure, atherosclerosis, and kidney dysfunction described above, anti-cancer effects of PPARγ ligands have recently been reported[59]. The usefulness and effectiveness of PPARγ ligands in the treatment of lifestyle-related diseases will be increasingly appreciated[59,85].

Further refinement of experimental strategies, group-specific chemical modification of potential compounds, and the development of specific and reliable translational models and biomarkers to better understand their safety and efficacy should all be of great assistance in the future clinical development of novel types of PPAR agonists[8]. Moreover, future efforts to further delineate the physiology, pharmacology, and molecular functions of the PPARs may identify additional novel targets that can also be exploited in the development of superior, efficacious, and tissue-/PPAR isotype-specific agonists for the treatment of hypertension[8].

There are clearly many uncertainties about the use of PPAR agonists in the treatment of cardiovascular disease. They have highly complex biologic effects resulting from the activation or suppression of dozens of genes, and the biologic effects of the protein targets for most of these genes remain largely unknown. Moreover, they possess different properties for different species[2]. Further efforts to completely investigate the effects of the PPARs and their agonists and the mechanisms by which they improve lifestyle-related diseases are required, including high blood pressure, in both human and animal models[2]. Additionally, the adverse effects of PPARγ agonists on cardiac function and water retention and the mechanisms responsible for these effects should be clarified in detail, particularly in humans[2]. Finally, the combination of PPARs with reagents or with other cardiovascular drugs such as diuretics and ARBs should be studied[2].

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (2): Coronary artery disease

Genetics of coronary heart disease with reference to *ApoAI-CIII-AIV* gene region

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Abstract

Cardiovascular diseases are affected by multiple factors like genetic as well as environmental hence they reveal factorial nature. The evidences that genetic factors are susceptible for developing cardiovascular diseases come from twin studies and familial aggregation. Different ethnic populations reveal differences in the prevalence coronary artery disease (CAD) pointing towards the genetic susceptibility. With progression in molecular techniques different developments have been made to comprehend the disease physiology. Molecular markers have also assisted to recognize genes that may provide evidences to evaluate the role of genetic factors in causation of susceptibility towards CAD. Numerous studies suggest the contribution of specific "candidate genes", which correlate with various roles/pathways that are involved in the coronary heart disease. Different studies have revealed that there are large numbers of genes which are involved towards the predisposition of CAD. However, these reports are not consistent. One of the reasons could be weak contribution of genetic susceptibility of these genes. Genome wide associations show different chromosomal locations which dock, earlier unknown, genes which may attribute to CAD. In the present review different *ApoAI-CIII-AIV* gene clusters have been discussed.

Key words: *ApoAI-CIII-AIV* gene cluster; Haplotype analysis; Single nucleotide polymorphism; Candidate gene study; Genome wide association studies

Core tip: Cardiovascular disease analysis requires holistic approach using genomic, epigenomic and exposomic techniques to improve the quality of life of patients and contribution towards personalised medicine.

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INTRODUCTION

Coronary artery disease (CAD), is mostly fatal if remain untreated result into atherosclerosis in the epicardial coronary arteries[1]. Atherosclerotic plaques progressively narrow the coronary artery lumen and impair antegrade myocardial blood flow. This reduction in coronary artery flow may lead to a myocardial infarction.

Cardiovascular disease is a multifarious disorder showing large diversity of phenotypes. The accurate, and analogous phenotypic evidences are crucial for detailed understanding of the affiliation between disease and genes, as well as understanding the role of various extrinsic factors on different component of various genotypes.

Table 1 Prevalence of coronary artery disease in different Indian surveys

City	Prevalence	Ref.
Urban population		
Chandigarh	(6.60%)	Sarvotham *et al*[49]
Rohtak	(3.80%)	Gupta *et al*[50]
Jaipur	(7.60%)	Gupta *et al*[51]
Delhi	(9.70%)	Chada *et al*[52]
Rural population		
Jaipur	(3.50%)	Gupta *et al*[53]
Ludhiana	(3.08%)	Wander *et al*[54]
South Indians		
Tamil Nadu	(14.30%)	Ramachandran *et al*[55]
Tamil Nadu	(11.00%)	Mohan *et al*[56]
Migrant Indians		
London, United Kingdom	(17.00%)	Bahl *et al*[57]
Illinois, United States	(10.00%)	Enas *et al*[3]

This complexity also contributes to difficulties in diagnosis and prognosis of the disease. Diagnostic difficulties also hamper the optimal and personalised treatment for patients. In recent years the role of genetic variability on the development of CAD has been extensively been studied[1,2] which is impacting upon our understanding of phenotypic outcomes and clinical complications. New developments in genomics, epigenomics and exposomics (environmental risk factors across the life span) would result into the improved understanding of the different phenotypes observed in CAD and would help in the better regimen of treatment. In the last century, there has been rapid increases in the global prevalence of CAD, which has become the important cause of cardiovascular mortality all over the world, is > 4.5 million deaths in the developing countries. By 2020, it is predictable that CAD will be the major source of disease burden universally[2]. The prevalence of CAD varies in different ethnic groups which may show higher/lower genetic and environmental susceptibilities. India has also witnessed consistent increases in the prevalence of CAD over the past few decades and could become the number one killer if appropriate interventions are not planned and implemented. In Table 1 the incidence of CAD is shown in different parts of India.

It has been reported that CAD is increasing in a linear fashion as it has increased from 4% in 1960 to 11% in 2001 *i.e.*, almost every 25th individual in 1960 was having CAD, while in 2001 every 9th individual was having CAD. The CAD is declining internationally among Indians settled abroad, whereas, these rates are growing in the Indian subcontinent. Presently, 10%-12% of metropolitan Indians have CAD compared to 3% of the United States population. Many studies document that Asian Indians are at 3-4 times greater risk of CAD than white Americans/Europeans, 6 times higher than Chinese, and almost 20 times higher than Japanese[3-7]. CAD prevalence has increased from 3.5% in the 1960s to 9.5% in the 1990s in urban populations of India[8]. Current studies recognized occurrence of CAD to be 13.9% in the urban south Indians, 9.6% in urban north Indians[9-11]. In 1990s, 33% of cardiovascular deaths have been reported from India[12] and it is likely that deaths from non-communicable diseases such as CAD will increase two times higher *i.e.*, 4.5 million in 1998 to 8 million in 2020 in India[13].

As CAD has a multifactorial nature and the occurrence of the familial clustering in CAD led investigators to start searching for susceptibility genes. In Figure 1 different risk factors involved in the causation of CAD are summarized.

It is vital to keep in mind that certain genes may show population specific effects. There are hundreds of genes known to have functional allelic variations that may be important for determining an individual's vulnerability to CAD. There is much argument on results of published epidemiological studies until now. The differences may be due to differences in the techniques used or the population used to calculate the incidence, prevalence and other risks. It has been proposed that if multiple markers are used for assigning the risk, the results would be more conclusive clinically. Most important reason of concern in developing countries like India is the incomplete detection, treatment and control of CAD risk factors. The benefits of addressing the root cause of CAD, such as inflammation, smoking and cholesterol, together with preventive methodology will be useful in improving quality of life and saving lives. This in turn may be translated into preventive approaches to help reduce the risk of CAD using genetic and epigenetic approaches. Although CAD mortality in the Indians is highest than other populations[14,15], the reason for increased risk, which has been recorded in both the Asian immigrants and among Indians in urban India; are not yet clear hence more systematic and comprehensive studies are required to understand the spectrum of genetic and epigenetic influences on CAD.

GENETIC BASIS OF CAD

Atherosclerosis involves multiple factors, hence understanding the genetic and environmental basis of this complex disease requires holistic approaches[16-18]. A range of candidate genes (*e.g.*, *APOE*, *APOB*, *LPL*, *iNOS*, *ACE*, *COX2*, *CD14*, *P-Selectin*, *E-Selectin*, *MTHFR*, *PON1*, *TNFα*) have been investigated in relation to initiation, development and progression of CAD[16-18]. A large number of studies using of candidate genes and genome-wide association analyses have shown some promising signals, but only a few have been confirmed to some extent which may be playing a role in CAD.

There are very few examples where single genes have played a role in causing atherosclerosis[19,20]. Mostly, CAD is caused by the environmental factors however the risk increases when some risk associated genes are also present. Research on identical twins consistently shows significant genetic effect in the development of CAD or its risk factors (Table 2). Heritability for CHD vary from 40% to 60%[21,22], suggesting a strong role of genes in the development of the disease. A detailed analysis of the many known CAD susceptibility genes and studies is be-

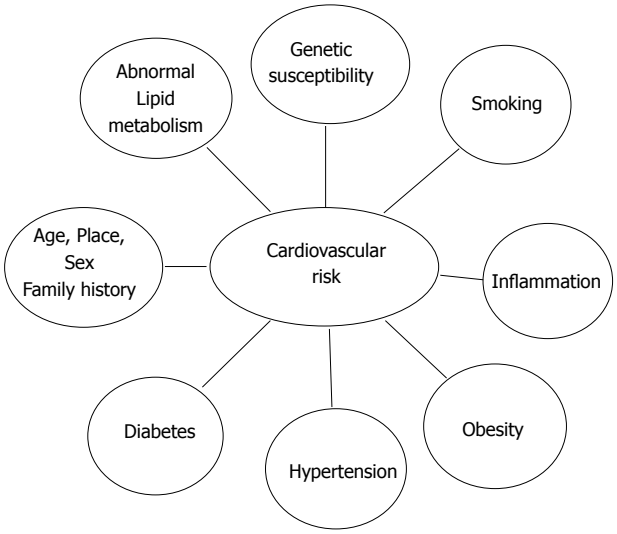

Figure 1 Cardiovascular risk factors.

yond the scope of this overview. This overview will focus on selected candidate genes in the *ApoAI-CIII-AIV* gene region.

SINGLE GENE DISORDERS AND CAD

Familial hypercholesterolemia

Familial hyper cholesterolemia (FH) is a classic genetic disease in which increased cholesterol, tendon xanthomas, and early heart disease segregates together. Joseph Goldstein and Michael Brown showed that FH results from mutations in the low-density lipoprotein (LDL) receptor, which leads to impaired binding, internalization and degradation of LDL. Dose dependent relationship was observed, homozygotes patients had higher levels of cholesterol (> 600 mg/dL), whereas heterozygotes had levels of approximately 400 mg/dL. This variable penetrance is modified by genes and other risk factors such as diet, smoking, and physical activity level[23]. Heterozygote frequency for this disease relatively high, approximately 1 in 500[24] in most populations, however DNA screening and effective treatments are available now[25,26].

Familial defective apolipoprotein B causing hypercholesterolemia

This comparatively common hypercholesterolemia (approximately 1 in 800), results from mutations in the major protein of LDL called Apolipoprotein B (ApoB). The mutations in ApoB prevent LDL binding to the LDL receptor. The majority of patients of this disorder carry a dominant mutation (codon 3500) and have lower cholesterol levels compared to FH patients. Other single-gene CHD/CAD traits are rare and of lower clinical/population significance[20].

CANDIDATE GENES AND CAD

During last 30 years, there have been many advancements in molecular genetic technology, development of sophisticated statistical tools and analyses which have contributed to improvements in human genetic research. One of the early developments was positional cloning technique, which allowed genetic mapping of many Mendelian diseases and traits. However for complex diseases, which involve many genes and environmental influences, this technique did not provide any major insights into genetic basis. Majority of our understanding of the genetic basis of CAD/CHD has been gained from studies of "candidate genes," and more recently genome wide association (GWA) studies. These population based studies have provided further insights into genetic susceptibilities/contributions to complex diseases. Some examples of these are given below.

Table 2 Genetic and environmental risk factors for coronary heart disease

Risk factors with a significant genetic component (heritability)
Elevated LDL and VLDL cholesterol (40%-60%)
Low HDL cholesterol (45%-75%)
Elevated triglycerides (40%-80%)
Increased body mass index (25%-60%)
Elevated systolic blood pressure (50%-70%)
Elevated diastolic blood pressure (50%-65%)
Elevated lipoprotein(a) levels (90%)
Elevated homocysteine levels (45%)
Type 2 diabetes mellitus (40%-80%)
Elevated fibrinogen (20%-50%)
Elevated C-reactive protein (40%)
Elevated homocysteine levels (45%)
Gender
Age
Family history
Environmental risk factors
Smoking
Diet
Exercise
Infection
Foetal environment
Air pollution (particulates)

Risk factors for coronary heart disease can be subdivided into those that are determined significantly by genetic differences and those that are largely environmental (Based on Lusis *et al*[58] 2004). VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein.

APOLIPOPROTEIN E AND *APOAI-CIII-AIV-AV* GENE CLUSTER

Apolipoprotein E (ApoE) is one of the extensively studied genetic locus as it plays a pivotal role in lipid metabolism and mediates the uptake of chylomicron and very low-density lipoprotein (VLDL) remnants. Utermann and colleagues[27] identified genetic polymorphism at ApoE locus and its association with cholesterol levels and type Ⅲ hyperlipidemia. The polymorphism and its CAD associations have been replicated in many global populations. E3 allele is the most common (approximately 60%) followed by E4 allele (approximately 30%) and E2

(approximately 10) in world populations. E4 allele carriers have increased plasma cholesterol levels compared to E3 allele carriers while E2 carriers have decreased plasma cholesterols. The allelic variation at ApoE locus explains approximately 5% of the variation in cholesterol levels[28]. Type Ⅲ hyperlipidemia, a relatively rare phenotype, are homozygous for the E2 allele, but not all E2 homozygous individuals have this disorder[29]. Therefore, genotype-phenotype relationships may require contribution of other genetic or environmental factors.

In addition to ApoE, there is now strong evidence that mutations in hepatic lipase influence the levels of high-density lipoprotein (HDL)[30], and the ApoAI-CIII-AIV-AV locus contributes to plasma triglyceride levels[31]. Many studies have shown that Lp(a) levels are strongly influenced by Apo(a) gene[32] In addition, both hepatic lipase and the ApoAI-CIII-AIV-AV cluster influence LDL particle size, which significantly contributes to CHD risk[33]. However, taken together, these genetic differences only explain a small amount of variation in plasma lipids and CHD/CAD phenotypes.

Dyslipidemia, a metabolic disorder, caused due to the defects in the synthesis, processing and catabolism of lipoprotein particles. Increased total cholesterol (TC)[34], triglyceride (TG)[35], LDL cholesterol (LDL-C)[36], and apolipoprotein (Apo)B[37], together with lower levels of ApoA1[37] and HDL cholesterol (HDL-C)[38] have been found to increase coronary artery disease (CAD) risk. Epidemiological and clinical studies have documented that above genetic factors/polymorphisms play a significant role in dyslipidemia[39] susceptibilities along with environmental factors. Twin and family studies suggest there are considerable genetic contributions in the inter-individual variation in plasma lipid phenotypes with the heritability estimates ranging from 40%-60%[40]. It has been suggested that understanding variation at these loci along with other newer genetic loci will provide a better understanding of the disease processes and contribution to personalized medicine.

ApoA1, is the main protein component of HDL-C, it functions in the activation of lecithin: cholesterol acyltransferase, and facilitates the reverse cholesterol transport from peripheral tissues[41]. ApoC3, is a 79-amino-acid protein formed mainly in the liver, is one of the major component of chylomicrons and VLDL and a minor component of HDL. ApoC3 prevents lipoprotein lipase and plays a key role in the catabolism of TG-rich lipoproteins. ApoA5 is detectable in very low-density lipoprotein, HDL, and chylomicrons and its concentrations are low compared to other apolipoproteins. Human *ApoA1/C3/A5* genes resides in the *ApoA1/C3/A4/A5* gene cluster on chromosome 11q23-q24[42-45]. The *ApoA1/C3/A4/A5* gene cluster has emerged as a significant risk factor for hypertriglyceridemia and atherosclerosis[41,42]. A number of studies have shown significant associations between single nucleotide polymorphisms (SNPs) in the *ApoA1/C3/A4/A5* gene cluster and raised plasma or serum lipid levels in humans, while others have reported negative or inconsistent results[42-46]. In addition there are many other SNPs involved in the inflammation and cell signalling with CAD and/or MI, some of these are summarized in Table 3.

One of the limitations of case control studies is that many false positive or false negative associations may emerge between different genetic markers and complex diseases like CAD. The reason for such results are: (1) controls are not properly selected; (2) sample size of both controls and cases because of which accurate power of the study is not generated and replication of results is not possible; and (3) position of single-nucleotide polymorphisms (SNP's) in terms of their effect on transcription of gene or protein expression. In general, results of small sample size studies (200-300 patients and control subjects) should be interpreted with caution and should be replicated with larger sample sizes. It is important to confirm that genotype distributions are not skewed, especially in the control group. Large deviations from the Hardy-Weinberg equilibrium, may suggest that the control group is not necessarily the representative of healthy and randomly sampled individuals. This departure may also highlight issues with genotype scoring. Recent genome-wide sequencing research has revealed extensive level of variation and heterogeneity between individuals and populations, which should be considered when choosing SNPs and interpreting SNP data. Some of the early SNP association studies failed to include the effect of the polymorphism on gene expression or protein function and genotype-phenotype correlations. This information could reveal if an SNP is the actual cause or solely a marker which may be in linkage disequilibrium another causal variant. These analyses could provide significant clues for understanding the pathophysiologic mechanisms behind clinical outcomes. It is important to correct/control for the age, gender, ethnicity, and other confounders in heart disease genetic association studies. There should be a holistic approach to understand the role of genes, environment and life style factors in CAD susceptibilities and progression.

Recently, genetic analyses have expanded to whole genome sequence analysis and genome-wide association studies (GWAS) as these analyses eliminates biases in the selection of the candidate genes. A number of GWAS studies have identified new loci in previously unsuspected genomic regions. These analyses have shown, novel biological pathways involved in the disease states and development of novel therapies. Many recent studies have shown only limited evidences may exist where the genetic variants may be associated with MI or only with CAD. A care has to be taken in interpreting the GWAS data as large number of variant alleles may be found but one should consider only elegant systems genetics approach to Plaisier *et al*[47] used similar approach and found that *FADS3* is a causal gene for familial combined hyperlipidemia (*FCHL*) and elevated triglycerides in Mexicans. The authors used network gene co-expression analysis and SNP data to assign a function to the genetic variants

Table 3 Example of association studies of factors involved in inflammation and cell signalling with coronary artery disease and/or myocardial infarction

Gene	Polymorphism	Ref.	Suggested results
CRP	1059G/C	Zee *et al*[59]	No significant association with non-fatal MI, stroke or cardiovascular death
ICAM-1	Lys-469-glu	Jiang *et al*[60]	Association with MI and CAD
E-selectin	Ser-128-Arg, Leu-554-phe, G98T	Wenzel *et al*[61]	Associated with angiographic proof of severe CAD in patients < 50 yr
	Ser-128-Arg, G98T	Herrmann *et al*[62]	No association with MI
		Zheng *et al*[63]	T allele more common in younger patients with angiographic CAD
	Ser-128-Arg	Ye *et al*[64]	Association with early-onset CAD
P-selectin	Pro715	Herrmann *et al*[65], Kee *et al*[66]	Possibly has a protective role from MI
	S290N, N562D, V599L, T715P, T741T	Tregouet *et al*[67]	Protective effect of the P715; S290N and N562D associated with MI, when carried by certain haplotype
	C-2123G, A-1969G, Thr715Pro	Barbaux *et al*[68]	Polymorphisms associated with P-selectin levels but not with MI
TNF-α and β	-863C/A, -308G/A (TNF-α), 252G/A (TNF-β)	Koch *et al*[69]	No association of TNF or IL-10 polymorphisms with MI or CAD
TNF-α	Five polymorphisms	Herrmann *et al*[65]	No association to MI or CAD
TNF-α and β	TNF- α 308 G/A , TNF- β 252 A/G	Padovani *et al*[70]	No association to MI
TNF-α and β	TNF- β 308 G/A , TNF- β 252 A/G	Keso *et al*[71]	No association to old MI by autopsy or CAD
TNF-α	308 G/A	Francis *et al*[72]	No association to angiographic CAD
IL-1 cluster	IL-1a (-889), IL-1b (-511), IL-1b (+3953), IL-1RA intron 2 VNTR	Francis *et al*[72]	No association to angiographic CAD IL-1RA VNTR allele 2 associated with single-vessel CAD
IL-1-RA	IL-1RA intron 2 VNTR	Manzoli *et al*[73]	No clear-cut association to CAD or MI
IL-1 cluster	IL-1b 511 C/T, IL-1RA intron 2 VNTR	Vohnout *et al*[74]	No association to angiographic CAD with either polymorphisms
IL-1-RA	IL 1RN-VNTR	Zee *et al*[75]	No association with risk for future MI
IL-1 β, IL-RA	IL-1b 511 C/T, IL-1RA intron 2 VNTR	Momiyama *et al*[76]	IL-1 β (-511)C/C and IL-1Ra (intron 2)2-or 3- repeat allele both associated with CAD, association with MI only in patient who are seropositive for Chlamydia pneumoniae
IL-6	IL-6 G (-174)C promoter polymorphism	Nauck *et al*[77]	No association with the risk for CAD or MI
	-174 (G/C), -572 (G/C), -596 (G/A), +528 I/D	Georges *et al*[78]	-174 C associated with MI (OR = 1.34)-174 C more frequent in patients with two or fewer stenosed vessels than in patients with three vessel lesions
IL-10	3 IL-10 promotor polymorphisms (1082G/A, - 819C/T and -592C/A)	Koch *et al*[69]	No association with MI or CAD
	7 polymorphisms	Donger *et al*[79]	No association with risk for MI
TGF-β1	29 T/C	Yokota *et al*[80]	T allele is a risk factor for MI in middle-aged Japanese men
	-509T	Wang *et al*[81]	No association with CAD
	7 polymorphisms	Cambien *et al*[82]	No association with degree of angiographic CAD, Pro25 allele associated with MI in some regions.
Stromelysin	5 polymorphisms	Syrris *et al*[83]	No association of either polymorphisms with CAD
(*MMP-3*)	5A-117/6A promoter polymorphism (5A/6A)	Schwarz *et al*[84]	No association with the risk for MI, 6A allele marker for progression of CAD
	5A/6A	Terashima *et al*[85]	5A allele associated with risk for MI
	5A/6A	Kim *et al*[86]	5A allele associated with stable angina
	5A/6A	Humphries *et al*[87]	6A genotypes at greater risk for CAD related events in nonsmokers, 5A/5A genotypes amplifies risk in smokers
	5A/6A	Ye *et al*[88]	Homozygosis for 6A associated with greater progression of angiographic CAD
PECAM-1	Val 125Leu, Asn563Ser and Gly670Arg	Sasaoka *et al*[89]	563Ser/Ser and 670Arg/Arg genotypes associated with MI
(*CD31*)	Val 125Leu, Asn563Ser	Wenzel *et al*[90]	125 Val and 563Asn associated with early onset of CAD (< 50 yr)
	Leu 125Val, Ser563Asn	Song *et al*[91]	125Val and 563Asn associated with CAD
	Val125Leu	Gardemann *et al*[92]	No association with MI; weak association of Val125 with CAD in low-risk patients without HTN or DM (OR = 1.54; 95%CI: 1.03-2.3)

CRP: C-reactive protein; DM: Diabetes mellitus; HTN: Hypertension; ICAM: Intercellular adhesion molecule; IL: Interleukin; MI: Myocardial infarction; MMP: Matrix metalloproteinase; PECAM: Platelet endothelial cell adhesion molecule; TGF: Transforming growth factor; TNF: Tumor necrosis factor; VNTR: Variable number of tandem repeats.

rs3737787 (1q21-q23) in *USF1* gene, which was previously identified to be associated with *FCHL*. It is envisaged that new methods like Network medicine[48] will play an important role in these analyses and the advancement of our understanding of pathophysiological mechanisms of diseases like CAD and MI.

CONCLUSION

This overview has highlighted some of the important

challenges regarding the use of genetic approaches to investigate complex diseases. The recent research using genomic, epigenomics and exposomic approaches is providing a range of patient centric tools which will help better classification of phenotypes and personalised medicine for CAD patients. The mechanisms underlying the association of these loci to CAD/MI remain largely unknown and the effects are relatively small. Hence the future challenges are (1) discovering new genetic variants through large-scale meta-analyses, using pathway-based approaches, and high throughput sequencing; (2) illustrating the mechanisms for the identified loci to CAD; and (3) translating the findings from CAD- GWASs and epigenetic analyses to novel and optimized therapeutic strategies.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Hypertrophic cardiomyopathy: Can the noninvasive diagnostic testing identify high risk patients?

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Abstract

Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden cardiac death (SCD) in the young, particularly among athletes. Identifying high risk individuals is very important for SCD prevention. The purpose of this review is to stress that noninvasive diagnostic testing is important for risk assessment. Extreme left ventricular hypertrophy and documented ventricular tachycardia and fibrillation increase the risk of SCD. Fragmented QRS and T wave inversion in multiple leads are more common in high risk patients. Cardiac magnetic resonance imaging provides complete visualization of the left ventricular chamber, allowing precise localization of the distribution of hypertrophy and measurement of wall thickness and cardiac mass. Moreover, with late gadolinium enhancement, patchy myocardial fibrosis within the area of hypertrophy can be detected, which is also helpful in risk stratification. Genetic testing is encouraged in all cases, especially in those with a family history of HCM and SCD.

Key words: Hypertrophic cardiomyopathy; Sudden cardiac death; Noninvasive diagnostic testing

Core tip: Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden cardiac death (SCD) in the young, particularly among athletes. Noninvasive diagnostic testing is important for risk assessment. Extreme left ventricular hypertrophy, documented ventricular tachycardia and fibrillation increase the risk of SCD. Fragmented QRS complex and T wave inversion in multiple leads are more common in high risk patients. Cardiac magnetic resonance imaging with late gadolinium enhancement, patchy myocardial fibrosis within the area of hypertrophy can be detected, which is also helpful in risk stratification. Genetic testing is encouraged in all cases, especially in those with family history of HCM and SCD.

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a common au-

tosomal dominant cardiac disease, affecting 1 in 500 people[1]. Cardiomyocyte hypertrophy, disarray, fibrosis and ventricular wall thickening are the pathological hallmarks of HCM. Although the majority of affected individuals present with mild symptoms or are asymptomatic, HCM is the most common identifiable cause of premature sudden cardiac death (SCD) in the young, especially the young athlete[1]. Since SCD can be the first manifestation in concealed cases and some symptomatic patients do bear a high risk of SCD, timely diagnosis and risk stratification for appropriate therapy and SCD prevention such as prophylactic implantable cardioverter-defibrillator (ICD) therapy are very important. The common risk factors associated with SCD are family history of HCM-related SCD, left ventricular wall thickness ≥ 30 mm, documented ventricular tachyarrhythmia such as frequent and/or prolonged bursts of non-sustained ventricular tachycardia (VT) and ventricular fibrillation (VF), as well as abnormal blood pressure response to exercise[2].

The diagnosis of HCM is based on echocardiography and/or cardiac magnetic resonance images (CMRI), wherein a non-dilated, hypertrophied left ventricle is found in the absence of any other systemic or cardiac event that explains the specific pathology, mostly arterial hypertension[3]. Since the outcome varies among affected individuals, the purpose of this review is to elaborate the usefulness of noninvasive diagnostic testing for identifying high risk patients with HCM.

ELECTROCARDIOGRAM

The electrocardiogram (ECG), the most basic test in cardiovascular disease management, is abnormal in the vast majority of patients diagnosed with HCM. In general, if patients meet the ECG criteria for left ventricular hypertrophy (LVH), the absence of an apparent cause should raise the suspicion of HCM[4]. The history and physical examination may be negative, and the signs of LVH on an ECG may occur earlier than the increase in the thickness of left ventricular wall detected by echocardiography[4,5]. Making a correct diagnosis in a timely manner is essential in SCD prevention.

QRS-ST-T changes in sinus rhythm

Presence of abnormal Q waves such as deep Q waves in multiple leads is common in patients with HCM[6]. Abnormal Q waves may appear prior to the increased QRS amplitude[7]. A deep Q wave is considered if the amplitude ≥ 3 mm or 1/4 of the R wave. In HCM deep Q waves are usually seen in more than two contiguous leads[8]. There are differences in terms of the significance of deep Q waves between the young and adults[6]. Presence of deep Q waves in children has yielded a higher specificity and sensitivity than adults in the diagnosis of HCM[6]. The mechanisms of deep Q waves in HCM are: (1) the electrical inactivation due to myocardial fibrosis; and (2) the altered direction of resultant initial QRS vector due to increased electrical forces of disproportionate hypertrophy of the basal septal and/or ventricular free wall, unopposed by apical forces[9]. Presence of deep Q waves in multiple leads is thought to be associated with an increased incidence of SCD[9].

Increased QRS amplitude is the most common ECG abnormality in HCM. It has been reported that increased QRS amplitude in the limb leads increases the likelihood of SCD in both children and adults with HCM[10]. Increased QRS duration (QRSD) is seen in septal and concentric HCM patients[11]. Ostman-Smith *et al*[10] measured QRS amplitude and duration in HCM subjects with and without cardiac arrest and SCD. They found that increased QRS amplitude-duration product is a better indicator of high risk HCM patients. Among the high risk patients that have undergone ICD therapy, there is a positive correlation between increased QRSD and defibrillation thresholds[12].

It is known that fragmented QRS complex (fQRS) in multiple ECG leads is associated with myocardial scarring or fibrosis in ischemic and non-ischemic cardiomyopathies. In the latter, patchy fibrosis are located in mid-myocardium or sub-epicardium, and predominantly in the perivalvular areas. Femenía *et al*[13] reported a female patient with recurrent syncope diagnosed with HCM at age 9 and had an ICD placed at age 16 after aborted SCD due to VF. Her ECG at age 16 showed fQRS in 12 leads. During a 2-year follow-up, this patient presented with sustained VT requiring anti-tachycardia pacing and ICD shocks[13]. In a large sample study, they found that fQRS located in the lateral area increases the likelihood of ICD therapy[14]. Therefore they postulated fQRS should be incorporated in multivariate models for SCD prediction, along with more classical risk factors[13,14].

ST segment elevation in HCM is viewed as a marker of disease progression[15]. Furuki *et al*[15] found a close correlation between convex ST elevation and left ventricular enlargement and wall motion abnormalities with a specificity of 85% and a sensitivity of 62%, respectively.

Ostman-Smith *et al*[10] found that HCM patients with high risk for SCD had negative T waves in the limb and precordial leads. Moreover, negative T waves in the precordial leads has a positive correlation with the extent of LVH in HCM[7]. On echocardiography, the maximum wall thickness was 19.2 ± 5.2 mm with negative T waves compared to 13.5 ± 5.1 mm without negative T waves[7]. Microvolt-T wave alternans (TWA), a surrogate for unstable ventricular repolarization properties, have been associated with an increased likelihood of VT/VF[16]. Momiyama *et al*[16] also demonstrated that among 7/17 HCM patients classified as high-risk individuals, only two of them did not show TWA.

Ventricular arrhythmia

Documented VT and/or VF are direct risk factors for SCD[17]. In HCM, VF may occur without the preceding VT. A study by Cha *et al*[18] revealed that sinus tachycardia or atrial fibrillation were the most common rhythms that initiated sustained VT followed by ICD discharges in high risk patients. Sustained VT is common in symptomatic individuals[19]. Medeiros *et al*[20] noted that the arrhyth-

mias with the highest prevalence according to their ICD storage recordings were sustained VT and VF.

Recurrent or repetitive non-sustained VT (>10 beats) is considered a risk for SCD in HCM[21]. On ambulatory ECG monitoring, non-sustained VT occurs in about 25% of HCM patients[22]. Gimeno *et al*[22] showed that exercise-induced non-sustained VT was associated with a 3.73 fold rise in SCD. 2D speckle tracking has also been used as an important tool to predict non-sustained VT in HCM patients[23]. According to one study, the results obtained by 2D speckle tracking are similar to the results obtained by ambulatory Holter ECG[23]. There have also been reports of the incidence of non-sustained VT by provocative maneuvers such as Valsalva[24]. Increased vagal tone has been considered a potential mechanism for the occurrence of non-sustained VT[24,25]. The current recommendation is consideration of ICD placement, even if non-sustained VT is the only risk factor[21].

ECHOCARDIOGRAPHY

Echocardiography is an integral diagnostic modality for HCM because it is highly reproducible and cost effective. LVH, the most important phenotypic characteristic of HCM, can be easily revealed by echocardiography. The extent of left ventricular wall thickness is associated with an increased risk of SCD. Maximum left ventricular wall thickness $\geqslant$ 30 mm is termed extreme left ventricular hypertrophy, and is an independent predictor of SCD in the young[26-28]. Spirito *et al*[26] observed 480 cases of HCM consecutively for an average follow-up of 6.5 years. Patients were divided into five groups according to the maximum left ventricular wall thickness: $\leqslant$ 15 mm, 16-19 mm, 20-24 mm, 25-29 mm, and $\geqslant$ 30 mm, respectively. They found that the 20-year cumulative risk for SCD was up to 40% in the group with left ventricular wall thickness $\geqslant$ 30 mm. Patients with extreme left ventricular hypertrophy were mostly young, with only mild symptoms or with no symptoms at all. Neither did they have any evidence of left ventricular outflow tract obstruction. Thus the authors suggest that young patients with extreme left ventricular hypertrophy ($\geqslant$ 30 mm), should consider prophylactic implantation of ICD regardless of the presence or absence of other risk factors. Elliott *et al*[27] found that in HCM patients with left ventricular wall thickness, the relative risk (RR) increased by 1.31 (95%CI: 1.03-1.66) for each additional 5 mm. In HCM microvascular dysfunction, cardiomyocyte hypertrophy and disarray can lead to myocardial ischemia and fibrosis[29]. The latter is a substrate for reentrant tachyarrhythmia and SCD[30]. HCM patients with extreme left ventricular hypertrophy indeed bear a higher risk of SCD and more frequent ICD discharges. Nevertheless, it does not necessarily mean that patients with left ventricular wall thickness < 30 mm are considered low risk. In the later stages of the disease when the left ventricular ejection fraction (LVEF) may be below 50% with left ventricular wall thinning, apical aneurysm and ventricular chamber dilatation[31], the risks of SCD and all-cause mortality increase[31,32].

CARDIAC CT

Although some of the newer systems are safe for ICD patients, MRI in general is hazardous to patients with implanted devices. As an alternative, Shiozaki *et al*[33] examined the value of delayed enhancement multidetector computed tomography (MDCT). They showed that myocardial fibrosis was found in 96.4% of patients with ICD using MDCT[33]. However, it must be noted that ICD cables caused artifacts and may have overrepresented the findings of myocardial fibrosis in these patients[33].

CMRI

Although echocardiography plays a central role in the assessment of HCM, it is sometimes limited by poor acoustic windows, incomplete visualization of the left ventricular wall, and inaccurate evaluation of left ventricular mass[34]. With excellent spatial resolution and border definition, CMRI provides complete visualization of the left ventricular chamber, allowing precise localization of the distribution of hypertrophy and measurement of wall thickness and cardiac mass (Figure 1). CMRI is superior to echocardiography for the detection of apical and focal basal anteroseptal variants and in recognizing noncontiguous areas of HCM[35]. CMR cine imaging provides evaluation of cardiac morphological information including systolic anterior motion of the anterior mitral leaflet with dynamic outflow tract obstruction, mitral regurgitation, apical aneurysms, and papillary muscle abnormalities. CMR stress perfusion imaging can identify areas of microvascular dysfunction or mismatch between left ventricular mass and coronary flow.

Based on CMRI findings in patients with HCM, distribution and extent of LVH is variable including asymmetrical septal, apical, localized, or concentric hypertrophy, but these usually are not extensive. Basal anterior left ventricular free wall and the contiguous anterior ventricular septum are the most commonly hypertrophied segments[34]. LVH can be focal (1-2 segments), intermediate (3-7 segments), or diffuse (> 8 segments). The number of hypertrophied segments is greater in patients with left ventricular outflow tract obstruction than without and was associated with an advanced New York Heart Association functional class. Left ventricular wall thickness was greater in segments with late gadolinium enhancement (LGE) than without. Segmental left ventricular hypertrophy largely confined to the anterolateral free wall, posterior septum, or apex were underestimated or undetected by echocardiography. These observations support an emerging role for CMR in the contemporary evaluation of patients with HCM.

Moreover, LGE plays a critical role in risk stratifying HCM patients (Figure 1). Myocardial fibrosis is present in up to 80% of patients with HCM, with a characteristic patchy pattern of LGE generally occurring in areas of hypertrophy[34]. In addition, the extent of fibrosis has been shown to correlate positively with regional hypertro-

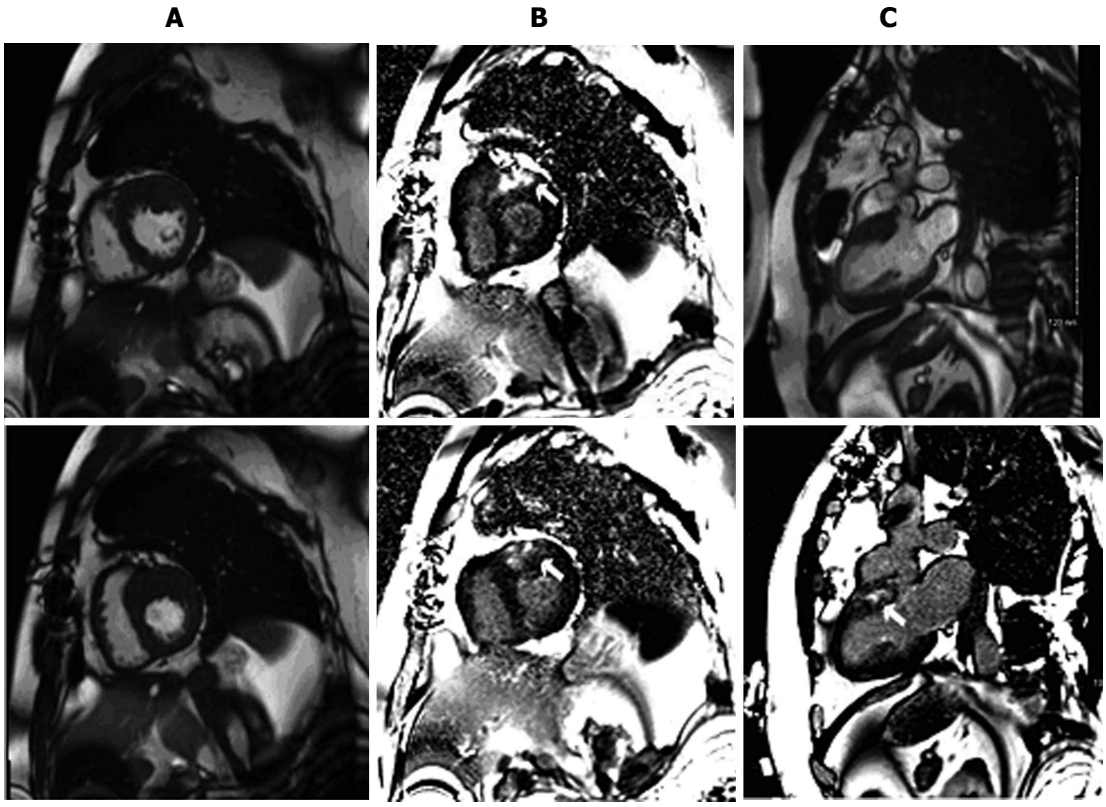

Figure 1 Basal anterior hypertrophic cardiomyopathy in a 45-year-old man with a history of syncope. Cardiac magnetic resonance imaging demonstrated severe thickening of basal anterior wall with a maximal measurement of 25 mm. A: Two chamber short-axis cine images; B: Late delayed gadolinium enhanced images; C: Two chamber long-axis cine images (top panel). Late delayed gadolinium enhanced images (bottom panel). Patchy, non-coronary artery disease scarring in the hypertrophied areas is indicated by white arrows.

phy and inversely with regional contraction. Consistently, in another clinical study[36] with 243 consecutive HCM patients, the presence of scar was an independent predictor of death, with an odds ratio of 5.47 for all-cause mortality and of 8.01 for cardiac mortality. Similarly, the risk of unplanned heart failure admissions, deterioration to NYHA functional class Ⅲ or Ⅳ, or heart failure-related death has been shown to be statistically greater in those with fibrosis[37]. In a study with 424 HCM patients[38], LGE-positive patients were more likely to have episodes of non-sustained VT, more episodes of non-sustained VT per patient, and a higher frequency of ventricular extrasystoles per 24 h, with all cases of SCD and appropriate ICD discharges occurring in LGE-positive patients. More recently, a meta-analysis[39] of four studies evaluating 1063 HCM patients over an average follow-up of 3.1 years demonstrated that there are significant relationships between LGE and cardiovascular mortality, heart failure death, and all-cause mortality in HCM patients. Additionally, LGE and SCD/aborted SCD displayed a trend toward significance. The assessment of LGE in HCM patients by CMRI has the potential to provide important information to improve risk stratification in clinical practice.

ROLE OF GENETIC TESTING

Since the pathogenic missense mutation in the β-myosin heavy chain gene (MYH7 R403Q) was revealed two decades ago, > 1400 mutations have been identified in putative HCM-susceptibility genes. The most common genetic subtype is sarcomeric-HCM, caused by mutations in genes encoding proteins in the myofilaments of the cardiac sarcomere[40]. Among patients with positive genetic tests, MYBPC3 (myosin-binding protein C) and MYH7 are, by far, the two most common HCM-associated genes with an estimated prevalence of 25%-35% for each gene, while other genes including troponin T, troponin I, α-tropomyosin, and α-actin each account for a small proportion of patients (1% to 5%)[41]. Collectively, the known causal genes account for about two-thirds of all HCM cases while one-third of the causal genes for HCM are yet to be identified[42].

Genetic testing for HCM has been commercially available for almost a decade. However, the low mutation detection rate and cost have hindered uptake[43]. Currently, genetic testing has been recommended for any patients with an established clinical diagnosis of HCM and for family members following the identification of the HCM-causative mutation in the index case[44]. Multivariate analysis advocates this recommendation by identifying female gender, increased left-ventricular wall thickness, family history of hypertrophic cardiomyopathy, and family history of SCD as being associated with greatest chance of identifying a gene mutation[43].

Including genetic testing in the diagnostic strategy is also more likely to be cost effective than clinical tests

alone when considering family screening and prevention of SCD[45,46]. The results of genetic testing identifies mutation carriers who will benefit from regular clinical investigation or early discussion of ICD. On the other hand, the result of genetic testing also identifies relatives without the causal mutation, who can be released without the need for long-term follow-up[47].

With rapid developments in genetic testing technology, a whole exome or a panel of HCM-related genes can now be tested by the next generation sequencing simultaneously, which provides an opportunity to detect multiple mutations in the same or different genes that are responsible for HCM. Emerging evidence documents that patients with HCM who carry more than one independent disease-causing gene mutation may be at a greater risk for severe disease expression and adverse outcome[48-51], especially in the absence of other conventional risk factors[52]. These observations support the emerging hypothesis that double (or compound) mutations detected by genetic testing may confer a gene dosage effect in HCM, thereby predisposing patients to adverse disease consequence[52]. It is observed that multiple mutation carriers are more likely to have suffered an out-of-hospital cardiac arrest or SCD[43]. For those patients who test positive for two or three mutations, frequent follow-up or early intervention may be required. Therefore, the integration of genetic testing into the current testing paradigm is likely to improve the general management of affected families.

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TOPIC HIGHLIGHT

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Alcoholic cardiomyopathy

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Abstract

Alcohol is the most frequently consumed toxic substance in the world. Low to moderate daily intake of alcohol has been shown to have beneficial effects on the cardiovascular system. In contrast, exposure to high levels of alcohol for a long period could lead to progressive cardiac dysfunction and heart failure. Cardiac dysfunction associated with chronic and excessive alcohol intake is a specific cardiac disease known as alcoholic cardiomyopathy (ACM). In spite of its clinical importance, data on ACM and how alcohol damages the heart are limited. In this review, we evaluate available evidence linking excessive alcohol consumption with heart failure and dilated cardiomyopathy. Additionally, we discuss the clinical presentation, prognosis and treatment of ACM.

Key words: Alcohol; Alcoholic cardiomyopathy; Dilated cardiomyopathy; Heart failure

Core tip: Cardiac dysfunction associated with excessive alcohol intake is a specific cardiac disease known as alcoholic cardiomyopathy. In spite of its clinical importance, data on alcoholic cardiomyopathy and how alcohol damages the heart are limited. In this review, we evaluate available evidence linking excessive alcohol consumption with heart failure and dilated cardiomyopathy. Additionally, we discuss the clinical presentation, prognosis and treatment of alcoholic cardiomyopathy.

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INTRODUCTION

Daily consumption of low to moderate amounts of alcohol has beneficial effects on cardiovascular health among both ischemic and non-ischemic patients[1-3]. In contrast, chronic and excessive alcohol consumption could lead to progressive cardiac dysfunction and heart failure (HF)[3].

HF is most frequently related to the presence of arterial hypertension and ischemic cardiomyopathy[4,5]. In younger individuals, however, where HF is less prevalent, a heterogeneous group of cardiac diseases, collectively known as cardiomyopathies, represent the leading cause of HF and heart transplant in the world[6]. Among cardiomyopathies, the variety that most often leads to HF and the first cause of heart transplant among young patients is dilated cardiomyopathy (DCM)[6]. DCM is defined as left ventricular systolic dysfunction and dilatation, which may or may not be associated with a similar right ventricular dysfunction. Excessive alcohol consump-

tion is prominent among the multiple aetiologies causing DCM and has been considered the major cause of non-ischemic DCM in Western countries[7-12].

Despite the key clinical importance of alcohol as a cause of DCM, relatively few studies have investigated the effects of alcohol on the heart and the clinical characteristics of DCM caused by excessive alcohol consumption (known as alcoholic cardiomyopathy). Moreover, conflicting results are available regarding several factors related to alcoholic cardiomyopathy (ACM), such as the precise amount of alcohol necessary to cause the disease, whether the long-term prognosis of ACM is similar to that of other forms of DCM, or whether complete alcohol abstinence is necessary to improve clinical outcomes.

In this review, we evaluate the available evidence linking alcohol consumption with HF and DCM. We also discuss the clinical presentation, prognosis and treatment of ACM.

HISTORICAL PERSPECTIVE

The depressing effect of alcohol on the heart has been known for some time. Indeed, the first account of the possible harmful effects of alcohol specifically on heart muscle was reported in the latter half of the 19th century. Expressions referring to "the heart of a wine drinker in Tubingen" and particularly a "Munich beer heart" were used and known in Germany during this time[13].

Bollinger, a pathologist in Munich in the late 19th century, was perhaps the first to suspect a possible link between excessive alcohol consumption and sudden death in young individuals, an occurrence that alarmed public opinion at the time. The diagnosis of the source of those deaths was found after performing autopsies and discovering the characteristic left ventricular dilatation and hypertrophy. The findings that led Bollinger to establish a causal relationship between alcohol consumption and these structural abnormalities were both of a clinical and an epidemiological nature. Thus, he identified that the incidence of alcohol-related DCM was much higher in Munich, where alcohol intake was greater, than in other German cities. Indeed, he found 42 cases of ACM from among 1500 autopsies performed in Munich, contrasting with a single case in Berlin from 809 hearts analysed. Also, he observed that these individuals often presented co-morbidities closely associated with alcohol consumption, including delirium tremens and cirrhosis of the liver, and that 22 of the 42 deceased individuals were regular patrons of beer houses in Munich, where they could drink from 6 to 12 L of beer per day[13].

Later, in 1902, William McKenzie, in his treatise on arterial and venous pulse and heart movements, described the existence of individuals who, in association with alcohol consumption, developed an accelerated heart pulse or swelling and engorgement of the veins, and according to his experience they had a poor prognosis with progressive heart failure. In their autopsies, he described finding dilated cavities of the heart and fatty degeneration of the ventricular walls[14].

Since those initial descriptions, reports on several isolated cases or in small series of patients with HF due to DCM and high alcohol intake have been published[15-17]. Some of these papers have also described the recovery of LVEF in many subjects after a period of alcohol withdrawal[15-17].

DEFINITION OF ALCOHOLIC CARDIOMYOPATHY

At present ACM is considered a specific disease both by the European Society of Cardiology (ESC) and by the American Heart Association (AHA)[18,19]. In the ESC consensus document on the classification of cardiomyopathies, ACM is classified among the acquired forms of DCM[19].

The diagnosis of ACM is usually one of exclusion in a patient with DCM with no identified cause and a long history of heavy alcohol abuse. According to most studies, the alcohol consumption required to establish a diagnosis of ACM is over 80 g per day during at least 5 years[9-12].

AMOUNT OF ALCOHOL REQUIRED TO PRODUCE ACM

Data on the amount of alcohol consumption required to cause ACM are limited and controversial.

The first study, which specifically focused on the amount of alcohol necessary to cause ACM, was conducted by Koide *et al*[20] in 1975. The authors examined the prevalence of cardiomegaly by means of chest x-rays and related it to alcohol consumption among a consecutive series of Japanese males of working age. They found that 2 of the 6 individuals (33%) whose alcohol consumption exceeded 125 mL/d had cardiomegaly. In contrast, an enlarged heart was found in only 1 of 25 subjects with moderate consumption (4%), in 6 of 105 very mild consumers (5.7%), and in 4.5% of non-drinking individuals.

A second set of studies that are quoted when addressing this topic are those conducted in individuals who started an alcohol withdrawal program[21-24]. In these studies, the authors estimated the amount and chronicity of alcohol intake and subsequently related the figures to a number of echocardiographic measurements and parameters. Although all of the studies reported an increase in left ventricular mass and volume, it cannot generally be stated that they provided the alcohol consumption dosage required to cause ACM.

Askanas *et al*[21] found a significant increase in the myocardial mass and of the pre-ejection periods in drinkers of over 12 oz of whisky (approximately 120 g of alcohol) compared to a control group of non-drinkers. However, no differences were found in these parameters between the sub-group of individuals who had been drinking for 5 to 14 years and the sub-group of individuals who had a drinking history of over 15 years. Kino *et al*[22] found

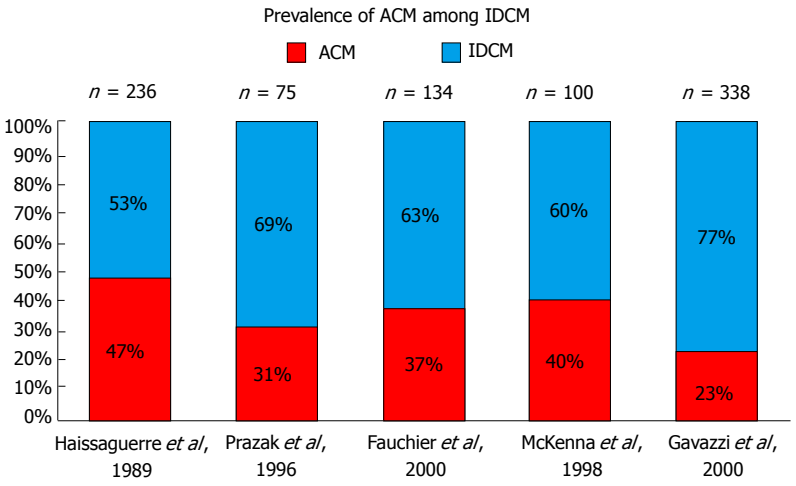

Figure 1 Prevalence of alcoholic cardiomyopathy among idiopathic dilated cardiomyopathy series. ACM: Alcoholic cardiomyopathy; IDCM: Idiopathic dilated cardiomyopathy.

increased ventricular thickness when consumption exceeded 75 mL/d (60 g) of ethanol, and the increase was higher among those subjects who consumed over 125 mL/d (100 g), without specifying the duration of consumption. In another study on this topic, Lazarević *et al*[23] divided a cohort of 89 asymptomatic individuals whose consumption exceeded 80 g/d (8 standard units) into 3 groups according to the duration of their alcohol abuse. Subjects with a shorter period of alcohol abuse, from 5 to 10 years, had a significant increase in left ventricular diameter and volume compared to the control group. However, a systolic impairment was not found as the years of alcoholic abuse continued.

Unfortunately Lazarević *et al*[23], as in most of these studies, systematically excluded patients with a history of heart disease or with HF symptoms. It is therefore possible that most of these studies may have also consistently omitted most alcohol abusers in whom alcohol had already caused significant ventricular dysfunction.

One of the exceptions in these accounts is the study conducted by Urbano-Márquez *et al*[24], in which 46 asymptomatic alcohol abusers who were beginning an alcohol withdrawal program were studied together with 6 alcoholics identified at the emergency department due to HF symptoms. This is the only study describing the existence of a direct linear relationship between accumulated alcohol consumption throughout life and left ventricular mass (r = 0.42), fractional shortening (r = 0.35), and ejection fraction (r = 0.46) (all $P < 0.001$). A large number of studies, however, never reproduced this relationship, and it has been suggested that this relationship could correspond to the existence of a threshold dose above which the risk of suffering this disease increases[25]. Kupari *et al*[25], after reviewing the research by Urbano-Márquez, suggested a lifetime cumulative cut-off dose of alcohol of 20 kg/kg of weight. Actually, in the research by Urbano-Márquez *et al*[24], slight dysfunction of the left ventricle had already appeared due to cumulative doses of 10 kg of alcohol per kg of weight.

Finally, it should be noted that a large majority of studies on the long-term prognosis of ACM used the cut-off point of 80 g/d for a minimum of 5 years to consider alcohol as the cause of DCM. Although this figure may be sufficient to cause the structural alterations described above, we must stress that this value is arbitrary and is not based on robust experimental or epidemiological data; also, the average consumption of the individuals included in the research was always much greater[9-12].

Additionally, the accepted ACM definition does not take into account a patient's sex or body mass index (BMI). As women typically have a lower BMI than men, a similar amount of alcohol would reach a woman's heart after consuming smaller quantities of alcohol.

EPIDEMIOLOGY OF ALCOHOLIC CARDIOMYOPATHY

For many decades, ACM has been considered one of the main causes of left ventricular dysfunction in developed countries. Specifically in the United States, ACM was declared the leading cause of non-ischemic DCM[7]; a fact related to the high consumption of alcoholic beverages worldwide, which is particularly elevated in Western countries[26].

Studies that have assessed the prevalence of ACM among IDCM patients have found high alcohol consumption in 3.8% to 47% of DCM patients. The lowest prevalence of ACM among DCM (3.8%) was obtained from a series of 673 patients admitted to hospital consecutively due to HF in the state of Maryland[27]. This study included not only DCM, but also all causes of left ventricular dysfunction, including hypertensive heart disease, ischemic cardiomyopathy and heart valve disease. Furthermore, the inclusion criteria for ACM were very strict and required a minimum consumption of 8 oz of alcohol (200 g or 20 standard units) each day for over 6 mo. In contrast, European studies focusing on the prevalence of ACM included only subjects diagnosed with DCM and applied the consumption threshold of 80 g/d for ≥ 5 years, finding an ACM prevalence of 23%-47% among idiopathic DCM patients[9-12] (Figure 1).

Finally, it should be noted that McKenna and coworkers, in one of the most frequently cited papers in the ACM field, reported an incidence of 40% in 100 individuals suffering from idiopathic DCM, but in this case

the consumption threshold used was only 30-40 g/d[8].

EVIDENCE LINKING EXCESSIVE ALCOHOL CONSUMPTION AND DCM

The existence of a direct causal link between excessive alcohol consumption and the development of DCM is a controversial issue. While some consider that this toxin alone is able to cause such a disease[18,19], others contend that it is just a trigger or an agent favouring DCM[3,21,22].

At present, however, ACM is considered to be a disease in its own right[18,19].

The evidence that allows this link to be established arises from 6 categories of research: (1) epidemiological studies; (2) experimental studies with controlled alcohol administration; (3) haemodynamic/echocardiographic studies analysing the effects generated by alcohol consumption on myocardial structure and function; (4) histological studies; (5) basic research studies identifying the mechanisms of alcohol-induced damage to the cardiomyocyte; and (6) studies analysing the positive clinical response to alcohol withdrawal.

Epidemiological studies

Epidemiological studies analysing the relationship between excessive alcohol consumption and the development of DCM have found the existence of a reciprocal link between both disorders.

In this respect, a higher prevalence of excessive alcohol consumption has been reported among individuals diagnosed with DCM than in the general population[8].

In 1986, Komajda *et al*[28] reported that DCM patients admitted due to HF had higher alcohol consumption levels than patients admitted to undergo surgical procedures (101 mL/d *vs* 64 mL/d; RR = 7.6, $P < 0.001$).

Furthermore, Gillet published a similar study in which a cohort of 23 patients with DCM reported higher average daily alcohol consumption (82 g/d *vs* 30 g/d; $P < 0.001$) and a greater duration of that consumption (34 *vs* 22 years, $P < 0.001$) than a second group of 46 individuals suffering from other forms of heart disease[29]. Also, in 1998 McKenna described an incidence of excessive alcohol consumption of 40% in a group of 100 DCM patients compared to 23% found in a control group of 211 healthy subjects[8].

Furthermore, Fernández-Solá *et al*[30], when analysing a population of alcoholics, found a higher prevalence of DCM in alcoholics than among the general population. Specifically, among alcoholics they found a prevalence of DCM of 0.43% in women and 0.25% in men, whereas the described prevalence of DCM in the general population is 0.03% to 0.05%[18,19].

Experimental studies

Experimental studies analysing the depressive properties of alcohol on the cardiac muscle invariably use similar approaches[31-39]. Accordingly, a given amount of alcohol is administered to volunteers or alcoholics, followed by the measurement of a number of haemodynamic parameters and, in some cases, echocardiographic parameters. Generally, following alcohol intake, healthy, non-drinking individuals showed an increase in cardiac output due to a decline in peripheral arterial resistance and an increase in cardiac frequency[31]. However, during the time that these haemodynamic changes appeared, some researchers identified a possible decrease in the ejection fraction and other parameters related to systolic function[32-39]. This was questioned by other authors, who pointed out that these conclusions could not be drawn, as alcohol itself also induces changes in the pre-load and after-load conditions, which influence cardiac contractility[35]. However, in this context, experimental *in vitro* studies using cardiomyocytes have shown that alcohol depresses the contractile capacity of the myocardium, regardless of the sympathetic tone and the haemodynamic conditions[36].

The capacity of alcohol to depress cardiac contractility became evident in studies carried out with chronic alcoholics and in patients with left ventricular dysfunction. In these patients, alcohol, in spite of causing vasodilatation and an increase in the heart rate, did not produce an increase in heart output or, if it did, it was lower than in healthy non-drinking individuals[32,34]. Together, this suggests a depressed contractile capacity. This was specifically addressed by Regan, who found that, after an intake of 81 g of alcohol, the heartbeat volume of a group of chronic alcoholics was reduced and the end diastolic pressure increased, indicating that in these individuals there was a reduction in the left ventricular contractile reserve[32]. This impairment of contractile capacity among chronic alcoholics was demonstrated in the same study using an after-load test with angiotensin. Results showed that the end diastolic pressure increased to a greater extent in alcoholics and was associated with a lower beat volume than in non-drinkers[32].

Echocardiographic and haemodynamic studies in alcoholics

Myocardial impairment following chronic excessive alcohol intake has been evaluated using echocardiographic and haemodynamic measurements in a significant number of reports. In these studies, haemodynamic and echocardiographic parameters were measured in individuals starting an alcohol withdrawal program. The findings were analysed taking into account the amount and chronicity of intake and they were compared with the same parameters measured in a control group of non-drinkers.

The majority of the echocardiographic studies performed on asymptomatic alcoholics found only mild changes in their hearts with no clear impairment of the systolic function. For example, a slight increase in the pre-ejection period/left ventricular ejection time ratio (PEP/LVET) was found by some authors, suggesting a sub-clinical impairment of systolic function[21,33]. Mathews and Kino found a small, but significant increase in left ventricular mass in individuals consuming at least 12 oz of whisky during 6 years and 60 g of ethanol per day, respectively[22,40]. More recently, Lazarevic found a modest

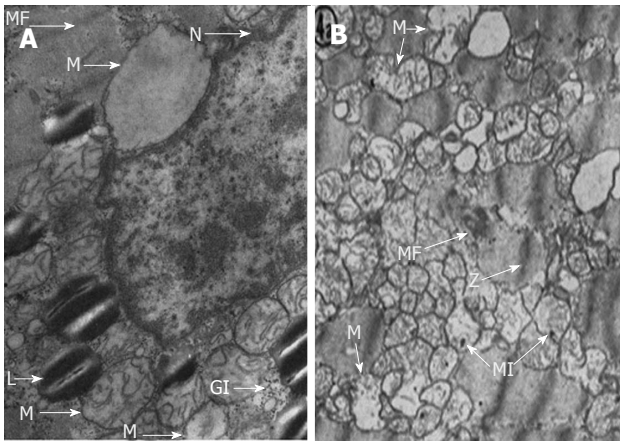

Figure 2 Cellular changes in alcoholic cardiomyopathy. L: Neutral lipids in the form of small cytoplasmic droplets; GI: Glycogen deposits; M: Mitochondria were swollen or oedema was present; N: Nucleus; MF: Myofibrils showed a progressively distorted structure (Z lines disrupted). Reproduced with permission from the American Heart Association[42].

increase in end-systolic and diastolic left ventricular volumes and a subsequent thickening of the posterior wall in a cohort of alcoholics consuming at least 80 g during 5 years[23]; however, no differences in systolic function were observed. Finally, only Urbano-Márquez *et al*[24] found a clear decrease in the ejection fraction, in a cohort of 52 alcoholics, which was directly proportional to the accumulated alcohol intake throughout the patients' lives.

Histological studies

Alterations caused by heavy alcohol intake have also been studied from the perspective of histopathology. Emmanuel Rubin analysed muscle biopsies from individuals who were previously non-drinkers and were submitted to a balanced diet with heavy alcohol intake during one month[41]. Although no significant changes were found using conventional microscopy, when electron microscopy was employed he discovered intracellular swelling, glycogen and lipid accumulation, and alterations in the structure of the sarcoplasmic reticulum and of the mitochondria (Figure 2). These changes, though subtle, were similar to those found by Ferrans and Hibbs in eight deceased individuals diagnosed with ACM[42,43]. On histological examination, various degrees of fibrosis, patchy areas of endocardial fibroelastosis, intramural blood clots and focal collections of swollen cells in both the epicardium and endocardium were found. Also, there were significant size variations in the myofibrils and they showed a relative decrease in the number of striations, in addition to swelling, vacuolisation and hyalinisation. Cell nuclei were larger than normal, morphologically difficult to define and they occasionally showed hyperpigmentation. The authors highlighted the presence of an extensive intracellular accumulation of neutral lipids, principally in the form of small cytoplasmic droplets. In a subsequent study using electron microscopy, the authors found histological features that could be superimposed onto those found in hearts that had suffered hypoxia, anoxia or ischemia[43]. Analogous to the sarcoplasmic reticulum, the mitochondria were swollen or oedema was present, with crest alterations and intra-mitochondrial inclusions suggesting degenerative processes (Figure 2). Moreover, myofibrils showed a progressively distorted structure, resulting in a homogeneous mass.

Despite these features, the structural changes do not seem to be specific, furthermore, they are not qualitatively different from those found in idiopathic DCM and they do not allow us to differentiate between the two conditions[44]. It also appears that the changes emerging in ACM patients only differ from idiopathic DCM in quantitative terms, with histological changes being more striking in idiopathic DCM than in ACM[44].

Basic studies on molecular mechanisms of myocardial damage

Basic research studies have described an abundance of mechanisms that could underscore the functional and structural alterations found in ACM. Because of this, their origin could be multifactorial and linked both to the alcohol molecule and to its main metabolite, acetaldehyde.

Coinciding with the histological studies mentioned above, the majority of research on molecular mechanisms describes dysfunctions of intracellular organelles prompting alterations in the lipid-energetic metabolism and in calcium homeostasis, which are especially relevant for the contractile activity of myofibrils.

In spite of numerous studies, the sequence of events that occur in alcohol-induced myocardial damage is still highly controversial. Although some authors contend that the initial event is the appearance of hypertrophy, the majority accept that the core event is the loss of cardiomyocytes.

The mechanisms described to date are shown in Figure 3 and they include: apoptosis[45,46], alterations of the excitation-contraction coupling in cardiac myocytes[47], structural and functional alterations of the mitochondria and sarcoplasmic reticulum[41-43], changes in cytosolic calcium flows[48], changes in calcium sensitivity of myofilaments[49,50], alterations of mitochondrial oxidation[37,38,46], deregulation of protein synthesis[51-53], decrease of contractile proteins and disproportion between the different types of myofibrils[54-56], changes in the regulation of myosin ATPase[51], up-regulation of the L-type calcium channels[57], increase of oxidative stress[58,59], induction of ANP and p21 mRNA expression in ventricular myocardium[45], and activation of the renin-angiotensin system and of the sympathetic nervous system[60-62]. Additionally, it has been proposed that mechanisms of a genetic nature play a determining role in the pathophysiology of this disease.

The suspicion that there may be an individual susceptibility to this disease is underscored by the finding that only a small group of alcoholics develop ACM, and that a proportional relationship between myocardial damage and alcohol intake has not been proven.

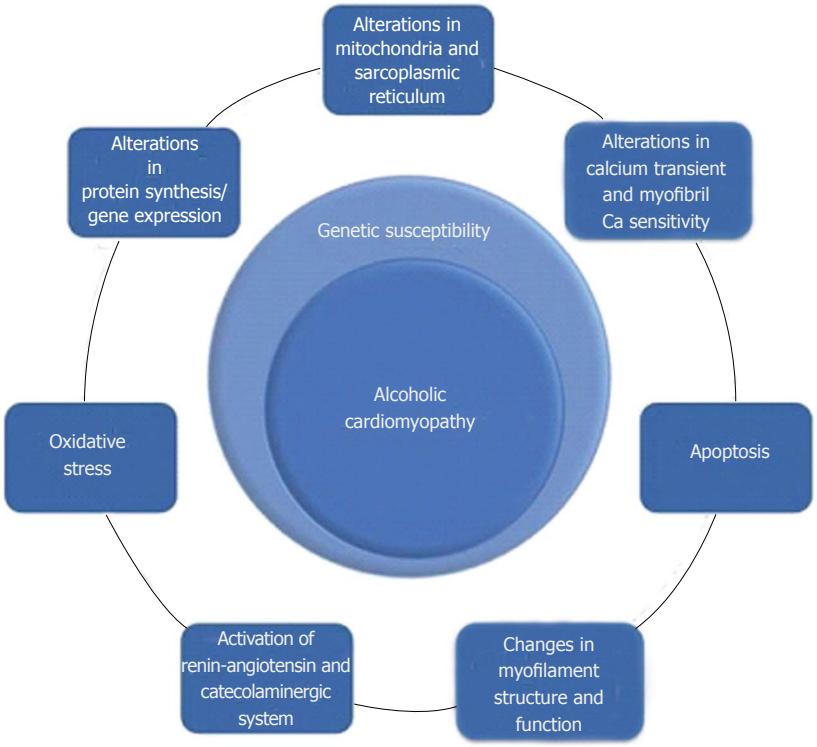

Figure 3 Alcoholic Cardiomyopathy. Pathophysiology.

Although some studies have detailed structural and functional damage in proportion to the amount of alcohol consumed during a patient's lifetime[24], a large majority of authors have discarded this theory[21-23,25]. Both the absence of a direct correlation and the theory of the existence of a threshold dose (above which some alcoholics develop ACM) require the presence of individual susceptibility to alcohol induced cardiac damage[63]. It is unknown whether individual susceptibility would be related to increased vulnerability at the myocardial level and/or to impaired alcohol metabolism.

One of the few papers analysing genetic susceptibility in ACM was published by Fernández-Solà *et al*[64] in 2002. He compared the prevalence of different polymorphisms of the angiotensin-converting enzyme gene in 30 ACM patients and in 27 alcoholics with normal ventricular function. The DD genotype was more frequent among ACM patients (56% *vs* 8%). Furthermore, 89% of the alcoholics with a DD genotype developed ACM, whereas only 13% of those with an II or ID genotype developed this condition. However, this individual susceptibility mediated by polymorphisms of the angiotensin-converting enzyme gene does not appear to be specific to ACM insofar as several diseases, including some that are not of a cardiologic origin, have been related to this genetic finding[65].

Regarding individual susceptibility based on alcohol metabolism, data are scarce, but provocative findings arose from a study published in 2002 which showed that the cardio-depressive power of alcohol in mice varied according to the activity of the enzymes involved in the metabolism of alcohol[66]. In this study, alcohol caused greater cardiomyocyte impairment in mice genetically modified with higher alcohol dehydrogenase activity. The mechanism by which cardiac damage occurred was not fully elucidated, but it was proposed that it was due to the accumulation of acetaldehyde. Furthermore, mice that received an aldehyde dehydrogenase inhibitor experienced an additional impairment in contractility[66]. Regrettably, the role of gene mutations in alcohol or aldehyde dehydrogenase and genetic polymorphisms including ADH1B (*) 2, ALDH2 (*) 2 in humans have not yet been studied.

Finally, it is worth stressing that a large majority of studies on the physiopathology and prognosis of ACM were conducted some years ago, prior to the development of our current understanding regarding the role of genetics in DCM[67]. According to recent data, a genetic form of DCM could be present in up to 50% of idiopathic DCM cases, and other specific forms of DCM such as peripartum cardiomyopathy have been shown to have a genetic basis in a significant number of cases[68]. It is therefore possible that patients with ACM could also harbour a genetic substrate that predisposes them to this form of cardiomyopathy.

Further research is required to determine the definitive role of genetics on ACM pathophysiology.

NATURAL HISTORY OF ALCOHOLIC CARDIOMYOPATHY

In spite of the high prevalence of excessive alcohol consumption and of its consideration as one of the main causes of DCM, only a small number of studies have analysed the long-term natural history of ACM. Unfortunately, all the available reports were completed at a time when a majority of the current heart failure therapies were not available (Table 1).

Furthermore, there are conflicting data among studies regarding the prognosis of the condition, with some

Table 1 Key studies on the long-term prognosis of alcoholic cardiomyopathy

Ref.	Definition alcohol intake/cardiopathy criteria	Number of patients	NYHA Ⅲ-Ⅳ	% Abstinent	Follow-up	Mortality or heart transplant
McDonald *et al*[69]	> 6 beers/d or 2 quarts of wine/wk or 1 fifth of whisky/wk (44 patients consumed > 6 yr) Cardiomegaly with HF signs or symptoms	48	N/A	N/A	N/A	40%
Demakis *et al*[70]	> 8 oz or 1 L wine or 2 L of beer (ca. 90 g) ≥ 5 yr < 50 years old, HF and cardiothoracic ratio > 0.5	57	100%	31%	40.5 mo	57% in persistent drinkers 24% in non-drinkers
Haissaguerre *et al*[9]	> 80 g/d; ≥ 5 yr LVEF< 55% or LVEF 55%-59% and LVEDV 115 mL/m^2	110	N/A	44%	38.8 mo	50% in persistent drinkers 6% in non-drinkers
Prazak *et al*[12]	> 80 g/d; ≥ 5 yr Heart failure and LVEF < 50%	23	52%	N/A	N/A	19% (10-yr survival)
Fauchier *et al*[11]	> 80 g/d; ≥ 5 yr DCM: WHO definition and hospitalization or arrhythmia	50	44%	45%	47 ± 40 mo	50% non-drinkers 70% in persistent drinkers
Gavazzi *et al*[10]	> 80 g/d; ≥ 5 yr or 100 mg/d 2 yr LVEF < 50 and HF or arrhythmia	79	35%	74%	59 ± 35 mo	Overall: 59% 55% in non-drinkers, 73% in persistent drinkers

HF: Heart failure; LVEF: Left ventricular ejection fraction; LVEDV: Left ventricular end-diastolic volume; DCM: Dilated cardiomyopathy; WHO: World Health Organization.

showing overall mortality near 60% and others showing a mortality rate of only 19% (Table 1).

The first paper to assess the natural history and long-term prognosis of ACM was published by McDonald *et al*[69] in 1971. He recruited 48 patients admitted to hospital with cardiomegaly without a clear aetiology and severe alcoholism. Patients were treated with diuretics, digitalis and vitamin B. During the follow-up, which varied significantly, 19 patients died (40%). The only factor to predict a poor outcome was the duration of symptoms before admission.

Demakis in 1974 recruited 57 patients with ACM[70]. The patients were drinkers of an amount of alcohol equivalent to > 90-100 g of alcohol per day for at least 5 years. During an average follow-up period of 40.5 mo, 24 deaths occurred among the 57 patients (42%). The adverse prognostic factors found in this study were lasting severe alcohol intake and the duration of HF symptoms.

In 1996, Prazak compared the evolution of a cohort of 42 individuals with idiopathic DCM and that of another group of 23 patients diagnosed with ACM who were seen between the years 1981 and 1992[12]. The populations were homogeneous and showed no clinical or haemodynamic differences at the beginning of the study. After 10 years of follow-up, the authors concluded that patients with ACM had better prognosis than patients with idiopathic DCM. Survival rates after 1, 5 and 10 years were 100%, 81% and 81%, respectively, in the ACM group, and 89%, 48% and 30% among those with idiopathic DCM. The predictive factors of poor prognosis that were identified were of a clinical nature: New York Heart Association (NYHA) functional class Ⅲ-Ⅳ, presence of hepatojugular reflux and congestion. The left ventricular volumes, ejection fraction and filling pressures were only predictors of prognosis among patients with idiopathic DCM.

The latest two papers to be published, unlike previous papers, reported worse outcomes for ACM patients compared to DCM patients. In the first of these studies, Fauchier *et al*[11] studied 50 patients with ACM and 84 patients with DCM between 1986 and 1997. Although up to 81% of ACM patients received an ACEI, none received beta-blockers and the use of spironolactone was not specified, although it was probably quite low. Also, current common cardiac therapies such as ICD and CRT devices were not used because of the period when the study was conducted. After a follow-up period of 47 mo, a significantly higher survival rate was observed among patients with DCM compared to patients with ACM. In this study, the only independent predictor of cardiac death was alcohol abstinence.

In the second study, Gavazzi led a multicentre study in which, from 1986 to 1995, 79 patients with ACM and 259 patients with DCM were recruited[10]. The average duration of follow-up was 59 ± 35 mo. Transplant-free survival after 7 years was worse among patients with ACM than among those with DCM (41% *vs* 53%). Among patients who continued drinking heavily, transplant-free survival was significantly worse than in non-drinkers (27% *vs* 45%). No other predictors were described.

Considering all the works conducted to date, it is clear that new studies on the natural history of ACM are needed, including patients treated with contemporary heart failure therapies. In light of the available data, new studies will help to clarify the current prognosis of ACM compared to DCM and to determine prognostic factors in ACM that might differ from known prognostic factors in DCM.

TREATMENT

To date, none of the ACM studies have proposed a treatment for ACM other than that recommended for DCM in current HF guidelines.

From the data provided in the available ACM studies, it appears that patients who received an ACEI globally showed improved prognosis. In contrast, beta-blockers, similar to aldosterone inhibitors, however beneficial they may be, have thus far not yielded sufficient data on their efficacy in relation to this disease.

Regarding ICD and CRT implantation, the same criteria as in DCM are used in ACM, although it is known that excessive alcohol intake is specifically linked to ventricular arrhythmia and sudden cardiac death[71]. As it is not uncommon in ACM for patients to experience a significant recovery of systolic function, it is particularly challenging in this disease to decide the most appropriate time to implant an ICD and whether it is necessary to replace a previously implanted device. Future studies in ACM should also address this topic, which has important economic consequences.

EFFECTS OF ALCOHOL WITHDRAWAL

Complete alcohol withdrawal is usually recommended to all patients with ACM. For tens of years, the literature has documented many clinical cases or small series of patients who have undergone a full recovery of ejection fraction and a good clinical evolution after a period of complete alcoholic abstinence. The need for complete withdrawal, however, is still disputed.

Demakis *et al*[70] in 1974 divided a cohort of 57 ACM patients according to the evolution of their symptoms during follow-up. The sub-group of patients in whom symptoms improved was made up of a larger proportion of non-drinkers (73%), compared to 25% in the group who did not improve, or 17% in the group whose condition worsened. However, a possible confusion factor was identified because the group with clinical improvement also exhibited a shorter evolution of the symptoms and the disease.

Guillo *et al*[17] in 1997 described the evolution of 9 ACM patients who had been admitted. He divided this cohort into two groups according to the evolution of the ejection fraction during 36 mo in which no deaths were recorded. The 6 subjects who experienced a clear improvement in their ejection fraction had fully refrained from drinking. Conversely, the 3 subjects recording a less satisfactory evolution had persisted in their consumption of alcohol. It should be noted that a moderate drinker included in this latter group showed an improvement of his ejection fraction.

The natural history and long-term prognosis studies of Gavazzi *et al*[10] and Fauchier *et al*[11] compared the evolution of ACM patients according to their degree of withdrawal. These authors found a relationship between the reduction or cessation of alcohol consumption and higher survival rates without a heart transplant.

Fauchier *et al*[11] found that after 47 mo of follow-up, the transplant-free survival of DCM patients was better than that of patients with ACM, but these differences were no longer significant when comparing the DCM group with the alcoholics who refrained from drinking or significantly reduced their alcohol consumption[11].

In the study by Gavazzi *et al*[10], ACM patients who continued drinking exhibited worse transplant-free survival rates after 7 years than those who stopped drinking alcohol (27% *vs* 45%)[10].

Ballester specifically analysed the effects of alcohol withdrawal on the myocardium using antimyosin antibodies labelled with Indium-111[72]. This radiotracer has been acknowledged as an indicator of irreversible myocardial damage. Of the 56 patients included in the study, 28 were former drinkers and 28 continued consuming alcohol during the study. Absorption levels of Indium-111 were high in 75% of patients who continued drinking and in only 32% of those who had withdrawn from consuming alcohol.

Of the 19 patients who were studied 9 ± 4 mo after withdrawal, the average absorption level decreased from an average HLR of 1.76 ± 0.17 to 1.55 ± 0.19 and this was associated with a significant improvement in the ejection fraction, from 30% ± 12% to 43% ± 16% ($P < 0.01$).

Data supporting the beneficial effect of continuing with alcohol intake at moderate levels in ACM patients arose from the observation that published studies evaluating the effect of alcohol abstinence included ACM patients who reduced their alcohol intake to low/moderate levels alongside ACM patients who stopped their alcohol intake altogether[9-12]. Also, low to moderate daily alcohol intake was proved to be a predictor of better prognosis for both ischemic cardiomyopathy and heart failure regardless of the presence of coronary disease[1,2].

Additionally, echocardiographic data suggest that subjects who do not fully withdraw from alcohol consumption, but who reduce it to moderate amounts recover LVEF in a similar manner to strict non-drinkers. Thus, Nicolás *et al*[73] studied the evolution of the ejection fraction in 55 patients with ACM according to their degree of withdrawal. The population was divided into 3 groups according to their intake volume during the follow-up period. At the end of the first year, no differences were found among the non-drinkers, who improved by 13.1%, and among those who reduced consumption to 20-60 g/d (with an average improvement of 12.2%). Conversely, those whose consumption remained in excess of 80 g/d showed an average decline of 3.8% in their ejection fraction.

Thus, although there is a certain degree of consensus regarding the recommendation of full alcohol withdrawal in ACM, it is yet to be resolved whether moderate alcohol consumption is sufficient to achieve an improvement in the prognosis of these patients.

Future studies with a strict classification of non-drinkers and drinkers will help clarify whether complete abstinence is mandatory for ACM patients. In the interim it seems appropriate to continue discouraging any alcohol consumption in these patients, as it would be difficult for them to maintain a limited alcohol intake considering their history of alcohol dependence and abuse.

LIMITATIONS OF ACM STUDIES

In all ACM studies, inclusion of patients is based on patients' self-reported alcohol drinking habits, which may lead to an underestimation of the prevalence of ACM together with problematic identification of patients who abstain and those who continue drinking. Although analytical markers of alcohol consumption, such as average erythrocyte volume and serum gamma-glutamyltranspeptidase levels, could be an aid to establish abstinence or persistence of alcohol intake in patients, the quantity of alcohol intake is dependent on the patients' report. Furthermore, in many of these reports, comorbid conditions, especially myocarditis and other addictions such as cocaine and nicotine, were not reported.

As pointed out before, the current accepted definition of ACM probably underestimates the number of women affected by the disease. Alcohol affects heart function and is dependent on the quantity of alcohol that the heart is exposed to. Women typically have a lower BMI than men, and therefore the same alcohol exposure can be achieved with lower alcohol intake.

CONCLUSION

ACM is an important clinical entity known since the 19th century. Epidemiological and experimental studies link excessive alcohol intake to the development of DCM. Although not based on solid experimental or epidemiological data, the currently accepted definition of ACM requires chronic exposure to > 80 g/d of alcohol for > 5 years. There is a surprising paucity of clinical data on ACM prognosis and particularly on ACM evolution under modern HF therapies. In the absence of robust data, current therapy of ACM should include complete alcohol abstinence along with all the therapies recommended to treat DCM. Further studies in the field of ACM are required.

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TOPIC HIGHLIGHT

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Interferon-γ and other inflammatory mediators in cardiomyocyte signaling during Chagas disease cardiomyopathy

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Abstract

Chagas disease cardiomyopathy (CCC), the main consequence of *Trypanosoma cruzi* (*T.cruzi*) infection, is an inflammatory cardiomyopathy that develops in up to 30% of infected individuals. The heart inflammation in CCC patients is characterized by a Th1 T cell-rich myocarditis with increased production of interferon (IFN)-γ, produced by the CCC myocardial infiltrate and detected at high levels in the periphery. IFN-γ has a central role in the cardiomyocyte signaling during both acute and chronic phases of *T.cruzi* infection. In this review, we have chosen to focus in its pleiotropic mode of action during CCC, which may ultimately be the strongest driver towards pathological remodeling and heart failure. We describe here the antiparasitic protective and pathogenic dual role of IFN-γ in Chagas disease.

Key words: Chagas disease; *Trypanosoma cruzi*; Interferon-gamma; Gene expression; Cardiomyopathy

Core tip: Chagas disease cardiomyopathy (CCC) occurs in 30% of those infected with the protozoan *Trypanosoma cruzi*, endemic in Latin America. It is an inflammatory cardiomyopathy with a worse prognosis than cardiomyopathies of other etiologies. Interferon (IFN)- γ is the main cytokine produced locally and induces strong signaling in cardiomyocytes. This review focuses on the pleiotropic protective and pathogenic effects of IFN-γ on CCC.

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INTRODUCTION

Chagas disease cardiomyopathy (CCC) is a particularly

aggressive inflammatory dilated cardiomyopathy that occurs decades after the initial infection with the obligate intracellular parasite *Trypanosoma cruzi* (*T.cruzi*) in 30% of infected individuals[1]. *T. cruzi* infection affects 10 million subjects in endemic areas of South and Central America and migratory waves have taken patients to the United States, Europe and Japan[2-4]. Patients with CCC have a worse clinical progression and survival than those with cardiomyopathy of other etiologies. The development of CCC is associated with inflammation and activation of the immune system, with a local increased cardiac production of cytokines by the heart-infiltrating T cells and other mononuclear cells[5]. These mononuclear cells infiltrating CCC heart tissue express predominantly interferon (IFN)-γ and tumor necrosis factor (TNF)-α, with lower levels of interleukin (IL)-2, IL-4, IL-6 and IL-10. Cytokines like IL-7 and IL-15, which promote T cell survival, are also found to have increased expression in CCC heart tissue[6,7]. Significant IFN-γ signaling was observed in the myocardium of CCC patients, including genes that are not ordinarily expressed by inflammatory cells[8]. A similar increase in IFN-γ and TNF-α expression is observed in cardiac tissue from animals infected with *T. cruzi*[9]. CCC patients have a progressive myocardial remodeling process with hypertrophy and fibrosis causing heart fiber damage, heart conduction abnormalities, arrhythmias, apical aneurysm, heart failure and sudden death[10,11]. Several hypotheses have been raised to explain the lesions in the myocardium of CCC, which includes persistence of the parasite or its antigens at the inflammatory site and autoimmune tissue damage[5,12]. There are two drugs available to treat the acute phase of the disease, nifurtimox (nitrofurane) and benznidazole (nitroimidazole). The use of these drugs to treat the acute phase of the disease is widely accepted. However, their use in the treatment of the chronic phase is controversial. There is no specific treatment, against the parasite, that can benefit patients at the chronic stage of Chagas disease[13]. The undesirable side effects of both drugs are a major drawback in their use, frequently forcing the physician to stop treatment. The treatment of chronic patients consists of control of the symptoms and improvement in quality of life, by preventing cardiovascular complications according to the guidelines for treating heart failure and arrhythmias[14]. Regardless of the mechanisms underlying the initiation and maintenance of the myocarditis, the bulk of the evidence indicates that the inflammatory infiltrate is a significant effector of heart tissue damage. Our group has demonstrated over the past several years that, aside from direct inflammatory damage, several cytokines and chemokines produced in the myocardium of CCC patients may also have a non-immunological pathogenic effect beyond direct inflammatory tissue damage, *via* modulation of gene and protein expression in cardiomyocytes and other myocardial cell types[5,7,15,16]. While IFN-γ acts as an immunological mediator during the acute stage of the disease suppressing overt parasitism, in the chronic phase of the disease it will both curtail parasitism and cause tissue damage through immunological and non-immunological effects entertaining the gradual progression to CCC.

IFN-γ IN HEALTH AND DISEASE

IFN-γ is a protein with 146 amino acid residues, the only member of the type Ⅱ IFN family, and in humans is encoded by a chromosomal locus separate from type Ⅰ IFNs, on chromosome 12q24.1 with approximately 5.4 kb and four exons[17]. IFN-γ is mainly produced by $CD4^+$ T helper cell type 1 (Th1) lymphocytes, $CD8^+$ cytotoxic lymphocytes, and natural killer (NK) cells, but can also be produced by other cells, such as B cells, NKT cells, and professional antigen-presenting cells (APCs). Cytokines secreted by APCs, most notably IL-12 and IL-18, control IFN-γ production and differentiation of cells capable of producing the cytokine. Interaction of macrophages and other APCs with pathogen-associated molecular patterns (PAMPs) induces secretion of IL-12 and chemokines. These chemokines attract inflammatory cells to the site of inflammation, and IL-12 promotes IFN-γ synthesis in these cells[18]. Negative regulators of IFN-γ production include IL-4, IL-10, transforming growth factor (TGF)-β, and glucocorticoids[19]. Animal models as well the analysis of different human diseases are good examples of the paradoxical roles of IFN-γ. Mice lacking IFN-γ and its receptor (IFNGR) showed no developmental defects, and their immune system appeared to develop normally[20]. However, these mice show deficiencies in natural resistance to infection. In humans, inactivating mutations of the human IFNGR1 or IFNGR2 chains show clinical presentation similar to the mouse models. At the same time IFN-γ can be beneficial in infectious diseases where it strengthens cellular defense mechanisms and favors the generation of specific immunity, and can be disease-promoting as described in non-infectious diseases. Reifenberg *et al*[21] have shown that SAP-IFN-γ transgenic mice, which constitutively express IFN-γ in their livers, developed chronic active myocarditis. These mice exhibited IFN-γ-mediated cardiotoxicity with left ventricular dilation and impaired systolic function, a true cardiomyopathy[21]. Morino *et al*[22] have reported a case of cardiomyopathy in a renal cell carcinoma patient treated with IFN-γ. In humans, IFN-γ is also implicated in the pathology of diseases such as systemic lupus erythematous[23], insulin-dependent diabetes mellitus[24] and multiple sclerosis[25]. Like other cytokines, the IFN-γ coding region is invariant with no reported polymorphisms. However, single nucleotide polymorphisms (SNPs) in intronic regions have been described and a microsatellite polymorphism consisting of a dinucleotide (CA) repeat in the first intron is the one most extensively studied as it is correlated with high IFN-γ production[26]. An association between IFN-γ SNPs and diseases like rheumatoid arthritis has been reported[27,28]. Nevertheless, as a cytokine with ambiguous effects, IFN-γ polymorphisms are correlated with increased longevity[25]. It has been proposed that a slightly dampened inflammatory status caused by an IFN-γ polymorphism, while not enough to significantly impact on

the individual's ability to clear infection, may prevent or defer inflammation-related diseases such as cardiovascular disease, neurodegeneration, osteoarthritis, osteoporosis, and diabetes[29]. In experimental *T. cruzi* infection, it has been shown by several investigators that IFN-γ can enhance macrophage killing of the parasite *in vitro* and increase resistance to an infectious challenge *in vivo,* an effect dependent on the *de novo* synthesis of TNF-α and NO by infected macrophages[9,30,31]. It has also been demonstrated that parasite-induced IFN-γ produced during *T. cruzi* infection by T and NK cells is involved in resistance to infection and protection in mice. This protection seems to be dependent on the IFN-γ/TNF-α pathway[31].

IFN-γ INDUCED SIGNALING IN CARDIOMYOCYTES INFECTED WITH *T.CRUZI*

Although infective *T. cruzi* trypomastigotes are capable of invading a wide variety of tissues and cell types in the vertebrate host, the majority of *T.cruzi* laboratory strains and isolates have tropism for cardiac tissue and or cardiomyocytes[32]. The establishment of a long-term infection in the heart and the development of a cardiomyopathy condition are directly related to the ability of *T. cruzi* to infect and persist within cardiomyocytes during the acute phase of infection[33,34]. Cardiomyocytes are differentiated cells that respond to *T.cruzi* infection by initiating adaptive strategies. These strategies can involve immunological and non-immunological events. For example, during *T.cruzi* infection cardiomyocytes reactivate an embryonic gene expression pattern[8] (*e.g.*, an increase in expression of atrial natriuretic factor), inhibit apoptosis[34], increase cell size by producing myofibrils (cardiac myosin heavy chain, several α-actin isoforms, smooth muscle myosin, actin-binding proteins, and collagen) and initiate a hypertrophic program, that are not related to an immunological response to the parasite[7]. However, these cells are actively integrated in the inflammatory process and can secrete chemokines such as C-C chemokine monocyte chemotactic protein 1 (*JE*/MCP-1/CCL2), chemokine (C-C motif) ligand 5 (RANTES/CCL5), keratinocyte chemoattractant (KC/CXCL3), macrophage inflammatory protein (MIP-2/CXCL2), Mig/CXCL9, and *cytokine*-responsive gene-2 (Crg-2/CXCL10), and the cytokines TNF-α, IL-1β and inducible NO synthase (iNOS)[35]. These chemokines will drive the early influx of leukocytes, and influence T-helper cell recruitment and local IFN-γ production defining the inflammatory infiltrate in the hearts during experimental *T.cruzi* infection and, presumably, also in acutely infected patients. It was recently demonstrated that there is a segregation of $CD8^+$ cell populations in the heart in *T.cruzi* infected mice into two groups: $CD8^+$ T cells producing perforin and no IFN-γ ($IFN\text{-}\gamma^{neg}\ Pfn^{+)}$ and perforin-negative and IFN-γ-producing cells ($IFN\text{-}\gamma^+\ Pfn^{neg}$). These data supported the idea that $CD8^+\ Pfn^+$ T-cells are involved in cardiomyocyte injury during *T. cruzi* infection, whereas $CD8^+$ $IFN\text{-}\gamma^+$ cells play a beneficial role in cardiomyocyte damage[36].

IFN-γ A DUAL ROLE IN CHAGAS DISEASE

A dual role in pathogenesis and protection during Chagas disease was described for IFN-γ and other cytokines, such as TNF-α[37]. Bahia-Oliveira *et al*[38], taking into account only the inflammatory actions of the cytokine, also described the dual role of IFN-γ during chronic Chagas disease. Our observations from the standpoint of the pleiotropic biological effects, both inflammatory and non-inflammatory, in Chagas disease made us remodel the concept as will follow in these review. During *T. cruzi* infection, once the inflammatory process starts, IFN-γ will be produced by Th1 cells and act as a prime inflammatory cytokine in different pathways of the immune system, such as upregulating MHC class I and class Ⅱ molecules, suppressing Th2 immune responses by antagonism of IL-4 production, inducing high levels of antigen presentation and activating macrophages[18]. Our group has demonstrated the importance of IFN-γ, TNF-α and several chemokines in CCC by showing that they play a role in the generation of the inflammatory infiltrate[8,15,39]. CCC patients have an increased peripheral production of IFN-γ and TNF-α when compared to patients with the asymptomatic/indeterminate form. On the other hand, IFN-γ has direct effects on cardiomyocytes and perhaps other cells of the myocardium[8]. In the following sections we describe in detail the dual mechanism of IFN-γ during Chagas disease (acute and chronic phases) as illustrated in Figure 1.

IFN-γ ACTS AS AN IMMUNOLOGICAL MEDIATOR INDUCING PROTECTION DURING THE ACUTE PHASE AND ALLOWING CONTROL OF CHRONIC PARASITISM

Data from animal models and from the earliest stages in a proportion of naturally infected individuals has shown that inflammatory cytokines such as IFN-γ play a central role in acute *T. cruzi* infection. During invasion, *T. cruzi* or its derived molecules like DNA and glycosylphosphatidylinositol-anchored mucin-like glycoproteins derived from trypomastigotes forms (tGPI-mucins) can stimulate the host cutaneous cells, macrophages, cardiomyocytes and dendritic cells (as seen in *in vivo* and *in vitro* infection) to produce mediators that will trigger a local inflammatory response[40]. This activation will induce these cells to promptly release pro-inflammatory cytokines such as IL-1, IL-6, IL-12, IL-18, IL-27 and TNF-α and further activate other inflammatory cells. These cytokines will participate in the control of the infection, killing the parasite with the help of NO production *via* iNOS/NOS2. *T. cruzi*-specific T cells will produce IFN-γ, which in conjunction with macrophages producing TNF-α will migrate with other blood leukocytes to the site of inflam-

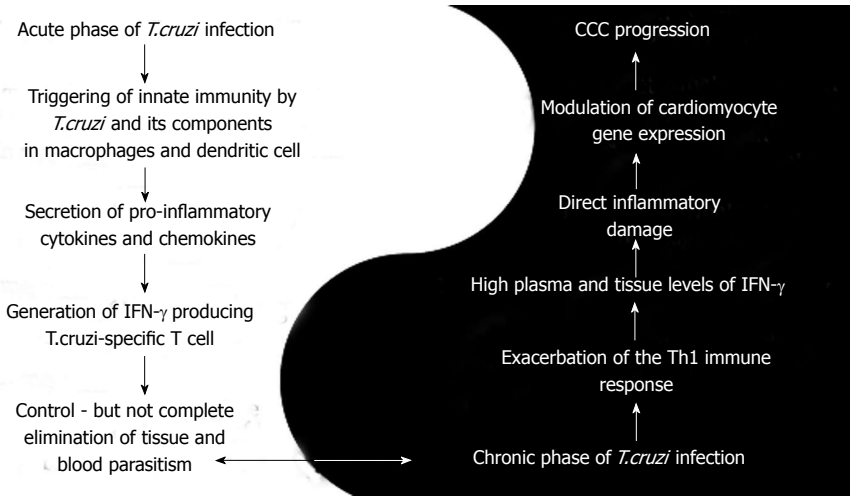

Figure 1 Interferon-γ a dual role in Chagas disease (acute and chronic phases). CCC: Cardiomyopathy; INF: Interferon.

mation in response to chemokines such as CCL2, CCL3, CCL4, CCL5, CXCL10 and CCR5[41]. The blockade of one, CCR5, by Met-RANTES significantly decreased the intensity of cardiac inflammatory infiltrate, suggesting that lymphocyte migration to the myocardium during acute infection is dependent on CCR5 ligands[42,43]. IFN-γ-inducible adhesion molecules, such as fibronectin and VCAM-1, can also be detected at high levels in cardiac tissue from *T. cruzi*-infected mice[44]. Few studies have investigated the immunology of the acute phase infection in human patients. It has been described that acutely infected children display increased expression of inflammatory cytokines, such as circulating IL-6 and TNF-α[45] and increased production of IFN-γ by mononuclear cells[46]. Serum C-reactive protein (CRP) and IL-6 concentrations have also been shown to increase in children infected with *T. cruzi* during the acute phase, but not in the chronic phase of Chagas disease[47].

IFN-γ INDUCES DISEASE PROGRESSION DURING THE CHRONIC PHASE ACTING AS A NON-IMMUNOLOGICAL MEDIATOR OF TISSUE DAMAGE

During the chronic phase of *T. cruzi* infection, CCC patients have an exacerbation of the Th1 immune response compared with those with the indeterminate form of Chagas disease. It was observed that CCC patients displayed greater cytokine production (Table 1) by mononuclear cells, higher plasma levels of TNF-α and IFN-γ and an increased number of IFN-γ-producing $CCR5^+CXCR3^+CD4^+$ and $CD8^+$ T cells, with reduced numbers of IL-10-producing and $FoxP3^+$ regulatory T cells[15,48-50]. It has been hypothesized that this increased production of IL-10 by regulatory T cells restricts Th1 T cell differentiation and IFN-γ production in the majority of chronically *T. cruzi*-infected individuals, leading to the asymptomatic form of the disease[15,48-50]. Aside from the delayed hypersensitivity type of tissue damage classically seen in tissue lesions induced by IFN-γ, with cardiomyocyte loss and fibrosis, the local production of IFN-γ in CCC heart lesions can induce profound changes in the cardiomyocyte gene expression pattern as observed by our group using cDNA microarrays. Significant IFN-γ signaling was observed in the myocardium of CCC patients, including genes that are not ordinarily expressed by inflammatory cells. We have observed that 15% of the genes selectively upregulated in CCC are IFN-γ-inducible genes, including inflammatory response genes expressed by the infiltrating inflammatory cells (*e.g.*, cytokine receptors, immunoglobulin, T cell receptor genes). Several IFN-γ modulated genes are not expressed by inflammatory cells, including angiotensin Ⅱ receptor 2, fatty acid-binding protein 5, cardiovascular 27-kd Hsp and genes encoding a number of proteins involved in oxidative phosphorylation and lipid catabolism in the CCC myocardium, compared with idiopathic dilated cardiomyopathy or donor myocardium[8]. cDNA microarray experiments in mice infected with *T. cruzi* showed changes in oxidative phosphorylation and depressed energy metabolism[51] and respiratory chain complexes with a reduced ATP-generating capacity[52]. Moreover, expression profiling in hearts of mice infected by *T. cruzi* also showed diminished myocardial energy metabolism and altered oxidative phosphorylation[51,53]. Significantly, mice infected for 100 d showed morphological alterations in the mitochondria and diminished expression of genes from the oxidative phosphorylation pathway, with a detectable reduction in OXPHOS-mediated mitochondrial ATP production[51]. Thus, both IFN-γ and *T. cruzi* infection can depress energy metabolism to reduce myocardial ATP generation, which has potential consequences for myocardial contractility, electric conduction and rhythm. Interestingly, one of the genes downregulated in CCC hearts, SERCA Ca^{2+}-ATPase is repressible by IFN-γ and is also involved in cardiac metabolism. *In vitro* experiments have shown that IFN-γ may induce profound changes in the cardiomyocyte gene expression program, including induction of atrial natriuretic factor and of the hypertrophic gene expression program, which can ultimately lead to heart dilation and heart failure[8]. Other inflammatory mediators and chemokines such as IL-18 and CCR7 ligands, up-

Table 1 Cytokine and chemokine expression in Chagas disease and animal models[80]

Cytokines/ chemokines	Phase (acute /chronic/IND /severe/ moderate CCC)	Host (mouse/ human)	Organ/cell type	Ref.
IFN-γ	Severe CCC	Human	Mononuclear cells	[15,49]
IFN-γ	Severe CCC	Human	Myocardium	[63,64]
IFN-γ	Severe CCC	Human	Heart-infiltrating T cells	[15]
IFN-γ	IND, Severe CCC	Human	Plasma	[15,65,66]
TNF-α	Severe CCC	Human	Mononuclear cells	[15,49]
TNF-α	Severe CCC	Human	Heart-infiltrating T cells	[15]
TNF-α	Severe CCC	Human	Myocardium	[63,64]
TNF-α	IND and Severe CCC	Human	plasma	[15,65,66]
IFN-γ	Acute/ chronic	Mouse	Heart	[67-69]
TNF-α	Acute/ chronic	Mouse	Heart	[70]
IL-6	Severe CCC	Human	Heart-infiltrating T cells	[15,63,64]
IL-2	Severe CCC	Human	Heart-infiltrating T cells	[15,63,64]
IL-4	Severe CCC	Human	Heart-infiltrating T cells	[15,63,64]
IL-10	Severe CCC	Human	Heart-infiltrating T cells	[15, 63, 64]
IL-7	Severe CCC	Human	Myocardium	[71]
IL-15	Severe CCC	Human	Myocardium	[71]
IL-12	Acute	Mouse	Mononuclear cells	[72]
IL-18	Acute	Mouse	Mononuclear cells	[73]
IL-10	Acute	Mouse	Mononuclear cells	[74-76]
TGF-β	Acute	Mouse	Mononuclear cells	[74-76]
IL-17	Chronic	Mouse	Mononuclear cells	[77]
CCL2, CXCL10, CXCL9 (mRNA)	Severe CCC	Human	Myocardium	[8]
CCR2, CXCR3 (mRNA)	Severe CCC	Human	Myocardium	[8]
CCR5, CXCR3	Severe CCC, IND	Human	Mononuclear cells	[48]
CCL5, CXCL9, CXCL10	Chronic	Mouse	Cardiomyocytes	[35]
CCR5	Chronic	Mouse	Heart	[43,78]
CCL5, CCL4, CXCR3 (mRNA)	Chronic	Dog	Heart	[79]

CCC: Cardiomyopathy; INF: Interferon; IL: Interleukin; TNF: Tumor necrosis factor.

regulated in the CCC myocardium[39], induce cardiomyocyte hypertrophy and molecules involved in the fibrotic process[54-56]. Transgenic mice overexpressing CCL2, TNF-α or IFN-γ in the myocardium develop myocardial hypertrophy and ventricular dilation[21,57,58]. Inflammatory cytokines may also affect myocardial energy metabolism, and ventricular dysfunction is associated with reduced energy metabolism[59,60]. Treatment of cardiomyocytes with IFN-γ inhibited oxidative metabolism and ATP production[61] and reduced gene and protein expression of creatine kinase, which is responsible for translocation of mitochondrial ATP to the sarcoplasm in cultured human skeletal muscle cells[62]. We observed that the myocardium of CCC patients displays reduced expression of some key energy metabolism enzymes, including isoforms of creatine kinases, Krebs cycle enzymes, and members of the ATP synthase complex, in comparison with the myocardium of patients with non-inflammatory cardiomyopathies and heart donors (unpublished observations), which could be partly due to IFN-γ inflammatory cytokine signaling. cDNA Microarray experiments in mice experimentally infected with *T. cruzi* showed changes in oxidative phosphorylation and depressed energy metabolism[51], and respiratory chain complexes with a reduced ATP-generating capacity[52]. Thus, both IFN-γ and *T. cruzi* infection can depress energy metabolism, reducing myocardial ATP generation, with potential consequences for myocardial contractility, electrical conduction and rhythm. Taken together, data show that, apart from the direct inflammatory damage, the non-immunological effects of IFN-γ in the myocardium may play a significant pathogenic role in CCC, resulting in disease progression observed by a high degree of heart failure-inducing hypertrophy and fibrosis. The in-depth understanding of these pathways may lead to the development of new therapies for CCC.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (4): Congestive heart failure

Innate immune receptors in heart failure: Side effect or potential therapeutic target?

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Abstract

Heart failure (HF) is a leading cause of mortality and morbidity in western countries and occasions major expenses for public health systems. Although optimal medical treatment is widely available according to current guidelines, the prognosis of patients with HF is still poor. Despite the etiology of the disease, increased systemic or cardiac activation of the innate immune system is well documented in several types of HF. In some cases there is evidence of an association between innate immune activation and clinical outcome of patients with this disease. However, the few large trials conducted with the use of anti-inflammatory medication in HF have not revealed its benefits. Thus, greater understanding of the relationship between alteration in the immune system and development and progression of HF is urgently necessary: prior to designing therapeutic interventions that target pathological inflammatory processes in preventing harmful cardiac effects of immune modulatory therapy. In this regard, relatively recently discovered receptors of the innate immune system, *i.e.*, namely toll-like receptors (TLRs) and nod-like receptors (NLRs)-are the focus of intense cardiovascular research. These receptors are main up-stream regulators of cytokine activation. This review will focus on current knowledge of the role of TLRs and NLRs, as well as on downstream cytokine activation, and will discuss potential therapeutic implications.

Key words: Heart failure; Innate immune system; Toll-like receptors; Inflammation

Core tip: Heart failure (HF) is a leading cause of morbidity and mortality despite of current medical and interventional treatment. Activation of the innate immune system leading to or contribute to advanced HF is focus of intense and growing research. This review will focus on the role of innate immune receptors in HF. We will discuss the current knowledge about the correlation of innate immune activation and the clinical course in HF. In addition, we will comment on potential therapeutic implications of modulating the immune system in this syndrome.

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INTRODUCTION

Heart failure (HF) is a one of the leading cause of mortality and morbidity. In developed countries, 1% to 2% of the adult population suffers from this syndrome[1]. In

patients ⩾ 70 years of age, the prevalence increases to more than 10%[2]. Although approximately 50% of HF patients have preserved left ventricular (LV) ejection fraction[1], this review will focus on systolic HF, owing to the lack of data on the influence of the immune system on HF with preserved LV ejection fraction.

The etiology of HF is manifold. Systolic HF arises in more than 60% of cases from coronary artery disease (CAD). Among others, dilated cardiomyopathy, myocarditis, alcohol abuse, and chemotherapy are relevant and often reasons for HF. Current treatment of systolic HF has been documented in a large number of randomized, controlled clinical trials[1]. These studies clearly demonstrate the benefits of drugs such as β-blockers, angiotensin, converting enzyme inhibitors, angiotensin receptor antagonists, mineralocorticoid receptor blockers, and new drugs such as ivabradine. These agents reduce mortality and/or improve clinical symptoms of chronic systolic HF by suppression of the renin-angiotensin, aldosterone system, neurohumoral activation and ion channels. In addition to medical treatment, mechanical interventions such as resynchronization therapy have also proven beneficial in selected patients[3]. However, despite current optimal HF treatment, the prognosis of these patients is still poor and is comparable to neoplastic diseases. This underscores the need for additional therapeutic options. Many different pathophysiological and therapeutic concepts are at the focus of intense current research. Despite various etiologies, there is a growing body of evidence in this context from more than two decades of research for innate immune activation-systemic and/or local-in a significant number of patients and in experimental studies[4]. The innate immune system represents the first line of host defense against pathogens. This system is composed of diverse cellular components including granulocytes (basophils, eosinophils and neutrophils), mast cells, monocytes/macrophages, dendritic cells, and natural killer cells[5]. These cells respond to noxious stimuli and conditions, including infections and tissue injuries that can trigger inflammatory responses[6]. Pro-inflammatory cytokines, which can be excessively produced by immune cells, have been identified over the last decades as "downstream effectors" of the innate immune system[7]. Moreover, several clinical studies that apply pharmacological cytokine inhibition have been carried out for various diseases[4]. However, in HF, suppression of the cytokine tumor necrosis factor (TNF) alpha has failed to show a benefit in patients[8]. One reason for this failure may be a general underestimation of the complexity of the innate immune system. The regulation of cytokines is indeed not well understood[7]. In this regard, the discovery of so-called pattern recognition receptors has substantially enlarged understanding of the innate immune system. Two families of receptors, *i.e.*, toll-like receptors (TLRs) and nod-like receptors (NLRs)-have been relatively recently discovered; they regulate the innate immune response[7,9]. This review will discuss the pathophysiology of TLRs and NLRs and their role as therapeutic targets in systolic HF.

TLRS AND NLRS

TLRs

The family of TLRs represents the best known receptor proteins in the innate immune system. The initially discovered TLR4 has by now been known and researched for nearly two decades[10]. Extensive research has led to discovery of ten functional TLRs in humans, and has enabled detailed decoding of the TLR pathway[11]. Still, the role of TLRs in autoimmune diseases has not yet been fully understood. All TLR share a cytoplasmic Toll/IL-R homology (TIR) domain[12]. They reside in different compartments of the cell, with TLR1, 2 and 4-6 on the plasma membrane and TLR3 and 7-10 on intracellular endosomes and lysosomes. In general, cell surface TLRs recognize microbial membrane lipids, and intracellular TLRs respond to microbial nucleic acids[13]. Furthermore, TLR2 recognizes peptidoglycans, TLR3 dsRNA, TLR4 LPS, TLR7 ssRNA, and TLR9 unmethylated bacterial CpG DNA[14]. Beneath their role in immune reaction against pathogens, TLRs can also respond to damage-associated molecular pattern molecules (DAMPs). DAMPs include cell, derived particles such as heat shock proteins (HSP) and high mobility group box (HMGB), particles from the extracellular matrix such as fibronectin, and other substances like oxidized low density lipoprotein and free fatty acids[15]. HSP60 has been shown to activate TLR2 and TLR4 in macrophages[16]. HSP70 also poses an endogenous stimulus to TLR, which leads to release of nitric oxide and tumor necrosis factor[17]. In dendritic cells, TLR2 is activated by hyaluronic acid derived from the extracellular matrix[18]. Upon activation, all TLRs except TLR3 engage the MyD88 pathway. Activated MyD88 forms a complex with IL-1R-associated protein kinases (IRAK4, IRAK1 and IRAK2) (schematic overview see Figure 1). Phosphorylation of IRAK1 leads to activation of tumor necrosis receptor-associated factor (TRAF) 6, which together with IRAK1 forms a new complex. Transforming growth factor-activated kinase (TAK)1, TAK1-binding proteins (TAB)1, TAB2, and TAB3 are recruited to this complex. Upon activation of TAK1 by ubiquitylated TRAF6 IKK-α, IKK-β, and NF-κB essential modulator (NEMO) form a complex, which degrades IκB. This leads to translocation of NF-κB to the nucleus[19]. NF-κB regulates transcription of proinflammatory genes, upregulation cell-adhesion molecules and chemokines, and increasing nitric oxide (NO)[14]. The MyD88-independent pathway is addressed by TLR3 and by TLR4 as an alternative pathway. TLR4 uses the adaptor protein TRIF-related adaptor molecule (TRAM) to activate TIR-domain, containing adapter-inducing interferon-β (TRIF). TRIF can either activate TRAF6-subsequently leading to NF-κB translocation, or can recruit TRAF3, TBK1, and IKKε. This complex phosphorylates interferon regulatory factor (IRF) 3, which induces its translocation to the nucleus and expression of type I interferone genes[19]. Several mechanisms aid in the function of TLR signaling. sCD14 has been known

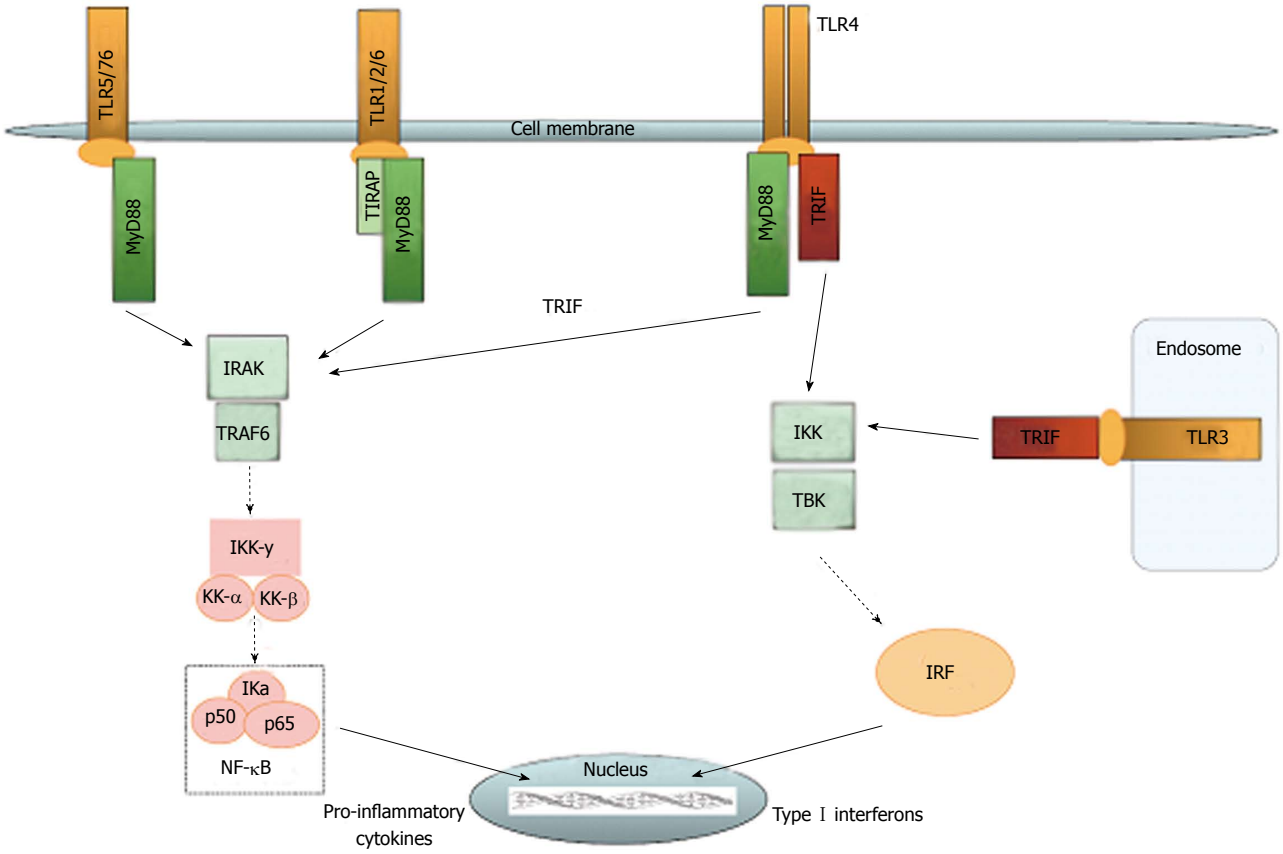

Figure 1 Toll-like receptor signaling. This figure summarizes schematically the complex signalling cascades of the Toll-like receptors. IRF: Interferon regulatory factor; IRAK: Interleukin-1 receptor-associated kinase; TRIF: TIR domain-containing adaptor inducing IFN-β; TLR: Toll-like receptor; IKK: IkB kinase; NF-κB: Nuclear factor κB; TBK: TANK binding kinase.

to chaperone lipopolysaccharide (LPS) from LPS binding protein to the TLR4/MD2 complex and thus support induction of TNF-α and interleukin-6 (IL-6) production. Recent research has shown that sCD14 is also capable of promoting internalization of TLR4 and activation of the TRIF-dependent pathway[20]. MHC class Ⅱ molecules also have the potential of addressing the TLR pathway in a rather unclassical manner. Together with CD40, MHC class Ⅱ can activate tyrosine kinase Btk, which leads to activation of both the MyD88-and the TRIF-pathways[21]. Another example for support of the proinflammatory TLR pathway is miR-155. This micro RNA interacts with Src homology 2 domain-containing inositol 5-phosphatase-1 (SHIP-1) and thereby restrains it from its control function[22]. A potent system such as the TLR proinflammatory pathway requires not only triggering but, perhaps more importantly, control. Recent years have seen establishment of possible control mechanisms for TLR signaling. SHIP-1 is upregulated after LPS stimulation, owing to increased production of transforming growth factor (TGF)-β, and inhibits PI-3 kinase, which consequently blocks TLR-MyD88 and MyD88 independent pathway[23]. IRAK-M functions as a decoy and prevents IRAK-1 from dissociating MyD88. It suppresses TLR-mediated inflammatory response. IRAK-M knock-out mice demonstrate an increase in inflammatory response and IL-1/TLR-signaling[24]. IRAK-M can also interfere with TLR2, although this downregulation is evidently IRAK-1 independent[25]. Other specific inhibitors are SHP2-which has been shown to inhibit only the TLR3 pathway-and sterile-α and armadillo motif-containing protein (SARM), which blocks only the TRIF-pathway without inhibiting MyD88 signaling[26,27]. An alternative splice variant of MyD88 is expressed after LPS stimulation. This variant, MyD88s, inhibits phosphorylation of IRAK1 by IRAK4, and leads to a suppression of the TLR pathway[28]. While microRNA is involved in the promotion of TLR signaling, it also plays an important role in anti-inflammation. miR-146- and miR-21-levels increase after LPS stimulation. miR-146 interacts with TRAF6 and IRAK1, which leads to decreased mRNA levels of both - whereas miR-21 inhibits PDCD4, which is an inhibitor of IL-10[29]. IKKβ, involved in the TLR-pathway, also has anti-inflammatory capacity by virtue of regulating the activation of the prosurvival kinase Akt1[30]. MHC class I also has a rather untypical function. It can be phosphorylated after TLR activation and can then activate Fps tyrosine kinase, which interferes with TLR signaling[31]. While evidence suggests a possible pro-inflammatory role of MHC class Ⅱ, MHC class I evidently supports anti-inflammatory effects.

NLRs

The nucleotide-binding and oligomerization domain

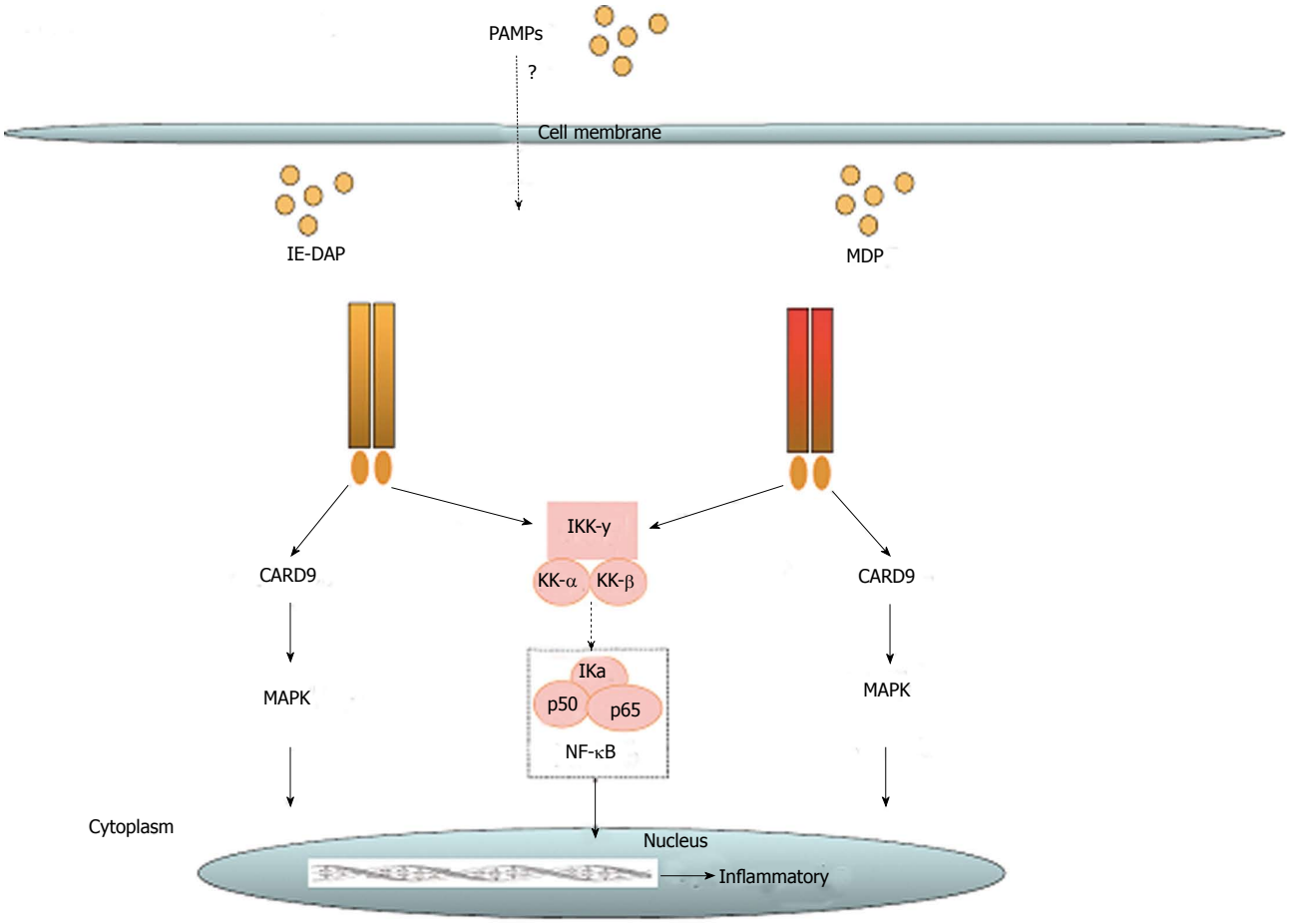

Figure 2 Nod 1 and nod 2 signalling. This figure summarizes schematically the signalling of the nod-like receptors nod 1 and nod 2. PAMP: Pathogen-associated molecular particles; IKK: IkB kinase; MAPK: Mitogen activated kinase; NF-κB: Nuclear factor κB; CARD: Caspase recruitment domain.

NLR family is defined by a common nucleotide binding domain and leucine-rich repeat series. All NLRs have a central ATPase region, which is called the NACHT domain[32]. Until now 22 NLRs have been identified in humans. They differ from each other by heterogeneous N-terminal effector domains and can be divided into four subgroups. Class Ⅱ transactivator (CIITA) is defined by an acidic transactivation domain (AD), and neuronal apoptosis inhibitor proteins (NAIPs) contain a baculovirus inhibitor of apoptosis protein repeat (BIR). The caspase recruitment domain (CARD) is common for NLRCs, including nod 1 and nod 2 (schematic overview see Figure 2). Finally, NLRPs share a pyridine domain (PYD). NLRs are important pattern recognition receptors in the intracellular compartment. Their activation leads to activation of innate immunity through nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), and interferon regulatory factors (IRFs)[33]. NLRPs contribute to innate immunity by formation of the inflammasome. This complex is formed by NLRP1, NLRP2, NLRP3, and NLRC4 upon recognition of physical damage to the plasma membrane or certain pathogen-associated molecular particles (PAMPs). Inflammasome formation can directly lead to caspase 1 activation, or it can recruit the adaptor protein ASC (apoptosis-associated speck-like protein containing a casapase recruitment domain) to activate caspase 1[34]. Serving another means of controlling infection, nod 1 and nod 2 can induce autophagy through activation of LC3-positive speckles[35]. In dendritic cells, nod 2-induced autophagy apparently plays a key role for bacterial elimination and antigen presentation[36]. The role of NLR has been implied for some diseases. In patients with early-onset Crohn's disease, a frameshift mutation of the nod 2 gene has been found, while in early-onset sarcoidosis a gain of function mutation of the NACHT domain has been identified[37]. There also is an indication for activation of NLRP3 in microglia in Alzheimer's disease by peptide amyloid-β[38].

Role of TLRs and NLRs in HF

The role of the innate immune system in HF has been controversially discussed. Inflammation plays an important role in most cardiac diseases, and receptor-mediated innate immunity is primarily investigated with respect to TLRs. The role of innate immune cells and NLRs is also subject to current research. All known human TLRs have been found in the heart. However, until yet, the best-characterized TLR in cardiovascular diseases is TLR4 (Figure 3). Their expression level, however, varies greatly. Expression of TLR4, TLR3, and TLR2 is at least 10 times higher than that of any other TLR in the heart[39]. Although TLRs were first known for their role in innate

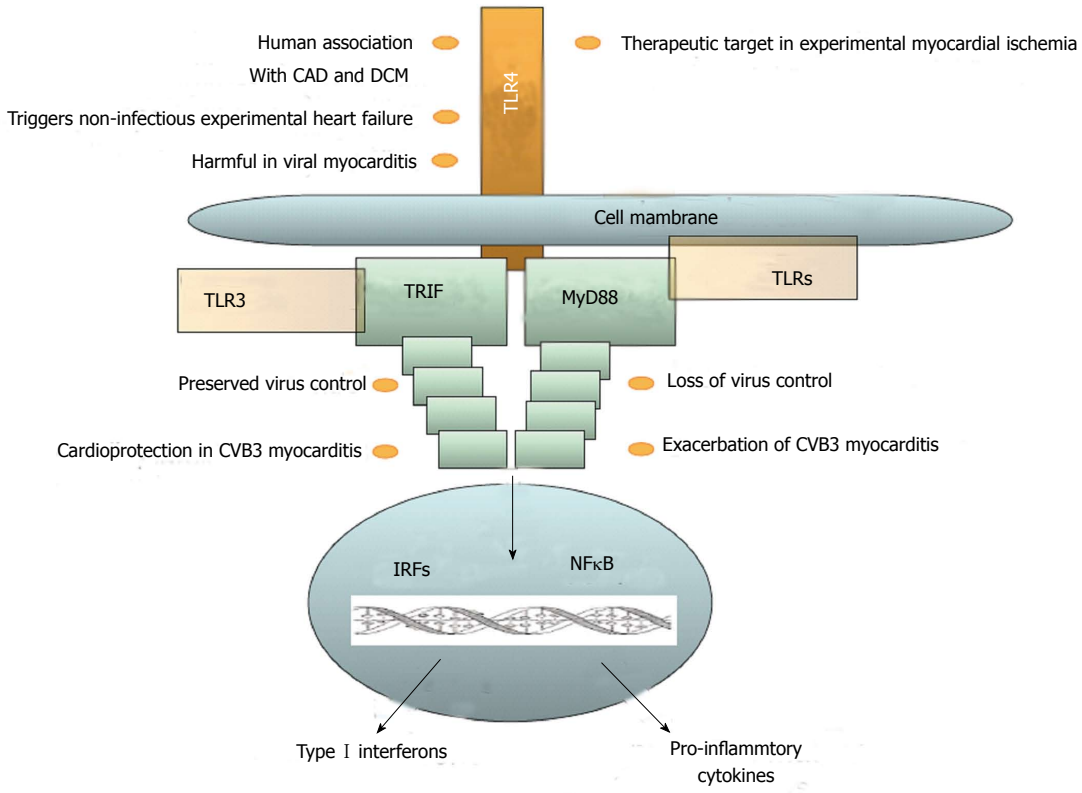

Figure 3 The role of Toll-like receptor 4 in heart failure. This figure summarizes current knowledge of the pathophysiological role of Toll-like receptor 4 (TLR4). CAD: Coronary artery disease; DCM: Dilated cardiomyopathy; TLR3: Toll-like receptor 3; TRIF: TIR domain-containing adaptor inducing interferon-β; MyD88: Myeolid differentiation factor 88; IRF: Interferon regulatory factor; NFkB: Nuclear factor kappa B.

immunity in their action against infection, inflammation in the heart is rarely caused by infectious agents. Other mechanisms lead to an inflammatory response, which often activates TLR pathways. Hemodynamic stress results in inflammation in the myocardium. Myocardial stress increases IL-6 production, which leads to an inflammatory response in the same manner as production of reactive oxygen species (ROS) due to mechanical strain. Macrophage infiltration is triggered by MCP-1 and TGF-β[40]. TNF-α is released by macrophages, mast cells, endothelial cells, and fibroblast. This secretion is triggered not only by infectious agents but also by tissue damage[41]. Necrosis in the myocardium leads to distribution of intracellular particles, which in turn activates the innate immune system. ROS activates innate immune response, but also directly impairs cardiac function. DAMPs activate the complement system and TLRs at the same time[42]. After activation of the TLR pathway, NF-κB induces the expression of pro-inflammatory cytokines and chemokines in endothelial cells, fibroblasts, leukocytes, and vascular cells[43]. Although research has disclosed little for the involvement of NLR in HF, studies have taken place on the effects of the inflammasome in the ischemia-reperfusion model. These results have revealed that mice deficient in caspase-1 or ASC have markedly reduced infarct formation, fibrosis, and cardiac dysfunction. It was further shown that inflammasome activation and IL-1β production occurred primarily in cardiac fibroblasts and leukocytes. This leads to the conclusion that NLRs do play a role in cardiac remodeling and may represent an interesting therapeutic target in the future[34]. Various immune cells evolve to serve functions in primary immune response to tissue damage in the heart, but may also perform a key function in limiting inflammation. Recent investigations have begun to unravel the complex system of macrophage subspecies and functions.

MACROPHAGES

Until now, two different phenotypes have been defined. M1 macrophages are described as first line of defense, with their increased microbicidal capacity as well as production of pro-inflammatory cytokines. M2 macrophages show increased phagocytic activity: they secrete the anti-inflammatory IL-10 and express IL-1 receptor antagonist[44]. The definition of just two subtypes is most likely oversimplified, and a functional perspective could prove more useful in distinguishing pro-inflammatory, regulatory, and reparative macrophages. The phenotype of macrophages is probably defined by a constantly changing variety of cytokines, chemokines, and growth factors, which enable great flexibility in the system[45]. Regulatory T cells (Tregs) have also been reported to possibly influence the macrophage phenotype. These regulatory cells suppress inflammation through IL-10 and TGF-β secretion, or by cell-cell contact. Mice lacking CCR5–which thus reduces Treg infiltration - show increased inflammation and MMP activity[46].

ISCHEMIA/REPERFUSION INJURY

To give some order to these many consequences of cardiac tissue damage, the example of ischemia/reperfusion injury can provide an overview of the immune response. Three phases can be determined that lead to adverse cardiac remodeling. First, neutrophils and pro-inflammatory macrophages migrate to the infarct site, with attraction by chemokines and cytokines secreted upon activation of innate immune pathways. Upon finishing the task of clearing the infarct site of necrotic cells, neutrophils go into apoptosis, which ends the inflammatory phase. Various macrophage subtypes migrate to the infarct in the proliferative phase. They activate endothelial cell growth and myofibroblast formation, with resultant production of a scar. In the final phase, more cells go into apoptosis and collagen cross-links, which possibly leads to ventricle dilation as the infarct matures[47].

During recent decades, intensive research has led to better understanding of ischemia/reperfusion (I/R) injury. I/R injury leads to rapid activation of the immune system, which in turn results in increased expression of TNF, IL-1β, IL-6, NO as well HSP[48,49]. These and other factors lead to infiltration of the infarct with neutrophil granulocytes. In canine and mouse models, infiltration ceased after 3-7 d, and the neutrophils went into apoptosis[50]. Early infiltration of the myocardium can cause more extensive cytotoxic injury to viable cardiomyocytes, which leads in turn to additional damage in the heart[51]. ROS generated by neutrophils may contribute to that adverse effect, as well as interaction with cardiomyocytes through intercellular adhesion molecule-1 (ICAM-1) and integrin[52]. To partially control the immune response, annexin and lactoferrin are transmitted by dying neutrophils to terminate further migration of neutrophils-but at the same time possibly attract macrophages to the site[53]. Furthermore, TNF-α, released at the infarcted area by resident mast cells, also promotes mononuclear cell infiltration[54]. These macrophages begin ingesting the apoptotic cells and, in turn, release cytokines as IL-10, TGF-β pro-resolving lipoxins, and resolvins[55]. Upregulation of IL-10 and TGF-β suppress production of adhesion molecules. Another process for inhibition of leukocyte adhesion is carried out by endogenous integrin ligands of endothelial cells[56]. Some experiments conducted on knock-out mice have provided further insights on the involved immune cells. The attempt to evade the effect of macrophage I/R injury was analyzed in monocyte chemoattractant protein (MCP) 1/CCL2 knock-out mice. MCP1 recruits pro-inflammatory and phagocytic macrophages to the infarct. Compared to wild-type mice, knock-out mice exhibited reduced dilative remodeling with equal infarct size[57]. Similar results were achieved for IL-1-deficient mice. Although infarct size did not vary, the extent of cardiac remodeling was reduced in deficient mice compared to wild type[58]. Those findings support the theory that the initial immune response does not pose the problem, but that long-term activation causes adverse effects. This could partly explain results for ICAM1-deficient mice. Once again compared to wild type, they showed no difference in infarct size, even after 1-3 wk[59]. The same applies to mice with ICAM1 and P-selectin deficiency. Neutrophil migration was decreased, but infarct size did not vary compared to wild type[60]. These results could also suggest that the role of neutrophils has been overestimated. ROS is a mediator among others secreted by neutrophils. It can activate complements, stimulate P-selectin expression promoting cell migration, and upregulate chemokine and cytokine synthesis through the NF-κB pathway[61]. ROS, as well as ATP and potassium abundance, may activate the inflammasome. The inflammasome is expressed by border-zone cardiomyocytes, white blood cells in the granulation tissue, and cardiac firboblasts. Inflammasome formation can be inhibited by P2X7 and cryopyrin, which leads to a decrease in infarct size[62]. Research on TLRs involved in I/R-injury focusses mainly on TLR2 and TLR4. TLR2 seems to play a key role. TLR2 knock-out mice demonstrate better contractile function after I/R injury, and they show similar infarct size, but less ventricular remodeling compared to wild type. Fibrosis is reduced in the non-infarct area, and TGF-β and collagene type 1 expression are lower in knock-out mice. The recovery of LV-developed pressure is also better in TLR2-deficient mice[63,64]. Further research has focused on the transmission of this effect to determine whether it entailed a central effect using TLR2 in the heart, or a peripheral effect involving white blood cells. Infarct size was compared for TLR2-deficient mice and wild-type mice with TLR2-deficient bone marrow. Infarct size did not differ significantly. When TLR2-deficient mice were injected with wild-type bone marrow, infarct size increased compared to purely TLR2-deficient mice. It was possible to inhibit this effect by administering an TLR2 antagonist-which resulted in smaller infarcts, enhanced overall cardiac function, and reduced inflammation and apoptosis[65]. We and others investigated the role of TLR4 in myocardial infarction. TLR4-deficient mice displayed an improved outcome and decreased cardiac inflammation, as also revealed by others[66,67]. Moreover, pharmacological inhibition of TLR4 using the antagonist eritoran led to beneficial effects, which suggests a potential new therapeutic strategy in myocardial ischemia, at least under experimental conditions[68]. Mice deficient in TLR4 also showed smaller infarct size after I/R injury[69]. Pre-treatment with LPS at 24 h before an I/R injury experiment results in better LV function compared to the sham group[70]. TLR2-TIRAP signaling mediates this effect, in which GSK-3β is subsequently inactivated–which prevents it from destabilizing mitochondria and leading to cell death[71].

VIRAL CARDIOMYOPATHY

The role of the innate immune system in viral cardiomyopathy has been primarily established by experiments using mice infected with coxsackievirus B3 (CVB3). In humans it is known that cardiac CVB3 infection needs intact interferon-I signaling[72]. TLR4-deficient mice exhibited higher titers of coxsackievirus B3 (CVB3) two

days after infection, but decreased titers and myocarditis in a 12-d follow up. The cytokines IL-1β and IL-18 were reduced in TLR4-deficient mice[73], Knock-out mice deficient for the TLR downstream adapter protein MyD88 are protected from CVB3 infection[74]. Interestingly, we found in TRIF knock-out mice a much higher susceptibility to CVB3 infection when compared to wild-type mice: to include induction of mortality, loss of virus control, and exacerbation of pro-inflammatory cytokine expression in heart tissue[75]. These data from MyD88 and TRIF knock-out mice suggest not only harmful effects of TLRs, but also cardioprotection in CVB3-induced myocarditis. TLR7 and TLR9 contribute to the susceptibility of MyD88-deficient mice in experimental myocarditis[76]. This is also strengthened by our finding that shows that MyD88 may contribute to the modulation of TLR9 in CVB3-induced myocarditis in mice[77]. In another study, infection of TLR3-deficient mice with encephalomyocarditis virus (EMCV)-a ssRNA virus-interestingly led to earlier death in knock-out mice, combined with increased viral replication and myocardial injury[78]. The mRNA expression of TNF, IL-1β, and IL6 was down-regulated, whereas IFN-β was up-regulated[78]. IRAK-deficient mice and MyD88-deficient mice both exhibit lesser degrees of myocarditis and viral replication after infection, as well as improved survival. Levels of IFN-β were higher in MyD88 knock-out, and IFN-α and IFN-γ were increased in IRAK knock-out. Overall inflammation was reduced[74,79]. Knock-out in cytokines/chemokines led to higher mortality, a greater extent of myocardial injury, higher viral titers for TNF knock-out and EMCV infection, as well as NO knock-out and CVB3 infection[80,81].

DILATED CARDIOMYOPATHY

Activation of the immune system is widely considered a pathophysiological mechanism in DCM[82-87]. For example, we disclosed that the initial white blood cell count upon initial hospital admission in DCM patients predicts long-term mortality in patients with DCM and severe LV dilation[84]. In addition, genetic variants of TLR4 are significantly associated with cardiac recovery in DCM patients, which suggests a potential role of receptor-mediated innate immunity in this disease[88]. Since TLRs are evidently involved in HF, and although viral or bacterial agents are much less frequently the cause than is ischemia, for example, it is interesting to examine a number of known DAMPs and their link to HF. For HSP60 and HSP70, a possible connection to HF has been evidenced. Both are increased in advanced HF. HSP60 trafficking through the plasma membrane is linked to apoptosis, and serum levels of HSP70 correlate with the severity of cardiac dysfunction[89]. Decreased levels of TLR2 and TLR4 have been defined in all subgroups of cardiomyopathy, ischemic cardiomyopathy (ICM), dilated cardiomyopathy (DCM), and viral cardiomyopathy (VCM), whereas TIRAP and IRAK4 are up-regulated[7]. A much wider overview of genetic alternations in cardiac disease allows fundamental compound analysis of innate immune signaling genes. It has showed that the failing heart shows a different expression plot when compared to non-failing heart tissue. Further gene expression in viral cardiomyopathy and idiopathic dilated cardiomyopathy is similar and is distinguished from ischemic cardiomyopathy. This phenomenon suggests different immunological involvement of VCM and DCM compared to ICM, and supports the theory that DCM may evolve from VCM.

THERAPEUTIC IMPLICATIONS

Early studies on the influence of the inflammatory response in HF confirmed the harmful effects of methylprednisolone administration in patients with myocardial infarction[90]. Since that time, many new options have evolved. Nevertheless, it is apparently no less difficult to achieve a positive result, even though current knowledge of innate immunity in HF is much more detailed. These difficulties are obvious in two studies on anti-TNF alpha therapy, with etanercept eventually proving not beneficial and even deleterious[91,92]. Another problem may lie in the limited comparability of humans and animal models. Whereas, in a canine model, antibody-inhibiting leukocyte adhesion acted in a protective manner to limit infarct size by 40% to 50%, there was no effect on infarct size in humans with STEMI administration of CD11b/CD18 integrin receptor inhibition[93,94]. There are, on the other hand, a number of promising substances. TLR4 antagonist eritoran significantly reduces infarct size[68]. New variations of lipid A have been found. They bind to TLR4 but demonstrate reduced agonistic activity (CRX-527, lipad-Iva). TAK-242 also inhibits TLR4 signaling, yet until now its target remains unknown. Ibudilast (AV411) is another TLR 4 antagonist, one that suppresses pro-inflammatory cytokines such as TNF and IL-6. It may induce IL-10 and is currently under trial for opioid dependence. OPN-401, a viral protein-derived peptide, inhibits TLR4 signaling but is still in development. OPN-305 is a promising monoclonal antibody-inhibiting TLR2 and is currently in orphan status for prevention of I/R injury after organ transplantation. AP177-DNA aptamer binds to TLR and antagonizes TLR2 ligand binding[95]. Anakinra, a IL1 receptor antagonist, suppresses post-infarct inflammation and has showed lower incidence of HF[96]. In summary, although knowledge of the pathophysiology of the innate immune system in HF has substantially increased and new therapeutic targets have been addressed under experimental conditions, future investigations, especially clinical trials and experimental research in human tissue–are needed to develop effective innate immune system modulating treatment in HF.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (5): Myocardial infarction

Atypical presentation of acute and chronic coronary artery disease in diabetics

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Abstract

In patients with diabetes mellitus, cardiovascular disease is the principal cause of mortality and chest pain is the most frequent symptom in patients with stable and acute coronary artery disease. However, there is little knowledge concerning the pervasiveness of uncommon presentations in diabetics. The symptomatology of acute coronary syndrome, which comprises both pain and non-pain symptoms, may be affected by traditional risk factors such as age, gender, smoking, hypertension, diabetes, and dyslipidemia. Such atypical symptoms may range from silent myocardial ischemia to a wide spectrum of non-chest pain symptoms. Worldwide, few studies have highlighted this under-investigated subject, and this aspect of ischemic heart disease has also been under-evaluated in the major clinical trials. The results of these studies are highly diverse which makes definitive conclusions regarding the spectrum of atypical presentation of acute and even stable chronic coronay artery disease difficult to confirm. This may have a significant impact on the morbidity and mortality of coronary artery disease in diabetics. In this up-to-date review we will try to analyze the most recent studies on the atypical presentations in both acute and chronic ischemic heart disease which may give some emphasis to this under-investigated topic.

Key words: Diabetes mellitus; Acute coronary syndrome; Acute myocardial infarction; Ischemic heart disease; Atypical presentation; Silent myocardial ischemia

Core tip: Atypical presentations of both acute and chronic ischemic heart disease in diabetic patients is one of the most under-investigated subjects despite extensive research into coronary artery disease even in major clinical trials. To date, according to available data from numerous studies, the impact of atypical presentation on outcome is highly controversial making definitive conclusions difficult. This may have a significant impact on morbidity and mortality of acute and chronic coronary artery disease in diabetics.

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INTRODUCTION

Cardiovascular morbidity is the main cause of death in diabetics. It is predicted that 366 million patients globally will have diabetes mellitus by 2030. As diabetes mellitus progresses, it results in endothelial dysfunction and changes in energy metabolism which lead to atherosclerosis in medium- and large-caliber arteries, creating lesions in coronary, cerebrovascular and peripheral arteries. Additionally, atherosclerotic plaques tend to develop much earlier, advance more swiftly and are more diffuse in diabetic patients than in non-diabetics. These factors

contribute to a two to four-fold higher risk of cardiovascular events in diabetics compared to non-diabetics, with cardiovascular disease being the main cause of death. The combined mortality rate due to cardiovascular disease and diabetes mellitus is 245/100000 population for adults aged 30 to 70 years according to World Health Organization report[1-3].

The overall frequency of coronary artery disease (CAD) among diabetics is 55%. To date, 90% of the published studies presenting data on the atypical presentation of chronic and acute ischemic heart disease are carried out in type 2 diabetics, while there are few data available on type 1 diabetics. Consequently, most of our conclusions in this review are for type 2 diabetes[4-6].

Diabetic patients frequently present with silent myocardial ischemia (SMI), and the absence of an imperative clinical "warning symptom". Statistics from the Framingham study showed that asymptomatic patients with various risk factors have an annual cardiac mortality rate of approximately 3%[4,5,7]. Such outcomes from these studies raise numerous questions regarding diabetes mellitus and CAD: Why is myocardial ischemia repeatedly atypical or silent in diabetic patients? In what way is it discovered? What is its aftermath? How do we deal with it? The current analysis will tackle these issues. We identified studies *via* searches in MEDLINE, PubMed, EMBASE, and Current Contents and by reviewing reference lists in all the studies performed in the last 30 years from both developed and developing countries using the following keywords: diabetes mellitus, acute coronary syndrome (ACS), acute myocardial infarction (AMI), ischemic heart disease, atypical presentation, and SMI. We attempted to provide conclusions and future perspectives on this under-evaluated topic according to up-to-date studies from different parts of the world.

POSSIBLE EXPLANATION FOR THE ATYPICAL PRESENTATION OF ACUTE CORONARY SYNDROME IN DIABETICS AND THE PROGNOSTIC IMPLICATIONS

Chest pain is the cornerstone symptom of ACS. However, data concerning the prevalence of atypical presentation among these patients and its relation to subsequent care is scarce. CAD has specificities in diabetics with pervasive atherosclerosis. Diabetic patients are also more frequently asymptomatic, with a wide range of atypical presentations which makes the diagnosis of CAD challenging. In addition, diabetic patients with CAD have poorer outcomes than non-diabetics. CAD is the foremost source of morbidity and mortality in diabetic patients with higher mortality after an acute cardiac event compared to non-diabetics. Such inconsistencies may be related to the degree of CAD in diabetics, the magnitude of left ventricular remodeling, and the occurrence of significant ventricular dysrhythmias[8-27].

Despite the fact that CAD is the primary vascular complication of diabetes, there is a significant gap in our knowledge and understanding on atypical ACS symptoms in diabetics. Conventional risk factors, such as, hypertension, diabetes, hypercholesterolemia and smoking have a significant impact on the symptomatology of ACS and stable angina, including both pain and non-pain symptoms. Although numerous investigations on diabetes management have been performed, only a few studies have focused on atypical ACS symptoms in patients with diabetes with contradictory results. Diabetics may have a diminished awareness of ischemic chest pain which could result in an uncharacteristic presentation. This may be explained by autonomic neuropathy and prolongation of the anginal perceptual threshold[28]. In addition, diabetic patients with SMI have evidence of a disseminated abnormality in metaiodobenzylguanidine uptake on positron emission tomography. A similar finding was also observed in asymptomatic diabetic patients on stress testing with a dipyridamole stress myocardial scan and contrast echocardiography in approximately 60% of diabetic patients, these findings reflect abnormal pain perception interrelated with sympathetic denervation[29]. SMI is seen more frequently in diabetic patients than in the general population. SMI may be the main atypical presentation observed in major clinical trials compared to other forms of atypically presented CAD in both acute and chronic forms. However, the exact prevalence of SMI remains unidentified[30]. In general, the frequency of silent CAD diverges according to the test used and the patient population investigated. The prevalence of silent CAD is 6%-23% in low-risk diabetics, and can be as high as 60% in high-risk diabetic patients. Recently it was recognized that silent CAD has a similar prognosis and adverse events rate when compared with symptomatic CAD[31]. Possible explanations for the dissimilar symptoms in patients with diabetes mellitus, comprise central mechanisms such as altered thresholds of pain sensitivity, beta-endorphin levels, in addition to autonomic neuropathy resulting in sensory denervation. The American Diabetes Association states that patients with symptomatic autonomic neuropathy are at increased risk of sudden death; however, it still controversial whether there is adequate scientific data available to indicate that cardiac autonomic neuropathy contributes to silent ischemia and whether specific diabetic patients might gain benefit from routine testing for occult ischemia[31].

In the last few years, diabetics have not experienced the same decline in CAD-related mortality as non-diabetics. The poor prognosis associated with diabetes after AMI has been witnessed in several studies despite adjustment for age, sex, coronary risk factors[12,13,15-20] and associated comorbidities[32]. Contradictory evidence is available concerning the morbidity and mortality of diabetic patients managed with insulin *vs* oral hypoglycemic agents or diet after AMI[12,18,27,32,33]. Similarly, uncertainty still exists regarding the negative prognostic implications of diabetes in patients with a different spectrum of ACS *i.e.*, unstable angina, non-ST and ST-segment elevated AMI. It is imperative to establish whether these patients

are consistently receiving proven cardiac interventions under current practices.

SILENT MYOCARDIAL ISCHEMIA AS A MODE OF ATYPICAL PRESENTATION IN DIABETICS (TABLE 1)

Silent myocardial infarction/ischemia (SMI) is more frequent than formerly thought. Up to 25% of patients with CAD have suffered silent SMI; the magnitude of the myocardium affected is on average 10% of the left ventricle muscle mass, and it is more prevelant in diabetics. The phenomenon of SMI is still debatable. The presence of cardiac autonomic dysfunction is the assumed factor that influences the frequency of SMI in diabetics[34]. Hence, the importance of identifying individuals with a high risk for cardiovascular events, prior to symptom onset may be of significance. Diabetes mellitus affects vascular endothelium, causing endothelial dysfunction[35]. A study assessed the frequency, scope, and independent predictors of SMI in 2 large independent cohorts of consecutive patients without a history of MI referred for rest/stress myocardial perfusion single photon emission computed tomography. One thousand six hundred and twenty-one patients were registered in the derivation cohort and 338 patients in the validation cohort. SMI was diagnosed in patients with a myocardial scar involving ⩾ 5% of the left ventricle. In the derivation cohort, 23.3% had SMI. The median infarct size was 10% [interquartile range (IQR) 5%-15%] of the left ventricle. The occurrence of SMI was 28.5% in diabetics *vs* 21.5% in non-diabetics (P =0.004). Diabetes mellitus was an independent predictor for the presence of SMI (OR = 1.5; 95%CI: 1.1-1.9; P = 0.004). In the validation cohort, the prevalence of SMI was 26.3%, with a higher incidence in diabetics (35.8%) compared to non-diabetics (24%; P = 0.049). The median infarct size was 11.8% (IQR, 5.9%-17.6%) of the left ventricle. After logistic regression analysis; diabetes mellitus was a noteworthy prognosticator of the presence of SMI confirming the derivation cohort result[36].

In a cross-sectional study involving 200 subjects (mean age; 46 ± 10 years, 31 had diabetes), the subjects underwent an exercise stress test. A positive test for silent ischemia was seen in 19% of diabetics and 13% of non-diabetics, which was not statistically significant (P = 0.397). Hypertension and obesity were found more frequently in diabetics (48% *vs* 27% and 35% *vs* 18%, respectively)[37]. Blood lipid levels may predict SMI in non-insulin dependent diabetes. A study included 220 asymptomatic diabetics who underwent laboratory tests and gated single-photon emission computed tomography with coronary angiography as the confirmatory test, when gSPECT detected ischemia. A higher level of total cholesterol was seen in gSPECT-positive diabetics, together with low-density lipoprotein (LDL), and triglycerides (P < 0.05). High-density lipoprotein (HDL) levels were lower in this group (P < 0.05). HDL was the most important normalized variable. This study included more men (33.3%) than women (24.8%). HDL levels were significantly lower in these patients. The association between low HDL and high triglycerides was a strong indicator of myocardial ischemia in type 2 diabetics without clinical cardiovascular signs[38]. A gated myocardial perfusion SPECT in asymptomatic diabetics with a high combination of cardiovascular risk factors detected SMI in a significant proportion of patients and this seemed to be related to future coronary events. Diabetic nephropathy may indicate a greater likelihood of abnormal studies[39].

A study evaluated the pervasiveness of SMI in 147 subjects in a diabetic Afro-Caribbean population. 23.1% had SMI; these patients had a personal history of cardiovascular disease similar to those without diabetes. On multivariate logistic-regression analyses, the adjusted odds ratio of SMI was considerably higher in patients with a personal history of cardiovascular disease (4.36, 95%CI: 1.36-13.96; P = 0.01) and left ventricular hypertrophy (LVH) (2.46, 95%CI: 1.03-5.86; P =0.04)[40].

Dobutamine stress echocardiography may be a useful diagnostic test for detecting SMI, especially in diabetic patients at high cardiovascular risk. A study of 79 diabetics (average age = 58.8 ± 11.8 years) revealed that 67.1% had a positive test, with a predominance of motion abnormalities in the anterior area (83%). Microalbuminuria (P = 0.0001), inactivity (P = 0.0001), dyslipidemia (P = 0.0002), arterial hypertension (P = 0.001), smoking (0.003) and male sex (P = 0.004) were the main cardiovascular risk factors associated with positivity[41].

In the detection of ischemia in asymptomatic diabetics (DIAD) study, the largest prospective study with a 4.8-year follow-up period included 1123 asymptomatic persons with type 2 diabetes who were randomized to either testing with stress myocardial perfusion scan or no testing. In this study, 53%-75% of participants with intermediate to high cardiovascular risk had a prevalence of inducible ischemia on screening that ranged from 21% to 24%, which was almost comparable to lower-risk patients (19%-23%). Patients with intermediate-/high-risk had higher rates of cardiac events (only significant for the UKPDS risk engine 4.2 *vs* 1.2%, P = 0.002). The yearly cardiac event rate was < 1% in all risk groups, apart from the high-risk UKPDS group (approximately 2% per year). Surprisingly the annual cardiac event rate for intermediate/high risk was low and not altered by standard testing for inducible ischemia[42].

High LDL level and higher carotid intima-media thickness are predominant issues that can indicate whether a patient with non-insulin dependent diabetes (NIDDM) is are at risk of SMI. A high carotid intima-media thickness is a substitute and dependable indicator of higher risk of CAD in non-insulin dependent diabetic patients, even in those without evident CAD[43].

Another study determined SMI in 90 unselected middle-aged asymptomatic NIDDM patients (48 men; mean age: 49 ± 6 years, mean diabetes duration of 4 ± 4.2 years (range 1-21 years) without CAD as documented by treadmill exercise test. Four percent of patients had a positive test. Diabetics with SMI were older (55 ± 3

years *vs* 49 ± 6 years, $P = 0.04$), had a higher fibrinogen level (372 ± 51 *vs* 307 ± 71 mg/dL, $P = 0.04$) and had lower total exercise time and peak workload (375 ± 30 s *vs* 474 ± 115 s, $P = 0.04$; 7.3 ± 0.5 *vs* 8.9 ± 1.9, $P = 0.04$, respectively). Insulin resistance is related to different atherosclerosis risk factors. Exercise test outcomes showed increased cardiac sympathetic activity and parasympathetic withdrawal in increased insulin resistance[44]. Left atrial surface area independently predicted SMI after adjustment for established echocardiographic and inflammatory risk factors in diabetics[45]. Age and differential pulse pressure may be predictors of SMI[46].

A study estimated the frequency of SMI in 353 asymptomatic Caucasian diabetic patients using the treadmill test with single-photon emission computed tomography and exercise testing or dipyridamole injection with coronary angiography as the confirmation test. Patients with SMI (8.5% were diabetics: 3 IDDM and 13 NIDDM) were older and had autonomic neuropathy, hypertension, dyslipidemia and higher microalbuminuria (613 ± 211 mg/d *vs* 72 ± 245 mg/d; $P < 0.05$)[47].

SMI may occur in more than 20% of asymptomatic patients with NIDDM. Conventional and evolving cardiac risk factors were not linked with abnormal stress tests, even though cardiac autonomic dysfunction was a resilient prognosticator of ischemia using adenosine technetium-99m sestamibi single-photon emission-computed tomography myocardial perfusion imaging in asymptomatic NIDDM patients and testing the efficiency of current American Diabetes Association screening guidelines. A total of 1123 patients, with no known or suspected CAD were randomly assigned to either stress testing and 5-year clinical follow-up or to follow-up only. In this study 22% had SMI; the strongest prognosticators for abnormal tests were abnormal Valsalva, male sex, and diabetes duration, but not traditional cardiac risk factors or inflammatory and prothrombotic markers. Choosing only patients who met the American Diabetes Association guidelines failed to detect 41% of patients with SMI[48]. Erectile dysfunction may become a possible indicator to identify diabetic patients with SMI during screening, particularly in patients with additional cardiovascular risk factors[49]. However, diabetics may have a higher prevalence of angina pectoris during daily activity than non-diabetics[50]. Using dobutamine stress echocardiography to detect SMI, significant CAD was identified in 9% of asymptomatic diabetics. Dynamic left ventricular outflow obstruction was detected in 59% of diabetics and in only 22% of non-diabetics, however, these results need to be investigated in future studies[51].

The association between SMI and cardiac autonomic neuropathy has been reported in a few studies (Table 1). Autonomic dysfunction is seen in 85.7% of diabetics with SMI *vs* 18.7% of diabetics without silent ischemia ($P = 0.001$). The incidence of SMI was higher in patients with autonomic neuropathy (40% *vs* 10%) $P < 0.001$. The duration of diabetes was greater (13 ± 1.59 years) in patients with autonomic neuropathy, and systolic blood pressure was predictive of silent ischemia in diabetics[52-54].

A few other studies[55-65] assessed different aspects of the association between SMI and diabetes (Table 1). Patients with SMI had higher ischemia in the working forearm compared to diabetic patients with and without neuropathy. There is a quantitative and qualitative difference in ischemic tolerance between patients with SMI and patients with diabetic neuropathy[57,58]. The role of beta endorphin in diabetic patients with SMI may be less substantial than in non-diabetics; therefore, diabetic neuropathy which affects the autonomic pain fibers that innervate the heart may be involved in the pathogenesis of SMI in diabetics and appears to be the most probable reason for the absence of pain[59,60].

ATYPICAL PRESENTATION OF ACUTE CORONARY SYNDROME IN DIABETICS

Many reports including major clinical trials and sporadic studies (Tables 2 and 3) have shown that diabetes mellitus is an independent predictor of atypical presentation of ACS with a controversial outcome[66]. Several studies reported that diabetic patients had less pain compared to non-diabetics[67-75], while other studies found no difference[76-81].

Studies which have shown diabetes mellitus is a predictor of the atypical presentation of acute coronary syndrome (Table 2)

In a nation-wide survey conducted in 2133 consecutive ACS patients who were separated into three age subgroups: < 65 years ($n = 974$), 65-74 years ($n = 500$), and ⩾ 75 years ($n = 639$), the incidence of no anginal pain/atypical symptoms on presentation increased with age in all ACS patients (14%, 21%, and 32%, in the three age subgroups, respectively; $P < 0.0001$). The occurrence of ST-elevation on admission electrocardiogram decreased with advancing age (59%, 46%, and 42%, in the three age subgroups, respectively; $P < 0.0001$), while ST-depression progressively increased (14%, 24%, and 28%, respectively; $P < 0.0001$). In a multivariate analysis, variables linked with no anginal pain/atypical symptoms on presentation were: history of heart failure, age, lack of past angina, diabetes, and non-smoking. ST-elevation was inversely associated with no anginal pain/atypical symptoms on admission (OR = 0.48; 95%CI: 0.37-0.63)[68].

A study by Culić *et al*[69] who performed subgroup analyses showed that diabetes was an independent prognosticator of "atypical" presentation of AMI in women. In this prospective, observational study of a large number of symptoms in 1996 patients, it was established that chest pain was more often reported by males, smokers, and hypertensive, non-diabetic, and hypercholesterolemic patients. Women frequently reported non-chest pain other than epigastric and right shoulder pain, along with a range of non-pain symptoms. The independent predictors of atypical AMI presentation in both men and women were diabetes mellitus ($P = 0.0002$ and $P = 0.002$,

Table 1 Studies on silent myocardial ischemia as a mode of atypical presentation in diabetics

Ref.	Study population	Study type/country	Silent ischemia %	Conclusion
Arenja *et al*[36]	1621 pts in the derivation cohort + 338 pts in the validation cohort	Derivation cohort/ Switzerland	23.3%- 28.5% in DM and 21.5% in non-DM	DM is an independent predictor for the presence of SMI (OR = 1.5; 95%CI: 1.1-1.9, P = 0.004). In the validation cohort, the prevalence of SMI = 26.3% (n = 89), while the prevalencea in diabetics (35.8%) *vs* non-diabetics was 24% (P = 0.049)
Sheikh *et al*[37]	200 subjects, 31 diabetics *vs* 169 non-diabetics	A cross-sectional study/Pakistan	(19%) diabetics *vs* (13%) non-diabetics	No significant difference in the frequency of SMI in diabetics *vs* non-diabetics
Peña *et al*[38]	220 asymptomatic NIDDM patients	A prospective, observational, analytical study /Havana	29.10%	Type 2 diabetics with ischemia had ↑ levels of total cholesterol, LDL and triglycerides. HDL levels were significantly ↓. The association of ↓ HDL with ↑ triglycerides was a strong indicator of SMI in NIDDM patients
Ruano Pérez *et al*[39]	56 asymptomatic diabetics	retrospective study	46.40%	Moderate-severe ischemia in 10.7%, necrosis with ischemia in 5.4% and necrosis in 7.1%, diabetic nephropathy was the only factor related to an abnormal SPECT (P = 0.043)
Blanchet Deverly *et al*[40]	147 NIDDM patients	cross-sectional study /France	23.10%	Multivariate logistic-regression analyses, the adjusted OR of SMI significantly ↑ in patients with a history of cardiovascular disease (4.36, 95%CI: 1.36-13.96, P = 0.01) and LVH (2.46, 95%CI: 1.03-5.86, P = 0.04)
Mbaye *et al*[41]	79 diabetics	Prospective/France	67.10%	Predominance of motion abnormalities in the anterior territory (83%). Cardiovascular risk factors associated with positivity of the test were microalbuminuria (P = 0.0001), inactivity (P = 0.0001), dyslipidemia (P = 0.0002), arterial hypertension (P = 0.001), smoking (0.003) and male sex (P = 0.004)
Bansal *et al*[42]	1123 NIDDM patients	Prospective/Detection of Ischemia in Asymptomatic Diabetics (DIAD) /United States and Canada (DIAD) study	21%-24% in the intermediate high risk group 19%-23% in the low risk group	Cardiac event rates ↑ in intermediate/high-risk. The annual cardiac event rate was ≤ 1% in all risk groups. In intermediate-/ high-risk participants randomized to screening *vs* no screening, 4.8-yr cardiac event rates were similar (2.5%-4.8% *vs* 3.1%-3.7%)
Agarwal *et al*[43]	77 NIDDM	Prospective study/ India	28.90%	The prevalence of SMI similar in males and females. Serum LDL levels > 140 mg % had a significant correlation with the prevalence of silent CAD (P = 0.04). The difference in CCA-IMT values was found to be statistically significant between the silent CAD and non-CAD groups (P = 0.019)
Ugur-Altun *et al*[44]	90 asymptomatic NIDDM patients	Prospective/Turkey	4%	Diabetics with SMI had ↑ fibrinogen level (372 ± 51 mg/dL *vs* 307 ± 71 mg/dL, P = 0.04), had ↓ total exercise time and peak workload (375 ± 30 s *vs* 474 ± 115 s, P = 0.04; 7.3 ± 0.5 *vs* 8.9 ± 1.9, P = 0.04, respectively)
Chico *et al*[47]	353 NIDDM asymptomatic Caucasians	Prospective/Spain	8.50%	SMI patients were older, had ↑ prevalence of autonomic neuropathy, microalbuminuria, hypertension, and dyslipidemia than those without
Wackers *et al*[48]	1123 NIDDM patients	Prospective/United States	20%	Predictors for abnormal tests: abnormal Valsalva, male sex and diabetes duration (5.2). Traditional cardiac risk factors or inflammatory and prothrombotic markers were not predictive. Ischemic adenosine-induced ST-segment depression with normal perfusion in women
Falcone *et al*[50]	618 patients with CAD	Prospective/Italy	58%	SMI during exercise seen in 58% of diabetics and 64% of nondiabetics. Both diabetics and non-diabetics with exertional SMI had ↑ heart rate values (P < 0.01), SBP (P < 0.01), rate-pressure product (P < 0.001), work load (P < 0.01) and maximum ST depression at peak exercise (P < 0.05)
Coisne *et al*[51]	49 diabetics and 63 non-diabetics	Prospective/France	9%	Significant CAD detected in 9% of asymptomatic diabetics. Dynamic left ventricular obstruction observed in 59% of the diabetic population and in only 22% in the non-diabetic population
Sukhija *et al*[53]	30 diabetics/30 non diabetics	Prospective/India	46.70%	Diabetics had ↑ heart rate and a greater number of supraventricular and ventricular ectopics, ↑ prevalence of multi-vessel involvement and diffuse disease compared to controls. 50% of diabetics and none of the controls had autonomic dysfunction. Autonomic dysfunction was present in 85.7% of diabetics with SMI *vs* 18.7% of diabetics without SMI (P = 0.001)
May *et al*[54]	240 diabetics	Prospective/Denmark	13.50%	Frequency of SMI did not differ significantly between diabetics and non-diabetics. Systolic blood pressure was predictive of SMI in diabetes
Tamez-Pérez *et al*[55]	60 NIDDM patients	Prospective/ Spain	17%	In a 2-yr follow-up, 4 diabetics developed symptomatic angina pectoris

Ahluwalia *et al*[56]	20 male diabetics	Prospective/India	50%	On exercise testing in diabetics, SMI was detected in 64% of the patients with 3 vessel disease, 50% of the patients with 2 vessel disease and 20% of the patients with one-vessel disease *vs* 18% of non-diabetic patients with three-vessel disease ($P < 0.05$) and in none of the patients with two- or one-vessel disease
Tanaka *et al*[61]	92 NIDDM patients	Prospective / Japan	38%	Diabetics with positive treadmill test were smokers, and had hypertension and ↑ triglyceride level compared to treadmill negative diabetics
Nesto *et al*[62]	30 diabetics with peripheral vascular disease	Prospective /United States	57%	57% had thallium abnormalities, with reversible thallium defects compatible with ischemia in 47% and evidence of prior, clinical SMI in 37%. Thallium abnormalities were seen more frequently in diabetics with concomitant hypertension and cigarette smoking ($P = 0.001$)
Koistinen *et al*[63]	136 diabetic subjects	Controlled study/ Finland	29%	Coronary angiography of 34 diabetics; 12 had significant coronary artery narrowing; seven had unimportant atherosclerosis; 15 had patent coronary arteries
Theron *et al*[64]	52 IDDM and 87 NIDDM subjects	Prospective /South Africa	See conclusion	No statistically significant relationship between any parameter and the presence of autonomic neuropathy. Atypical infarctions not limited to subjects with autonomic neuropathy, the incidence much ↑ than the general population
Touze *et al*[65]	50 black African diabetics	Prospective /Africa	10%	SMI was ↓ among black African diabetics compared with white diabetics. The coronary lesions were mostly limited. Proximal narrowing and one-vessel disease mostly encountered-

↑: Increase/higher; ↓: Decreased/lower. CAD: Coronary artery disease; IDDM: Insulin dependent diabetes mellitus; NIDDM: Non-insulin dependent diabetes mellitus; MI: Myocardial infarction; HDL: High density lipoprotein; LDL: Low density lipoprotein; SMI: Silent myocardial ischemia/infarction; CCA-IMT: Common carotid artery intimal medial thickness

respectively), lower creatine kinase-MB fraction level ($P < 0.0001$ and $P = 0.0003$, respectively), older age ($P = 0.001$ and $P = 0.01$, respectively), and absence of smoking in men ($P = 0.005$). The independent predictors of non-pain symptoms in both men and women were higher levels of creatine kinase-MB fraction ($P = 0.01$ and $P = 0.049$, respectively) and diabetes mellitus ($P = 0.048$ and $P = 0.005$, respectively), while hypercholesterolemia ($P = 0.01$) in men was the predictor of atypical presentation[69].

A recent study in South Korea evaluated the risk factors associated with atypical presentation according to age. In this study, diabetes and hyperlipidemia predicted atypical symptoms in the younger (< 70 years) age group. Comorbid illnesses such as stroke or chronic obstructive pulmonary disease were positive predictors in the older (> 70 years) age group[70].

Statistics from a prospective clinical trial of patients with symptoms indicating ACS in 10 United States hospitals during emergency assessment compared patient demographics, clinical variables, and outcomes. Of 10783 subjects, a definitive diagnosis of long-established ACS was made in 24% of patients, of which 35% had AMI and 65% had unstable angina. Sixty-two percent of ACS patients and 9.8% of AMI patients had no pain. Patients with painless ischemia were older, and more frequently females with more cardiac and related diseases. Patients with painless AMI were less likely to be admitted to critical care units. Among patients with acute infarction, logistic regression predicting lack of pain categorized age, heart failure and diabetes as the main predictors with only age and heart failure in those with ACS. After controlling for clinical features, silent acute ischemia predicted augmented hospital mortality[72].

In the National Registry of Myocardial Infarction 2 (NRMI 2): a prospective observational study in the United States, which included 434877 patients with MI, 33% had no chest pain on presentation to the hospital and were 7 years older than those with chest pain (74.2 years *vs* 66.9 years), more likely to be female (49.0% *vs* 38.0%), have diabetes mellitus (32.6% *vs* 25. 4%) or previous cardiac failure (26.4% *vs* 12.3%) and have delayed presentation (mean, 7.9 *vs* 5.3 h). These patients were less likely to be diagnosed with SMI and were less likely to undergo thrombolysis or primary angioplasty (25.3% *vs* 74.0%), and treatment with aspirin (60.4% *vs* 84.5%), beta-blockers (28.0% *vs* 48.0%), or heparin (53.4% *vs* 83.2%). SMI patients had higher in-hospital mortality compared to symptomatic patients (23.3% *vs* 9.3%)[73,74].

Many sporadic studies from different parts of the world both in developed and developing countries have assessed the atypical presentation of ACS in different communities. Such studies have shown diverse results (Table 2). A study assessed 9509 healthy adults over 5 years who had an average annual incidence of 3.6/1000 persons with unrecognized infarcts and 5.3/1000 persons with clinical infarcts. Patients whose electrocardiograms were initially read by cardiologists as non-infarcts, but by the computer as infarcts, had a high rate of unrecognized infarcts in the subsequent 5 years and a markedly higher 7-year mortality rate in the unrecognized infarct group *vs* the non-infarct population, but significantly lower than those who developed a clinical infarct. In this study, age, left axis deviation, left ventricular hypertrophy, cigarette smoking, systolic or diastolic blood pressure, and peripheral vascular disease were significant risk factors for unrecognized myocardial infarction on multivariate analysis. Cholesterol, diabetes, anxiety, and psychosocial problems, do not play a significant role in unrecognized infarcts[75].

The Global Registry of Acute Coronary Events (GRACE study), is the largest multinational, prospective,

Table 2 Studies which have shown that diabetes mellitus is a predictor of atypical presentation of acute coronary syndrome

Ref.	Study population/	Study type/country	Atypical presentation %	Conclusion
Stern *et al*[68]	2113 ACS patients	Nationwide survey/ Israel	21.7% had no chest pain	In multivariate analysis, variables associated with no anginal pain/atypical symptoms on presentation (in ↓ order): history of heart failure, age, no past angina, diabetes and non-smoking. 18.7% of male patients had no chest pain on presentation vs 29.7% of females
Culić *et al*[69]	1996 MI patients	A prospective, observational study/ Croatia	14.8% had no chest pain	The independent predictors of atypical presentation in both gender; ↓ levels of CK-MB fraction ($P < 0.0001$ and $P = 0.0003$, respectively), NIDDM ($P = 0.0002$ and $P = 0.002$, respectively), older age ($P = 0.001$ and P = 0.01, respectively), and no smoking in men ($P = 0.005$)
				The independent predictors of the presence of non-pain symptoms; DM ($P = 0.048$ and $P = 0.005$, respectively), ↑ levels of CK-MB ($P = 0.01$ and $P = 0.049$, respectively) and hypercholesterolemia (P =0.01) in both men and women
Hwang *et al*[70]	931 newly diagnosed as ACS	Retrospective/ South Korea	7.8% of younger pts and 13.4% of older pts	A logistic regression analysis after adjustment for gender and ACS type indicated that diabetes and hyperlipidemia significantly predicted atypical symptoms in younger patients
MacKenzie *et al*[71]	64 (12 women with DM)	Descriptive, cross-sectional/ Canada	See conclusion	Less chest pain in diabetics *vs* non-diabetics ($P = 0.02$)
				No difference in pain intensity in diabetics with MI *vs* non-diabetics ($P \geqslant 0.05$)
				Diabetics with UA or MI were more likely to report mid-sternal chest pain ($P = 0.04$) and chest pain that radiated to the back of the left arm ($P = 0.01$) than non-diabetics
				Diabetics with UA or MI reported more SOB (53.1% *vs* 31.3%; NS)
				In diabetics with UA or MI, SOB was a factor in deciding to seek care
Coronado *et al*[72]	2541 (1058 women, 410 women with DM);	Secondary analysis of multisite a prospective clinical trial/United States	6.2% of patients with ACS and in 9.8% of AMI.	DM independent predictor of painless presentation in acute MI, but not in the ACS group. Diabetes more common in non-pain ACS (35% *vs* 26%; $P = 0.01$)
				Shortness of breath most common in the painless presentation group (72%) and women were more likely to have painless ACS (53%) ($P =$ 0.007)
Vaccarino *et al*[73]	384878 patients	Prospective, observational study/ National Registry of MI/United States	33%	Atypical presentation patient: older, ↑ proportion of women and diabetics without a significant interaction between sex and diabetes ($P = 0.30$). HF comorbidities and less likely to have coronary intervention with ↓ chance of anticoagulants, aspirin and β blocker usage
Canto *et al*[74]	434877 MI ptsJune 1994-March 1998	Prospective observational study United States	33% had no chest pain	Patients without chest pain on presentation:
				Likely to be diabetics (32.6% *vs* 25. 4%)
				Older (74.2 yr *vs* 66.9 yr). Likely to be female (49.0% *vs* 38.0%)
				Likely to have prior HF (26.4% *vs* 12.3%)Had a longer delay before hospital presentation (mean, 7.9 h *vs* 5.3 h)
				Less likely to be diagnosed with confirmed MI at the time of admission (22.2% *vs* 50.3%)
				Less likely to receive thrombolysis or PCI (25.3% *vs* 74.0%), aspirin (60.4% *vs* 84.5%), BB (28.0% *vs* 48.0%), or heparin (53.4% *vs* 83.2%).
				23.3% in-hospital mortality *vs* 9.3% in patients with chest pain
Medalie *et al*[75]	9509 healthy adult subjects	Israeli Heart Attack study, cohort/ Israel	3.6 unrecognized MI/ 1000 persons and 5.3 clinical MI/1000 persons	By multivariate analysis, age, left axis deviation, LVH, cigarette smoking, systolic or diastolic BP, and PVD were the most significant risk factors. Cholesterol, DM, anxiety, and psychosocial problems, do not play a significant role in unrecognized MI
Brieger *et al*[76]	20881 ACS patients	Global Registry of Acute Coronary Events/multinational, prospective, observational study (in 14 countries)	8.4% presented without chest pain	23.8% not initially recognized as having an ACS, < 33% of the population with atypical symptoms were diabetics.
				Less likely to receive effective cardiac medications
				↑ hospital morbidity and mortality (13% *vs* 4.3%, respectively; $P < 0.0001$)
				↑ hospital mortality rates in patients with presenting symptoms of pre-syncope/syncope. Nausea or vomiting, dyspnea and in those with painless presentations of UA

↑: Increase/higher; ↓: Decreased/lower. MI: Myocardial infarction; UA: Unstable angina; AMI: Acute myocardial infarction; ACS: Acute coronary syndrome; DM: Diabetes mellitus; SOB: Shortness of breath.

Table 3 Studies which have not shown that diabetes mellitus is a predictor of atypical presentation of acute coronary syndrome

Ref.	Study population/	Study type/country	Atypical presentation %	Conclusion
Meshack *et al*[77]	589 patients, aged 25 to 74 yr, with AMI	A community-based surveillance program/ United States	Sweating (64.2%), fatigue (62.6%), dyspnea (60.3%), and arm or jaw pain (58.2%).	Adjusting for age, DM, gender, and relative to non-Hispanic whites, Mexican Americans were more likely to report chest pain, upper back pain, and palpitations, and less likely to report arm or jaw pain
Richman *et al*[78]	216 (19 women with DM); AMI	A prospective, observational study/ United States	No statistical difference in diabetics vs non-diabetics in terms of the presence chest pain	No difference in the frequency of chest pain or associated symptoms by diabetic status ($P \geq 0.05$); No chest pain symptoms was more common in diabetic patients (NS)
Kentsch *et al*[79]	1042 (330 women; 155 women with DM) with STEMI	Secondary analysis of MITRA PLUS (18786 pts.; North German Registry, NGR, 1042 pts.)/ Germany	16.9% of DM and 15.0% of non-DM	No difference in the frequency or intensity of chest pain by diabetic status Patients with DM reported significantly more dyspnea than those without DM (29.5% *vs* 19.5%; $P < 0.01$)
DeVon *et al*[80]	100 (50 women, 23 women with DM); DM	rospective secondary analysis; descriptive, cross-sectional; structured interview/United States	3%	No difference in the frequency and severity of chest pain in diabetics vs non-diabetics ($P \geq 0.05$) No differences in UA symptoms by diabetic status Patients with DM reported weakness as the second most common symptom and more likely to describe chest pain as squeezing ($P = 0.02$) or aching ($P = 0.04$) than non-diabetics Diabetics had ↑ frequency of hyperventilation ($P = 0.04$) and ↓ frequency of nausea ($P = 0.04$) than non-diabetics
Thuresson *et al*[81]	N = 1939 (480 women, 82 women with DM)	Descriptive, cross-sectional study/ Sweden	See conclusion	No difference in chest pain or other ACS symptoms by DM status Women reported more tiredness/weakness, anxiety/ fear, vomiting, back pain, left arm pain and neck or jaw pain than men ($P = 0.01$).

↑: Increase/higher; ↓: Decreased/lower. STEMI: ST elevation myocardial infarction; UA: Unstable angina AMI: Acute myocardial infarction; ACS: Acute coronary syndrome; DM: Diabetes mellitus; PVD: Peripheral vascular disease.

observational study and involves 14 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, France, Germany, Italy, New Zealand, Poland, Spain, the United Kingdom, and the United States). Of the 20881 patients included, 8.4% had no chest pain, and 23.8% were not initially recognized as having ACS. These patients had higher hospital morbidity and mortality (13% *vs* 4.3%, respectively; $P < 0.0001$) and were less likely to receive effective cardiac medications than patients with typical presentation. After adjusting for potentially confounding variables, excluding diaphoresis, higher in-hospital mortality rates were seen in patients who presented with pre-syncope/syncope (OR = 2.0; 95%CI: 1.4-2.9), nausea or vomiting (OR = 1.6; 95%CI: 1.1-2.4), and dyspnea (OR = 1.4; 95%CI: 1.1 to 1.9), than in those with painless presentations of unstable angina (OR = 2.2; 95%CI: 1.4-3.5) and ST-segment elevation MI (STEMI) (OR = 1.7; 95%CI: 1.2-2.2). In patients with unstable angina and non-ST elevation MI, 5.7% and 12.3% had atypical symptoms, respectively. In addition, patients with atypical presentation had less coronary angiography and subsequent revascularization, anticoagulant, antiplatelet and B-blocker therapy. These patients were also less likely to receive aspirin, B-blockers, or statins after discharge, this was seemingly linked to the failure to identify the diagnosis initially. Bearing in mind the higher baseline risk of the population presenting without chest pain, those with atypical presentation frequently had in-hospital complications. On the other hand, the excessive mortality rate seen in the GRACE study was marked with almost 20% in-hospital mortality in the silent STEMI patients. Nevertheless, the absence of chest pain resulted in a greater probability of in-hospital death in all patients with ACS, and, even after multivariate analysis, the excessive mortality rate persisted among patients with unstable angina and STEMI[76].

Studies which did not show that diabetes mellitus is a predictor of atypical presentation of acute coronary syndrome (Table 3)

Numerous studies[77-81] have shown that diabetes mellitus is not a predictor of atypical presentation of ischemic syndrome. A study examined the disparities between Mexican Americans and non-Hispanic whites in the described symptoms of AMI. The symptoms in patients in a community-based surveillance program were determined to establish the differences between groups in relation to ethnicity, gender, and diabetic status. Information concerning the symptoms of 589 patients hospitalized and identified as having either definite or possible AMI (aged 25 to 74 years) was obtained. Chest pain was the most frequent complaint (83.2%), followed by chest

pressure or discomfort (67.6%), sweating (64.2%), fatigue (62.6%), dyspnea (60.3%), and arm or jaw pain (58.2%). After adjusting for age, diabetes mellitus, and gender, and relative to non-Hispanic whites, Mexican Americans frequently reported chest pain, upper back pain, and palpitations, but were less likely to report arm or jaw pain. Similarly, women predominantly reported fatigue, dyspnea, dizziness, upper back pain, palpitations, and cough, but less frequently reported chest pain. Substantial differences were observed in older compared to younger patients' symptoms[77].

Diabetics with AMI may present similar to non-diabetics. In a prospective, observational study in patients with typical and atypical symptoms consistent with cardiac ischemia, 216 diabetic and non-diabetic patients with AMI were compared, 24% were diabetic, with no significant difference in age ($P = 0.13$), female gender ($P = 0.13$), and time to presentation from symptom onset (192 ± 238 min *vs* 251 ± 456 min, $P = 0.41$). For diabetic *vs* non-diabetic with AMI, hypertension was more common in diabetic compared with non-diabetic patients with AMI (77% *vs* 50%, $P = 0.001$), and the same applied to elevated cholesterol (48% *vs* 33%, $P = 0.06$). No significant differences between diabetics and non-diabetics in terms of the frequency of chest pain (OR = 1.04; 95%CI: 0.95-1.14, $P = 0.30$), associated symptoms, and diagnostic ECGs (OR = 1.16; 95%CI: 0.76-1.79, $P = 0.53$) were observed[78].

Data from 2 registries of AMI patients presenting in hospital (MITRA PLUS with 18786 patients; North German Registry, NGR), analyzed AMI symptoms in 1042 diabetic and non-diabetic patients. Diabetics were significantly older and more often female than non-diabetics. No difference in the incidence of pre-infarction angina between the 2 groups (Mitra Plus) was observed. In the NGR, severe angina during AMI was perceived in 49.8% of diabetics *vs* 46.3% of non-diabetics (P = NS). In addition, 16.9% of diabetics and 15.0% of non-diabetics (P; NS) had SMI with no disparity in extra-thoracic pain, dizziness, nausea, sweating, palpitations, radiation of angina and localization of radiating pain in diabetics *vs* non-diabetics. Severe dyspnea occurred in 29.5% of diabetics and 19.5% of non-diabetics patients ($P = 0.003$). In this analysis, apart from a higher frequency of severe dyspnea in diabetics, no differences in the clinical symptoms of AMI patients with and without diabetes mellitus were noted. Silent or minimally symptomatic AMI was more common in non-diabetics[79]. A study determined the differences in symptoms in patients (50 women and 50 men) with and without diabetes during an episode of unstable angina. In this study diabetics were more frequently hypercholesterolemic (83% *vs* 60%), had a past cardiac history (85% *vs* 65%), and prior angiogram (85% *vs* 67%). Diabetics had less nausea (20% *vs* 40%), less squeezing (25% *vs* 48%) and less aching (25% *vs* 45%) pain, with more frequent hyperventilation as the presenting symptoms (27.5% *vs* 11.7%). With no difference in other cardiac symptoms seen in the two groups[80].

SILENT AND ATYPICAL MYOCARDIAL ISCHEMIA IN DIABETICS: TO SCREEN OR NOT?

Assuming a greater risk of cardiovascular events and more frequent silent CAD in diabetics compared to non-diabetics, screening asymptomatic diabetic patients for CAD is an attractive concept. Nevertheless, there are many elements against instigating a wide-ranging screening program. Of note is the paucity of confirmed data indicating that a prospectively utilized screening program has a positive prognostic impact in asymptomatic diabetic patients. From the above reviewed studies the incidence of atypical SMI is highly variable. Measures should be taken to manage hypertension and hyperlipidemia exclusively on the basis of diabetes status, devoid of diversity based on the presence or absence of recognizable CAD. From the above available data the studies which used stress single-photon emission computed tomography imaging showed around 50% abnormal images and 20% high-risk images, respectively. However, the DIAD (Detection of Ischemia in Asymptomatic Diabetics) study[42] described a considerably lower percentage of abnormal SPECT images (16%) and images with a very large (≥ 10% of the left ventricle) defect of 1%. We think that it is wise for the clinician to investigate silent and/or atypical myocardial ischemia and this applies to stable CAD in high risk diabetic patients, *i.e.*, patients with long-standing diabetes and diabetic complications such as diabetic neuropathy which may frequently present atypically. We suggest using a test which has high specificity and sensitivity for the detection of myocardial ischemia such as a myocardial perfusion scan and SPECT scan as shown in the above studies. The massive fiscal consequences of investigating all asymptomatic diabetic patients at intermediate and high risk using clinical scoring systems should be considered. Undoubtedly more investigations are required to address these issues.

CONCLUSION

Not all diabetics have the same coronary risk, therefore, it is important to determine which investigations to perform and for which patients. This strategy is reasonable as it allows identification of patients who require a medical or an invasive (angioplasty *vs* CABG) procedure, as these interventions may improve the prognosis. Patients with more than two risk factors may need further investigations with exercise stress testing which may provide supporting diagnostic and prognostic data. When exercise stress testing is sub-maximal or non-diagnostic, a second investigation with perfusion myocardial scintigraphy may be warranted bearing in mind that in diabetics this test may not have the same diagnostic accuracy as in the general population, but it is of prognostic value. Ischemia involving over 20%-25% of the myocardium justifies therapeutic investigation. Stress echocardiography is

comparable to scintigraphy.

The greater incidence of SMI in diabetics seems to be due to the increased frequency of ischemic heart disease in diabetics. The importance of cardiac autonomic neuropathy in SMI is still debatable, but is the most acceptable cause of SMI, as discussed in the above review, nevertheless studies are sporadic. The risk factors associated with SMI and atypical ischemic syndrome are the usual traditional factors *i.e.*, age, male gender, hypercholesterolemia, hypertriglyceridemia, hypertension, smoking, a family history of cardiovascular disease, insulin therapy (for type II diabetes), proteinuria, retinopathy, and peripheral occlusive arterial disease. Upcoming studies should determine possible approaches to augment the patient subgroup that will possibly benefit from screening with judicious cost-effective analyses. Currently, there are no data to support the use of anti-ischemic medication to improve CAD in diabetic patients.

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REVIEW

Renal sympathetic nervous system and the effects of denervation on renal arteries

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Abstract

Resistant hypertension is associated with chronic activation of the sympathetic nervous system resulting in various comorbidities. The prevalence of resistant hypertension is often under estimated due to various reasons. Activation of sympathetic nervous system at the renal- as well as systemic- level contributes to the increased level of catecholamines and resulting increase in the blood pressure. This increased activity was demonstrated by increased muscle sympathetic nerve activity and renal and total body noradrenaline spillover. Apart from the hypertension, it is hypothesized to be associated with insulin resistance, congestive heart failure and obstructive sleep apnea. Renal denervation is a novel procedure where the sympathetic afferent and efferent activity is reduced by various techniques and has been used successfully to treat drug-resistant hypertension improvement of various metabolic derangements. Renal denervation has the unique advantage of offering the denervation at the renal level, thus mitigating the systemic side effects. Renal denervation can be done by various techniques including radiofrequency ablation, ultrasound guided ablation and chemical ablation. Various trials evaluated the role of renal denervation in the management of resistant hypertension and have found promising results. More studies are underway to evaluate the role of renal denervation in patients presenting with resistant hypertension in different scenarios. Appropriate patient selection might be the key in determining the effectiveness of the procedure.

Key words: Resistant Hypertension; Sympathetic nervous system; Sympathectomy; Renal denervation; Radiofrequency ablation

Core tip: Resistant Hypertension is a serious condition that could result in various comorbidities, if left untreated. The pathogenesis involves activation of sympathetic nervous system at the renal level and systemic level. Surgical therapy targeted at the systemic level has serious systemic side effects. Renal denervation offers an unique way of mitigating the chronic activation of sympathetic nervous system and controlling the high blood pressure.

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INTRODUCTION

American Heart Association[1] and Joint National Committee[2] define resistant hypertension as blood pressure

that remains uncontrolled with the patient remaining compliant to 3 or more drugs, one of them being a diuretic. Care should be taken to differentiate resistant hypertension from uncontrolled hypertension, as the latter may be due to sub-optimal therapy, non-adherence to medications and secondary hypertension. The prevalence of resistant hypertension is often under estimated due to various reasons including inadequate sample size, exclusion of patients with resistant hypertension in larger studies[3,4]. Kaplan *et al*[3] have estimated that up to 5% of patients in general medicine clinics and approximately 50% of patients seen in renal clinics have resistant hypertension.

An important consideration in defining a patient with resistant hypertension is the frequent mislabeling of secondary hypertension as resistant hypertension and not addressing the issue of non-adherence to optimal therapy. This has been frequently reported in the literature including white-coat hypertension[5], non-compliance[6], secondary hypertension[1], and isolated systolic hypertension[7].

ROLE OF SYMPATHETIC NERVOUS SYSTEM IN HYPERTENSION

Renal sympathetic efferent and afferent nerves, which lie adjacent to the wall of the renal artery, are crucial for production of catecholamines contributing to hypertension. Surgical sympathectomy, targeted at removing sympathetic ganglia, to control hypertension has been reported even before the advent of newer antihypertensives[8]. Due to its profound side effects and the introduction of pharmaceutical sympatholytic agents, surgical sympathectomy is not a preferred procedure anymore. Renal denervation is a novel technique, which involves selective ablation of renal sympathetic nerve fibers and has demonstrated promising results in controlling resistant hypertension. The renal nerves are sensitive to ablation techniques such as s radiofrequency and ultrasound.

RENAL SYMPATHETIC DENERVATION AND HYPERTENSION

Various types of primary and secondary hypertension, including essential hypertension[9], renovascular hypertension[10], hypertension associated with disordered sleep breathing[11], hypertension associated with Cushing's syndrome[12] and primary aldosteronism, and preeclampsia, have been shown to have an association with sympathetic nervous system in various human and animal models.

Initially postulated to control circulation, sympathetic nervous system has been found to play a crucial role in initiation and maintenance of systemic hypertension through its effects on renal blood flow and perfusion[13,14].

Renal sympathetic nervous system consists of afferent and efferent sympathetic nerve fibers adjacent to the adventitious layer of the renal arteries. Efferent sympathetic nerves, when stimulated, have multitude of effects including increased renin secretion, decreased renal blood flow and increased renal tubular sodium absorption[15]. These changes contribute to the increased fluid retention and sustenance of vascular hypertension. Such sympathetic nerve fiber stimulation also contributes to increased renin mediated Angiotensin-Aldosterone activity further augmenting the hypertension.

The physiological effects of sympathetic nervous system in initiation, and maintenance of blood pressure makes it an excellent therapeutic target for drug and procedure based intervention in the management of hypertension. In animal models, Roman *et al*[16] has demonstrated that denervation resulted in leftward shift in the pressure natriuresis curve implying increased excretion of both water and sodium with no change in renal perfusion pressure.

Various types of hypertension have been shown to be ameliorated by renal denervation in different experimental models[14]. These experiments explored the role of efferent sympathetic nerve fibers in pathophysiology of development and maintenance of hypertension. Renal afferent sensory fibers act through a different mechanism in maintaining sodium and water homeostasis. These fibers are found primarily in the renal pelvic wall and they act through substance P and calcitonin gene related peptide, both of which act as primary neurotransmitters[17]. These fibers, by responding to changes in pressure in renal pelvis (mechanoreceptors) and chemical characteristics (chemo sensitive receptors) of urine, increase diuresis and natriuresis[18].

Activation of the efferent renal sympathetic nerve fibers can occur in response to augmented afferent signaling from renal sensory nerve fibers caused by various stimuli such as renal ischemia, hypoxia, and oxidative stress[19,20].

Renal afferent nerve fibers send signals to the hypothalamus and stimulate sympathetic outflow, causing hypertension and increased systemic vascular resistance[21,22]. Hausberg *et al*[23] reported similar effects of increased activity of sympathetic outflow due to renal afferent nerve signaling in end stage renal disease. In a recent study, Ceral *et al*[24] measured the serum drug levels of prescribed antihypertensive drugs to evaluate the adherence in individuals with difficult-to-control hypertension. In 65% patients, non-adherence was diagnosed. In upto 34 patients, no drugs were detected underscoring the importance of recognizing non-adherence in this population.

Such effects are also observed in patients with chronic kidney disease including end stage renal disease. Significant decreases in sympathetic activity have been demonstrated in patients with bilateral nephrectomy[25]. Converse[26] recorded the rate of sympathetic-nerve discharge to the muscular blood vessels in patients with chronic kidney disease with and without renal transplantation. He reported significant sympathetic over activity in End Stage Renal Disease (ESRD) patients with and without renal transplantation compared to normal subjects and in ESRD patients who had undergone nephrectomies. It has also been demonstrated that there is upto 30% sympathetic nerve re-innervation even in transplanted kidneys.

Further evidence of association of sympathetic over

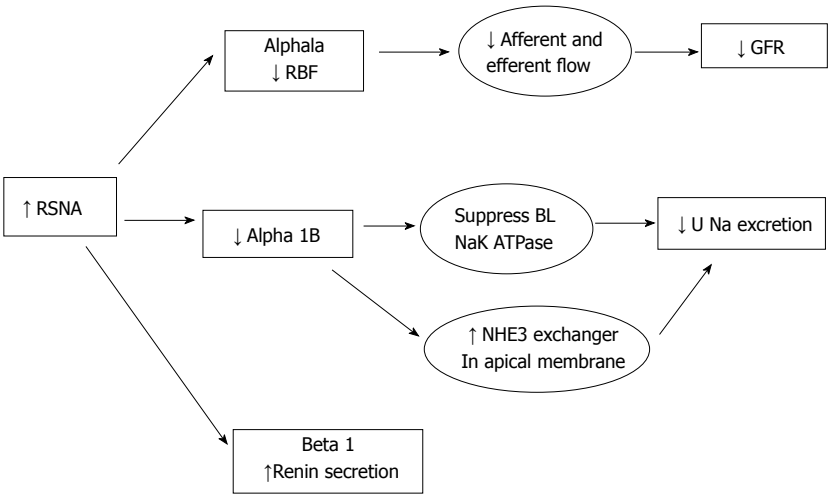

Figure 1 Showing intra-renal mechanisms of renal sympathetic activity. RSNA: Renal sympathetic nerve activity; RBF: Renal blood flow; GFR: Glomerular filtration rate; NHE: Sodium hydrogen transporter; U Na: Urinary sodium; BL: Baso-Lateral membrane.

activity with hypertension was seen in patients with obstructive sleep apnea. Marshall[27] and Cooper *et al*[28] elucidated that hypoxia seen in OSA results in increased sympathetic outflow to renal, cardiac and splanchnic beds and associated hypertension.

INTRA-RENAL FUNCTIONS OF THE RENAL SYMPATHETIC NERVOUS SYSTEM

It is elucidated that various physiological aspects of kidneys are regulated by sympathetic nervous system. Activation of sympathetic nerve fibers at the renal level results in locally increased release of norepinephrine and renin. This leads to renal vasoconstriction, decreased renal blood flow resulting in decreased glomerular filtration rate and increased renal tubular reabsorption of sodium and water at the tubular level[13]. Figure 1 shows intra-renal mechanisms of renal sympathetic activity

It is important to understand the physiological effects of sympathetic nervous system on the different ultra structural components in the kidneys to be aware of the outcomes of sympathetic denervation.

Renal blood flow: there is a decrease in renal blood flow with increased renal sympathetic nervous system activity. This decrease in flow is primarily mediated through increased afferent renal arteriolar vasoconstriction. There is also efferent arteriolar constriction, which helps sustain effective filtration pressure to sustain glomerular filtration rate (GFR).

Renal tubules

There is extensive sympathetic innervation in the entire renal tubule. The innervations are most dense in the thick ascending loop of Henle (TALH) followed by the proximal tubule, distal tubule and the cortical collecting duct. Activation of the SNS suppresses the NA+K+ ATPase at the basolateral membrane, which provides the energy for most of the transcellular transport that occurs across the luminal side of the tubules. There is also increased activation and expression of the NHE3 exchanger in the apical membrane which leads to increased Na retention across the tubules. The NKCC2 transporter at the TALH is also activated with SNS activation, which enhances salt absorption at this segment further increasing salt retention.

Renin secretion

Activation of the ERSNS increases rennin mRNA and therefore increases plasma and renal renin secretion. The increased renin secretion is partially mediated through the effects on the baroreceptors at the afferent renal arterioles. This increased renin secretion happens at low renal perfusion pressure even with minimal sympathetic nervous activation. The baroreceptor mediated renin release does not occur at high renal perfusion pressure states.

Reno-renal reflex

Increased pelvic pressure or high salt intake activates ARSN and thereby inhibits the ERSNA hence decreases salt retention and decreases blood pressure in normal kidneys. However in ischemic kidneys or chronic hypertension there is a reversal of the reno-renal reflex and ARSN activity further enhances the sympatho excitatory state and increases salt retention and hypertension. This reversal of the reno-renal reflex is significant since there is a greater expression of the afferent sympathetic nerves in patients with hypertension when compared to normotensive controls.

The above mechanisms enunciate the intricate significance of SNS and renal physiology in the development and maintenance of hypertension.

The increased sympathetic fiber traffic can be measured by microneurography- a clinical method of measuring multi[29] - and single[30] fiber activity in skeletal muscle fibers in humans. The measurement of microneurography allows direct and accurate measurement of NE activity when compared to measurement of plasma catecholamines.

Organ specific (for example, cardiac and renal) norepinephrine release can be quantified by "Norepinephrine Spillover" technique, which involves measuring organ specific outward flux of endogenous norepinephrine[31].

Although, renovascular hypertension and hyperten-

Table 1 Different techniques of renal denervation

Approach	Technique	Device	Study	Follow-up	Outcome
Invasive	RF ablation	Balloon:			
		OneShot	Renal hypertension ablation system trial[69]	12 mo	Average reduction in BP = 30.6 ± 22.0
		Vessix	REDUCE-HTN	Ongoing	
		Non-balloon:			
		Simplicity	SIMPLICITY I [35]	24 mo	32/14
			SIMPLICITY II [62]	6 mo	32/12
			SIMPLICITY III	Ongoing trial	
		Spiral	Renal hypertension ablation system trial[69]	12 mo	Average reduction in BP = 30.6 ± 22.0
		EnligHTN	EnligHTN I trial[70]	6 mo	Reduction in BP-26/10
	Ultrasound	Paradise	REALISE[71]	3 mo	Reduction in BP 22/12
		TIVUS	TIVUS I	Ongoing study	
Non-invasive	Ultrasound	Verve			
	Chemical	Cisplatin	Salman[72]	Animal study	
		Vincristine	Silva	Animal study	
		Guanethidine	Koistinaho[73]	Animal study	
		Neurotoxin	Apex nano nanomagnetic therapy	Animal studies	
	Other	Beta radiation cath	Novoste[74]		

sion due to chronic kidney disease are separate clinical entities than essential hypertension, they somehow share a common pathway with enhanced sympathetic nervous activity and activation of Renin Angiotensin Aldosterone System.

RENAL DENERVATION

Several experimental models have explored the role of renal sympathetic efferent and sensory afferent nerves in systemic and renal function by renal denervation. These experiments were done by surgical ligation and by surgical ablation of the renal nerve with phenol application in the adventitia of the renal arteries. The role of renal denervation was explored in clinically significant medical conditions such as hypertension[32], chronic kidney insufficiency[25] and in chronic heart failure in the past decades[33]. Dibona[13] elucidated the role of bilateral renal denervation in decreasing the sympathetic nerve fiber activity in various animal models including reno-vascular hypertension and chronic renal failure to reducing hypertension.

Renal denervation not only reduces renal sympathetic efferent activity selectively, but also decreases in whole body efferent sympathetic activity. Schlaich *et al*[34] and Krum *et al*[35] reported a considerable reduction in renal nor adrenaline spillover and a reduction in plasma renin activity[34]. Renal denervation also has shown to reduce whole-body noradrenaline spillover, evident by reduced sympathetic nerve signaling to the skeletal muscle vasculature. In a recent study, Hering *et al*[36] found substantial and rapid reduction in firing properties of single and multiple sympathetic vasoconstrictor nerve fibers.

The role of sympathetic nervous system in renovascular hypertension is well studied in animal models[37, 38]. Although, no study has been done in human models to evaluate the role of renal denervation in renovascular hypertension, the critical association of SNS activity and resistant hypertension is well established[38]. Table 1 shows different techniques of renal denervation.

SURGICAL SYMPATHECTOMY

As previously mentioned, sympathectomy was considered an effective modality of controlling hypertension as early as 1930s[39]. Splanchnicectomy, which includes sympathectomy of abdominal organs, was poorly tolerated due to its significant side effects including orthostatic hypotension, palpitations, anhidrosis and ejaculation defects[40]. Later, more conservative surgeries were performed at the level of thoracic vertebra[41]. Although a satisfactory blood pressure control and improvement of survival was seen in almost 50% of patients, it was not widely performed due to its adverse systemic effects. Advent of novel anti hypertensive medications has shifted the focus towards drug therapy in controlling severe hypertension. Sympathectomy has been reserved for severe resistant hypertension not responsive to medications.

CLINICAL STUDIES ON RENAL DENERVATION

Renal denervation offers the advantage of sympathectomy, yet involves denervation at the renal level largely avoiding the adverse effects of sympathectomy. Table 2 shows different clinical trials on renal denervation.

We present the human data that has demonstrated favorable reduction in blood pressure after renal denervation.

In SIMPLICITY I trial, 45 patients were included and radiofrequency ablation was done using a treatment catheter (Simplicity by Ardian Inc, Palo Alto, CA, United States). This is a non-randomized, prospective proof of concept study. Patients were eligible if they had systolic blood pressure of 160 mmHg or more, despite optimal

Table 2 Different clinical trials on renal denervation

Trial	Mean followup	Reduction in SBP/DBP	Location	Type	Primary outcome	Safety data
SIMPLICITY I 2009, (n = 50)[35]	6 mo	22/11	Australia/Europe	Catheter-based	Substantial and sustained BP reduction w/o serious adverse events	One case of Renal artery dissection
	12 mo	27/17			Substantial and sustained BP reduction w/o serious adverse events	
SIMPLICITY I F/u study 2011[42] (n = 153)	24 mo	32/14	Australia/Europe/ United States	Catheter-based	Substantial BP reduction	Groin pseudoaneurysms
SIMPLICITY II 2010, (n = 106)[43]	6 mo	32/12	Australia/Europe/ United States	Catheter-based	Meaningful reduction in BP	Hypertensive emergency in 3 cases
Mahfoud 2013, (n = 245)[48]	3 mo	19/13				
(n = 236)	6 mo	17/12				
(n = 90)	12 mo	16/10	Australia/Germany	Catheter-based	RDN imporved BP relevantly in office and ambulatory scenarios	No adverse events reported
Witowski *et al*[49]	6 mo	34/13	Poland/United States	Catheter-based	Improvement in severity of sleep apnea, glucose tolerance and BP	No adverse events reported
Brandt *et al*[75] 2012 (n = 110)	6 mo	29/8	Austria/Germany	Catheter-based	Improves BP, arterial stiffness and central hemodynamics	No adverse events reported
Davies *et al*[76] 2012, (n = 7)	6 mo	7/0.6	United Kingdom/ United States	Catheter-based	Improvement in symptoms and exercise capacity	No adverse events reported
Esler *et al*[62] 2012 (n = 106)	24 mo	32/12	Australia/Europe/ United States	Catheter-based	Saftety and continues benefit with denervation	Hypotension after denervation
Hering *et al*[64] 2012 (n = 15)	6 mo	32/15	Australia/Europe/ United States	Catheter-based	Safe and BP beneficial in resistant HTN and CKD stage 3-4	No peri- or postprocedural complications reported
	12 mo	33/19			Safe and BP beneficial in resistant HTN and CKD stage 3-4	
Mahfoud *et al*[77], 2011	3 mo	28/10	Germany	Catheter-based	Reduction in BP and glycemic control	None reported
Lambert *et al*[78] 2012, (n = 40)	3 mo	16/6	Australia/Europe	Catheter-based	Quality of life improved after denervation but not directly associated to BP reduction	None reported
Mahfound *et al*[77] 2011, (n = 37)	1 mo	28/10				
	3 mo	Dec-32	Australia/Germany	Catheter-based	Improvement in glucose levels and insulin sensitivity in addition to BP reduction	No significant adverse events reported
Ott *et al*[63] 2013, (n = 19)	6 mo	16/7	Germany/United States	Catheter-based	Significantly improvement in peripheral and central BP	No changes in renal function and perfusion
Schlaich *et al*[61] 2013, (n = 9)	3 mo	18/4	Germany/Australia/ Poland/United States	Catheter-based	RDN causes sustained lower BP in ESRD	One patient developed femoral pseudo-aneurysm
(n = 8)	6 mo	16/6				
(n = 6)	12 mo	28/5				
Steinberg *et al*[51] 2013, (n = 13)	12 mo	25/10	United States	Catheter-based	RDN patients displayed a significant reduction in systolic and diastolic pressure and maintained	No Adverse events reported
Ukena *et al*[50] 2012, (n = 2)	6 mo	No Info	Germany/ United States	Catheter-based	Ventricular tachyarrhythmias significantly improved after RDN	No complications reported
Vaclavik *et al*[79] 2013, (n = 1)	3 mo	No effect in this unilateral procedure	Czech Republic	Catheter-based	Unilateral Renal sympathetic denervation does not lower BP	No complications reported

CKD: Chronic kidney disease.

therapy with three antihypertensive drugs or more (including a diuretic). The primary endpoint was safety and reduction in blood pressure after the procedure and secondary endpoints were effects of the procedure on renal noradrenaline spillover and renal function. Patients with secondary hypertension including reno-vascular hypertension were excluded. The follow-up period was 1 year. There was a significant reduction in systolic and diastolic blood pressure at 1- and 3-mo follow up which remained consistent through out the follow-up period. In this proof of principle study, they found that the reduction in blood pressure was consistent suggesting neither significant nerve fiber recovery nor the development of any counter-regulatory mechanisms. Six patents did not have any response to treatment suggesting a possible different mechanism in the development of resistant hypertension

or inadequate therapeutic renal denervation.

The same study[42] was continued for a total of 24 mo and a sustained reduction in blood pressure was seen in the cohort.

SIMPLICITY Ⅱ[43] trial is a multicenter, prospective, randomized trial, which compared 52 patients who underwent renal denervation with 54 controls. During the follow-up of six months, patients with renal denervation had a decrease of blood pressure upto 32/12 mm HG, whereas control group had no reduction in blood pressure. This study included 11 patients with early stage 3 CKD (GFR 45-60 mL/min per square meter) and found no significant worsening of renal function. Ambulatory blood pressure monitored in 20 patients in the study population showed a reduction of 11/7-mmHg.

Brinkmann *et al*[44] nalyzed a small subset of patients (n = 12) who underwent renal denervation. Mean follow up period was 6 mo. Only 3 patients had clinically significant reduction in blood pressure (157 ± 7/85 ± 4 mmHg before and 157 ± 6/85 ± 4 mmHg after renal denervation). No significant reductions in sympathetic nerve fiber activity were noted either [prior to denervation- 34 ± 2 bursts per minute and after denervation -32 ± 3 bursts per minute (P = 0.6)]. In 7 patients, post denervation blood pressure was actually higher compared to the pre denervation blood pressure. Interestingly, 5 out of 12 patients did not meet the criteria for resistant hypertension (pre denervation BP was less than 140/90.

In a recent study done on patients with resistant hypertension sent for renal denervation workup, Fadl Elmula *et al*[45] reported only 6 of 18 patients met criteria for resistant hypertension that underwent renal denervation. Twelve patients did not meet the criteria of resistant hypertension for different reasons, including one patient with primary hyperaldosteronism, one with renal artery abnormality and, five patients with normalized ambulatory blood pressure after witnessed drug intake. Out of those 6 patients, only 2 had decreased blood pressure that was sustained for 6 mo.

In a recent report, Vonend *et al*[46] reported a brief reduction in blood pressure followed by resurgence of hypertension and occurrence of renal artery stenosis after the renal denervation.

Savard *et al*[47] reported that only a fraction of patients with resistant hypertension referred for renal denervation actually qualified for denervation based on the strict guidelines laid out by European Society of Hypertension.

Mahfoud *et al*[48] reported the results observed in 303 resistant hypertensive patients and followed them for a period of 12 mo. At 3, 6, and 12 mo follow-up, office systolic blood pressure (SBP) was reduced by 21.5/23.7/27.3 mmHg, office diastolic blood pressure (BP) by 8.9/9.5/11.7 mmHg, and pulse pressure by 13.4/14.2/14.9 mmHg (n = 245/236/90; P for all < 0.001). Response to RDN has been defined as a reduction in office SBP ⩾ 10 mmHg 6 mo after treatment.

The most recently concluded SIMPLICITY 3 trial in North America has demonstrated that the primary outcomes were not met on initial analysis of the results. However the study has demonstrated safety in the patients who underwent the denervation procedure. The study was a randomized single blinded case controlled study, which included a sham procedure on the control arm. The primary outcome was decrease in office blood pressure and the secondary outcome was reduction in ambulatory blood pressure at the end of the 6 mo follow-up. Despite the failure of this trial to demonstrate a significant reduction in blood pressure when compared to a sham procedure, an inference cannot be drawn that renal artery denervation is not an effective therapeutic modality anymore. The efficacy of the denervation with this catheter has not been compared to other devices, which use other modalities of generating energy (ultrasound, laser, *etc*.) to ablate the renal nerves. Also the magnitude of denervation has not been assessed in the SIMPLICITY 3 trial. This raises the question if the denervation achieved in the SIMPLICITY 3 trial was adequate to achieve the clinical benefits seen in some of the European and Australian studies. Further analysis from this study would enlighten most of us with the reasons for the lack of benefit from renal artery denervation in this well performed study.

RENAL DENERVATION IN OTHER CONDITIONS

Obstructive sleep apnea

Witkowski *et al*[49] studied 10 patients with refractory hypertension and sleep apnea who underwent renal denervation and were evaluated at 3- and 6-mo after the procedure. Changes in ambulatory blood pressure and polysomnography were monitored during the follow-up period. Three and 6 mo after the denervation, decreases in median office systolic and diastolic BPs were: -34/-13 mmHg at 6 mo. In addition to the reduction in blood pressure, there was also an improvement in glycemic control and decrease in apnea-hypopnea index.

Arrhythmias

Ukena *et al*[50] first reported the role of renal denervation in successfully treating two patients with refractory ventricular tachycardia storm.

In a recent study, Steinberg *et al*[51] enrolled 27 patients with atrial fibrillation (14 randomized to Pulmonary Vein Isolation alone and 13 randomized to Pulmonary Vein Isolation with Renal denervation. The follow-up period was 12 mo after ablation. At 12 mo, the reductions in systolic and diastolic blood pressures were successfully and significantly maintained (P < 0.001 *vs* pulmonary vein isolation only) resulting in a fall from baseline of 25 ± 5 mmHg and 10 ± 2 mmHg, respectively. This effect was thought to be due to increased atrial stretching and dilation (*i.e*., atrial substrate), when blood pressure is elevated resulting in deleterious atrial electrical consequences that promote AF. With the ablation of afferent renal nervous input, central sympathetic output is decreased and autonomic triggers and substrate potentiators of AF are at-

tenuated.

The results were similar to another study[52] that demonstrated a deceased incidence of AF recurrences in patients that underwent both pulmonary vein isolation (PVI) and renal artery ablation over time compared with the control PVI-only group.

Chronic kidney disease

Renal denervation has been studied in chronic kidney disease (CKD) - another subset of patients known to have resistant hypertension. Although the decreased clearance of catecholamines was thought to be a factor, the theory was not proven by enhanced clearance upon postural changes[53]. Levitan *et al*[54] demonstrated that clonidine (acting as a sympatholytic) significantly decreases norepinephrine secretion and mean blood pressure when compared to controls in patients with chronic kidney disease. Various mechanisms including increased catecholamine sensitivity[55], renal ischemia[56] and decreased oxygen supply[57] have been proposed as the reason for refractory hypertension in CKD patients. Nevertheless, sympathetic hyperactivity is prevalent in CKD and its role in organ damage is well substantiated.

Zoccali *et al*[10] demonstrated that sympathetic over activity in ESRD is an independent predictor of fatal and non-fatal cardiac events. Hering *et al*[58], had performed bilateral renal denervation in 15 patients with resistant hypertension and stage 3-4 CKD (mean GFR of 31 mL/min per 1.73 m^2) and found consistent reduction in blood pressure with an average systolic and diastolic blood pressure decrease of 34 mmHg and 19 mmHg respectively. They also reported considerable reduction in nocturnal BP control. This is of much importance as nocturnal BP has been shown to predict cardiovascular mortality in hypertensive patients[59,60].

Hering *et al*[58] performed bilateral renal denervation in 15 patients with resistant hypertension and stage 3-4 CKD. Mean changes in office systolic and diastolic BP at 1, 3, 6, and 12 mo were -34/-14, -25/-11, -32/-15, and -33/-19 mmHg, respectively.

In another study, renal denervation was performed in 12 ESRD patients[61] and significant reduction in blood pressure was seen in all subjects.

Few other studies[62,63] also found similar effects in patients with chronic renal disease.

Insulin resistance and obesity

In a recent study, Hering *et al*[64] found reduction in fasting glucose and insulin levels in patients treated for resistant hypertension by renal denervation.

Obesity related hypertension has been associated with resistant hypertension[65]. Holecki *et al*[66] surveyed approximately 5000 patients with obesity and hypertension and found an increased need in the number of anti-hypertensive medications used with an increase in BMI. Approximately 12% of patients with body mass index (BMI) between 30 and 34.9, 16% of patients with BMI between 35 and 39.9 and 26% of patients with BMI > 40% were found to have resistant hypertension. Although the absolute pathophysiology of hypertension in obese patients has not been well elucidated, experimental and clinical studies conducted in the past few decades have demonstrated an association between increased sympathetic activity and obesity. This association was demonstrated by Rumantir[67], who found twice normal increase in mean renal noradrenaline spillover in normotensive as well as hypertensive obese patients compared to non-obese patients. The effect of renal denervation in reducing the blood pressure was studied in obese dogs with hypertension[68]. Renal denervation decreased plasma renin activity and abolished the hypertension in those dogs but failed to suppress systemic sympathetic activity.

COST-EFFECTIVENESS

Most of the renal denervation procedures are performed in Europe, as the device for catheter-based denervation has not been approved by FDA for clinical use in the United States. The average cost of the catheter is 2000 to 3000 United States dollars. The average cost for the procedure in Germany is 4000 to 5000 United States dollars. Patients are admitted to the hospital and observed overnight after the procedure. All the studies published so far have demonstrated blood pressure lowering affects few days to months after the procedure, there have not been reports of a precipitous immediate reduction in blood pressure after the procedure. Hence the reason for an overnight stay is difficult to justify for a procedure that is similar to most catheter based procedures performed through a femoral arteriotomy. Over 60 million population in United States are estimated to have hypertension. Cost and appropriate patient selection would be a major determinant next to patient outcomes in implementing denervation program for hypertensive patients in the United States. Practices with certified hypertension specialists and a suitable arrangement for performing these procedures as an outpatient would be ideal for appropriate patient selection and reducing cost.

CONCLUSION

Renal nerve denervation has been explored as a modality of treatment for resistant hypertension for several decades. Targeting renal nerves through a non-surgical approach has generated more interest in pursuing denervation as an option for hypertension refractory to conventional medical management. Despite controversies in the true prevalence of resistant hypertension, the existence of such a disease is beyond clinical doubt. Long-term patient outcomes including mortality and renal outcomes are yet to be substantiated with evidence from on-going and future trials with hard outcomes. Though the SIMPLICITY 3 trial did not reach primary outcomes, there could be other systemic benefits secondary to sympathetic denervation, which is yet to be proven in clinical trials. Also the effectiveness of denervation with the SIMPLICITY catheter has not been compared to other devices capable of denervating the renal arteries. With the established procedural safety from the SIMPLICITY

3 trial it might be safe and cost-effective to perform these procedures in an outpatient setting for a few selected patients who may still benefit from renal nerve denervation.

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REVIEW

Is reversal of endothelial dysfunction still an attractive target in modern cardiology?

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Abstract

Although the endothelium has a number of important functions, the term endothelial dysfunction is commonly used to describe impairment in its vasodilatory capacity. There have been numerous studies evaluating the relationship between endothelial dysfunction and cardiovascular disease, however assessment of endothelial function is perhaps still primarily thought of as a research tool and has not reached widespread clinical acceptance. In this review we explore the relationship between endothelial dysfunction and cardiovascular disease, its prognostic significance, methods of pharmacological reversal of endothelial dysfunction, and ask the question, is reversal of endothelial dysfunction still an attractive target in modern cardiology?

Key words: Endothelium; Vascular; Nitric oxide; Atherosclerosis; Risk factors; Flow-mediated dilatation

Core tip: There is an abundance of evidence suggesting that endothelial dysfunction is present throughout a wide spectrum of cardiovascular disease and is an independent indicator of adverse prognosis. Despite this, perhaps due to lack of standardisation of investigative techniques, endothelial function assessment is not yet routinely undertaken, despite a number of therapies which have been shown to have beneficial effects on the endothelium. More studies are required to judge whether assessment of endothelial function can impact on clinical management and prognosis.

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INTRODUCTION

For many years the vascular endothelium was thought of as simply a selectively permeable barrier between the intra- and extravascular compartments. However, discovery by Furchgott *et al*[1] that the large blood vessels of mammals only dilate if the endothelium is intact due to its response to nitric oxide (NO) was the first step in our understanding that the endothelium is a key modulator of cardiovascular health. Indeed, the integrity of the vascular endothelium is essential for providing adequate blood flow and antithrombotic activity. While these are key functions of the endothelium, in the context of cardiovascular health, the key function of the endothelium is maintenance of vasodilatation in response to NO. The healthy human endothelium maintains a vasodilated state as a baseline, in part due to NO production from L-arginine by nitric oxide synthase. NO then diffuses into the endothelium, leading to increased cyclic guanosine monophosphate (GMP) production and vasodilatation[2]. Damage to the endothelium, whether anatomical or functional can cause a disturbance of this pathway leading to endothelial dysfunction. There are three potential mecha-

nisms that can lead to endothelial dysfunction (either in isolation or combination): reduced production of NO[3]; reduced availability of NO[4] or antagonism of NO by endothelium derived contracting factors[5]. Indeed, although NO is the main endothelium-derived relaxing factor there are other factors active on the endothelium, all of which play a key role in its health. Other endothelium-derived relaxing factors include prostacyclin and endothelium-derived hyperpolarizing factor, both of which can show increased activity in response to a decrease in NO. Meanwhile, there are several endothelium-derived contracting factors causing vasoconstriction such as endothelin-1, thromboxane A_2 and prostaglandin H_2. Nevertheless, the majority of clinical studies have concentrated on NO, and this will be the focus of our review.

NO has a number of vascular protective roles including inhibition of platelet aggregation and leucocyte adhesion, however endothelial dysfunction can be simply described as the imbalance of vasodilatation and vasoconstriction caused by vasoactive substances acting on the endothelial cells[6]. Endothelial dysfunction is present in a number of cardiovascular conditions such as diabetes, hypercholesterolemia and hypertension and seems to be an important feature in the pathogenesis of the atherosclerotic disease process.

In this review, we will discuss the association of endothelial dysfunction with the cardiovascular disease, its prognostic relevance, methods for reversing endothelial dysfunction and their impact on outcome.

HOW DO WE QUANTIFY ENDOTHELIAL FUNCTION CLINICALLY?

Theoretically endothelial function can be measured in any artery. In most methods the endogenous NO-dependent vasodilation is measured using a pharmacological agonist such as acetylcholine (Ach) or other substances which stimulate endogenous NO production. Comparison is then made with NO-independent vasodilatation using substances such as glyceryl trinitrate. Invasive measurement of the coronary artery response to acetylcholine is a validated measurement of coronary artery endothelial function and was the first method used to demonstrate endothelial function[7,8]. Using quantitative coronary angiography the change in diameter of the artery can be measured in response to Ach. In dysfunctional coronary arteries Ach causes reduced vasodilatation or apparently paradoxical vasoconstriction due to the unopposed direct smooth muscle muscarinic action of Ach at apparently high concentrations[9].

Non-invasive measures include what is considered by many as gold standard, venous occlusion plethysmography. This technique is used to assess forearm blood flow (in the brachial artery) in response to an inflated blood pressure cuff. The inflation of the cuff occludes venous return (but not arterial inflow) thus creating a "reservoir" of blood within the anatomically isolated limb region (forearm). The rate of vessel swelling can be measured as a surrogate for vascular resistance while the volume increases in proportionally in relation to the forearm blood flow[10]. Endothelial function, which is closely related to NO bioactivity, can be measured by constructing dose-response curves to escalating doses of Ach and measuring the rate of change in arm swelling by strain gauge. One advantage of this technique is that measurement of forearm blood flow in the contralateral arm can be used as a further within patient control, allowing optimal reproducibility[11]. Nevertheless, the requirement for arterial cannulation may limit patient tolerability and repeatability.

Flow-mediated dilation (FMD) is probably the most common method of endothelial function assessment. This technique involves using ultrasound to measure the peripheral arterial response (again, usually the brachial artery) to temporary ischemia caused by inflation and release of a cuff. Release of the cuff causes an increase in blood flow and therefore shear stress which stimulates NO release and leads to vasodilatation. The increase in diameter of the blood vessel from baseline can be measured by two dimensional ultrasound and is related (but not exclusive) to NO bioavailability, giving an excellent measure of endothelial function which can again be compared to dilatation using endothelium-independent vasoactive substances[12]. Of note, FMD has been shown to have excellent correlation with coronary endothelial function[13].

A more recently developed method of assessment is peripheral arterial tonometry (PAT). This technique allows non-invasive measurement of vasomotor function by measuring plethysmographic changes in the fingertip pulse. Again, the endothelium-dependent response can be ascertained by arterial cuff occlusion[14]. PAT has also been shown to correlate well with both coronary endothelial function and FMD[15,16].

WHAT IS THE CLINICAL RELEVANCE OF ENDOTHELIAL DYSFUNCTION?

While several methods have been developed to assess endothelial function in different arterial beds, there can only be any benefit to quantification of endothelial dysfunction if there is evidence that it can be used to identify groups with an adverse prognosis.

Several studies have shown a relationship between endothelial dysfunction, coronary disease risk factors and atherosclerosis. One of the earliest studies revealing this relationship was carried out by Ludmer *et al*[8] who discovered that in patients with both mild and advanced coronary artery disease (CAD) there was paradoxical vasoconstriction induced by acetylcholine. Evidence of endothelial dysfunction has also been noted in patients with risk factors for CAD but without angiographically significant CAD, suggesting that endothelial dysfunction may indeed predate the development of clinically significant atherosclerosis[17,18]. Age[19], diabetes mellitus[20-22], smoking (both active and passive)[23-25], hypertension[26] and hyperlipidemia[27,28] have all been associated with endothelial

dysfunction prior to the development of clinically significant CAD. Furthermore, patients with a combination of risk factors (such as smoking and hypercholesterolemia) have been shown to have worse endothelial function than those with a single risk factor[29].

The presence of endothelial dysfunction has been shown to be a predictor of cardiovascular events independent of the arterial bed studied or method of assessment[30,31]. Much of this effect is due to the fact that endothelial dysfunction is invariably present whenever there is end-organ damage. This is clinically manifested as atherosclerosis, left ventricular hypertrophy, small vessel brain ischemia and renal impairment, leading to significant morbidity and mortality[32-34]. Not unreasonably, endothelial function assessment could be considered as the barometer of vascular health[35]. Large studies investigating the prognostic value of endothelial function assessment using FMD are summarized in Table 1.

So why has endothelial dysfunction assessment not been adopted more widely clinically? As we have discussed, FMD appears to be the most robust and widely used technique, yet it very rarely appears in any clinical guidelines. One reason may be that although FMD does have predictive value, there are of course several other risk factors that may be easier to assess which are also predictors of adverse cardiac outcome[31]. Secondly, although many studies have reported the excellent reproducibility and variability of FMD measurement in multiple institutions[36-39], these studies all rely on following an "ideal" protocol for obtaining FMD measurements. According to a recent paper published by the European Society of Cardiology, this includes 10 min rest for the patient prior to measurement, correct cuff placement, an occlusion time of 5 min and measurement 45-60 s after cuff release[40]. Clearly, following this prescribed methodology takes some time and is prohibitive to its use within the clinical setting, however, not using these techniques can lead to inaccurate measurements, thus diluting the utility of FMD measurements. Automated analysis software may well overcome some of the difficulties regarding standardization of results[37], however, when it is much simpler to check a cholesterol level or measure a blood pressure, it is easy to see why FMD has perhaps not yet penetrated the clinical realm. Also, FMD is strongly influenced by baseline brachial artery diameter, and changes in FMD tend to vary based on this[41]. Finally, the absence of normal values makes it difficult to provide any clinically relevant recommendations to non-experts in the field of endothelial function assessment.

ENDOTHELIAL DYSFUNCTION IN ASYMPTOMATIC PATIENTS

In asymptomatic patients, most clinicians use the assessment of risk factors, such as the Framingham Risk score, to assess cardiovascular risk[42]. Studies looking at the independent prognostic value of FMD in prediction of adverse events in asymptomatic patients have shown mixed results. Suzuki *et al*[43], in the Northern Manhattan Study, evaluated 819 patients with cardiovascular risk factors and showed that patients with metabolic syndrome and endothelial dysfunction (measured by FMD) were at a higher risk for stroke, myocardial infarction MI or cardiovascular death than those without endothelial dysfunction. In one of the largest studies to date, Yeboah *et al*[44] reported that in 2792 patients with 5 years of follow up, FMD was an independent predictor of a poor outcome, however it did not appear to add much to the overall predictive model. Further large cohort studies have also shown that FMD is an independent predictor of adverse events, although there is some question as to whether the small incremental increase in prediction provided by the assessment of endothelial function mandates the routine clinical use of FMD[31,44-46]. Indeed, other large studies have not found incremental predictive value with use of FMD. A large study of 842 asymptomatic patients in the Northern Manhattan Study found that although FMD did predict adverse outcomes it was not an independent predictor when included in a multivariable analysis including traditional cardiac risk factors[47]. Two further studies found that while FMD was not an independent predictor of adverse events, several components of endothelial function measurement, such as hyperemic velocity and assessment of resistance artery endothelial function, were[48,49]. In general, there is still doubt that endothelial dysfunction is a predictor of adverse cardiovascular events in asymptomatic patients.

ENDOTHELIAL DYSFUNCTION IN ESTABLISHED CAD (CHRONIC STABLE CAD)

Endothelial dysfunction in the coronary arteries is closely related to systemic endothelial dysfunction[13]. In patients with CAD the presence of severe endothelial dysfunction has been shown to be a predictor of cardiac death, myocardial infarction or revascularization[50]. These results have been replicated in other large studies[51-53]. Endothelial dysfunction has also been related to adverse plaque characteristics (such as lipid-rich necrotic cores) in this group of patients[54,55]. FMD has also been shown to be an independent predictor of in-stent restenosis in patients with single vessel coronary artery disease undergoing percutaneous coronary intervention[56]. Elsewhere in the vascular tree, FMD has also been shown to be a predictor of post-operative MACE in patients with hypertension[57], early peripheral arterial disease[58] and those undergoing vascular surgery[59].

ENDOTHELIAL DYSFUNCTION IN ACUTE CORONARY SYNDROMES

Over the past decade there has been an increasing realization that acute coronary syndromes (ACS) cannot be predicted simply by risk factors or even the presence of ob-

Table 1 Large studies evaluating the prognostic value of flow-mediated dilation

Ref.	Number of patients	Cohort	Asymptomatic Patients?	Length of follow-up (mo)	Outcome	Result	Independent value of FMD?
Rossi *et al*[45]	2264	Post-menopausal women	Yes	45 ± 13	CV death, MI, revascularisation, TIA, stroke	FMD was a predictor of MACE independently of traditional cardiac risk factors.	Yes
Patti *et al*[56]	136	Patients with single-vessel coronary artery disease undergoing PCI	No	6	In-stent restenosis	Patients with impaired FMD were more likely to suffer in-stent restenosis.	Yes
Gokce *et al*[59]	187	Patients undergoing vascular surgery	No	1	CV death, MI, unstable angina, ventricular fibrillation, stroke, raised troponin	FMD was an independent predictor of MACE in the immediate post-operative period.	Yes
Brevetti *et al*[58]	139	Patients with peripheral arterial disease	No	23 ± 10	CV death, MI, revascularisation, TIA, critical limb ischaemia	FMD was an independent predictor of events over the follow-up period.	Yes
Chan *et al*[53]	152	Patients with coronary artery disease	No	34 ± 10	CV death, MI, revascularisation, claudication	FMD was a strong independent predictor of risk even accounting for carotid plaque burden.	Yes
Shimbo *et al*[47]	842	Asymptomatic multi-ethnic cohort	Yes	36	Vascular death, MI, stroke	FMD was able to predict adverse events but not independently.	No
Suzuki *et al*[43]	819	Asymptomatic multi-ethnic cohort including patients with metabolic syndrome	Yes	81 ± 21	Vascular death, MI, stroke	Patients with the combination of metabolic syndrome and endothelial dysfunction had a significantly worse outcome.	No
Yeboah *et al*[44]	2792	Mixed cohort of patients > 65 yr	No	60	CVD death, MI, stroke, congestive heart failure, claudication, revascularisation	FMD was an independent predictor of risk but added little to traditional risk stratification.	Yes
Muiesan *et al*[57]	172	Hypertensive patients	No	95 ± 37	CV death, MI, revascularisation, arrhythmia, TIA, critical limb ischaemia, retinal artery occlusion	FMD below median was independently associated with adverse outcome.	Yes
Shechter *et al*[46]	618	Healthy subjects (mixed)	Yes	55.2 ± 21.6	CV death, MI, stroke, congestive revascularisation	FMD predicted adverse outcome independently.	Yes
Katz *et al*[77]	259	Heart failure patients (LVEF < 40% and NYHA class 2-3)	No	28	Death or cardiac transplantation	FMD is associated with increased adverse outcome in ischaemic and non-ischaemic heart failure.	Yes

PCI: Percutaneous coronary intervention; MACE: Adverse major cardiovascular events; MI: Myocardial infarction; TIA: Transient ischaemic attack; FMD: Flow-mediated dilation.

structive CAD[60,61]. The development of the "vulnerable plaque" concept that leads to ACS (and sudden cardiac death) is influenced by omnipresent endothelial dysfunction *via* several methods. Endothelial dysfunction leads to reduced expression of anti-inflammatory mediators, leading to plaque destabilization[62]. In particular, Endothelin-1, a potent vasoconstrictor, is released significantly more by the dysfunctional endothelium as well as directly at the site of unstable coronary plaque lesions[63]. The predominant vasoconstriction of the dysfunction coronary artery may cause plaque rupture directly[64]. Finally, the dysfunctional endothelium also has reduced antithrombotic tendency allowing thrombus formation[65].

Endothelial dysfunction is also a predictor of adverse outcome in patients after ACS. Improvement of endothelial function post-ACS is associated with improved prognosis[66,67]. Endothelial dysfunction has also been shown to lead to adverse remodeling post-ACS[68].

ENDOTHELIAL DYSFUNCTION IN HEART FAILURE

There is ample evidence to suggest that endothelial function is impaired in patients with both acute and chronic heart failure[69]. NO has been shown to be involved in myocardial relaxation[70], and reduction in NO availability (for the same reasons as seen in the vasculature) can impair left ventricular relaxation, causing diastolic dysfunction. The presence of diastolic dysfunction is associated with impaired FMD in patients with established CAD[71]. The presence of endothelial dysfunction has also been associated with perfusion defects and reduced coronary

flow in patients with suspected coronary artery disease thus potentially leading to impaired ventricular function[72,73]. In chronic heart failure there may be a vicious circle effect, by which the reduction of cardiac output leads to a decrease in vascular shear stress and NO production, therefore causing further worsening of endothelial function[74]. FMD has also been shown to be a predictor of adverse outcome in heart failure patients[75-78].

In acute heart failure, there is also a reduction in NO availability leading to vasoconstriction and increased vascular stiffness, increasing afterload. There is also increased endothelin-1 production and oxidative stress, again placing further strain on the heart and vasculature[79,80]. Coronary artery endothelial dysfunction has been shown to predict progression of allograft vasculopathy and mortality in patients with orthoptic heart transplantation[81,82].

Endothelial dysfunction is associated with adverse outcome in patients with LV dysfunction[83-85]. It has also been shown to be a good predictor of response to cardiac resynchronization therapy (CRT)[86].

CAN ENDOTHELIAL DYSFUNCTION BE REVERSED?

We have shown that there is substantial evidence to support the role of endothelial dysfunction in the development and progression of cardiovascular disease and its prognostic role. Because of this there has been a significant interest in finding methods to ameliorate endothelial dysfunction. Despite many drug classes being evaluated, only a few have shown concrete benefits on the endothelium. Large clinical studies evaluating pharmacological endothelial dysfunction reversal are summarized in Table 2.

Some of the most studied drug classes are those that act on the renin-angiotensin system, namely angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin-Ⅱ receptor antagonists (ARBs). These drugs have several anti-oxidant and anti-inflammatory effects, reducing superoxide (thus reducing oxidative stress) and endothelin-1 activity[87]. Angiotensin Ⅱ stimulates angiotensin type 1 receptors (AT1) to mediate arteriolar vasoconstriction and remodelling, superoxide anion production, renal sodium reabsorption, aldosterone secretion and endothelin-1 release[88]. Many of these actions affect the vascular endothelium adversely. On the other hand stimulation of the angiotensin type 2 (AT2) receptor by angiotensin has mainly opposing actions to those of AT1 stimulation and recently has been shown to contribute to endothelial NO release[89]. AT2 production can be reduced by angiotensin converting enzyme inhibitors which also increase both tissue and plasma bradykinin by inhibiting kininase II[90]. By stimulating the B2 receptors, bradykinin mediates the release of NO, prostacyclin and the endothelial hyperpolarizing factor; agents that produce vasodilation[91-93]. The large TREND study provided evidence that quinapril was able to reverse endothelial dysfunction[94]. The beneficial effects of ACEIs have been replicated by several other studies[95-98]. Angiotensin-Ⅱ receptor antagonists have also shown similar results[99,100].

Spironolactone and eplerenone, which have mineralocorticoid receptor antagonist activity have received much attention recently. They have been reported to improve NO bioactivity in patients with heart failure[101]. The mechanism(s) by which aldosterone impairs endothelial function is unclear. Aldosterone enhances vascular responsiveness to pressor agents such as norepinephrine and angiotensin Ⅱ[102]. Also, aldosterone can cause direct vascular smooth muscle contraction *via* a non-genomic pathway that has not yet been characterised. Both drugs have however been shown to improve endothelial function in patients with heart failure and hypertension[103-106].

Beta-blockers and diuretics have generally been shown to have no effect on endothelial function however, newer beta-blockers such as nebivolol and carvedilol have shown some beneficial effects on reversal of endothelial dysfunction[107-109]. Nebivolol has a direct effect on NO synthase while carvedilol has some antioxidant properties. Calcium channel antagonists also improve endothelial dysfunction by several pathways, particularly in the coronary microvasculature by indirectly increasing in intracellular smooth muscle cell cGMP, which is the second messenger of NO and mediates vasodilation[110,111]. Two additional mechanisms have been described to explain the effects of calcium channel blocker in the forearm circulation. The first explanation is that most calcium channel blockers have antioxidant activities, reducing production of superoxide anions[88,89]. The second explanation involves a reduction in endothelin-1 release by calcium channel blockers. Endothelin-1 is a potent vasoconstrictor and it is released from the endothelium[112]. Normally, there is a balance between vasoconstrictive and vasodilating substances in the vasculature but in hypertension, the bioavailability of endothelin might be increased in parallel with a reduction in NO bioactivity. It has shown that calcium channel blockers improved NO bioactivity by reducing endothelin release[100,101]. In addition, Cardillo *et al*[113] have recently shown that in patients with essential hypertension, the increased endothelin activity is partly responsible for the increased vascular tone. Hence, in a model where vasoconstrictive activity is increased, such as hypertension, a reduction of endothelin release would improve NO bioactivity. CCBs may also improve other aspects of endothelial dysfunction, reducing tissue plasminogen activator activity, thus reducing thrombogenic risk by decreasing platelet activation[114].

Statins also have proven beneficial effects on endothelial dysfunction in addition to their effects on lipids[115-118]. Reduction in LDL-cholesterol is thought to be the main method by which statins improve endothelial function, however, they also enhance expression and activity of NO synthase and reduce C-reactive protein (which has deleterious effects on the endothelium)[119,120]. On a similar, intriguing, theme of non-antihypertensive therapies improving endothelial function, recent studies have also suggested that drugs such as metformin[121], ranolazine[122] and allopurinol[123] may also improve endothelial function.

Table 2 Selected studies examining pharmacological reversal of endothelial dysfunction

Ref.	Drug	Cohort	Design	Results
Mancini *et al*[94]	Quinapril	105 normotensive patients with coronary artery disease	Randomised double-blind, placebo controlled	Quinapril improved endothelial function compared to placebo as measured by coronary artery diameter response to acetylcholine
Higashi *et al*[96]	Various ACE inhibitors, beta-blockers, calcium channel blockers and diuretics	296 hypertensive patients	Multi-centre cohort study	ACE inhibitors significantly improved endothelial dependent vasodilatation compared to other drug classes as measured by forearm blood flow
Wassmann *et al*[97]	Candesartan, felodipine	47 patients with high cholesterol	Randomised double-blind, placebo controlled	Candesartan improved forearm blood flow compared to felodipine or placebo
Ghiadoni *et al*[98]	Nifedipine, amlodipine Perindopril, telmisartan, atenolol, nebivolol	168 patients with hypertension	Randomized, single-blind, parallel-group	Only perindopril improved FMD (although perindopril, telmisartan, nifedipine and amlodipine reduced oxidative stress and increased plasma antioxidant capacity)
Tzemos *et al*[99]	Valsartan, amlodipine	25 hypertensive patients	Randomised double-blind, crossover	Valsartan improved forearm blood flow
Takagi *et al*[100]	Telmisartan	Mixed; 398 patients	Meta-analysis of 7 studies	Statistically significant increase in FMD by 48.7%
Farquaharson *et al*[101]	Spironolactone	10 patients with NYHA class I-II heart failure	Randomised, double-blind placebo-controlled crossover study	Spironolactone improved forearm blood flow compared to placebo
MacDonald *et al*[103]	Spironolactone	43 patients with NYHA class I-II heart failure	Randomised, double-blind crossover study	Spironolactone improved forearm blood flow compared to placebo
Abiose *et al*[104]	Spironolactone	20 patients with NYHA class III-IV congestive heart failure	Cohort study	Spironolactone improved FMD at 4 wk with a sustained improvement at 8 wk
Tzemos *et al*[107]	Nebivolol, atenolol	12 hypertensive patients	Randomised, double-blind crossover study	Only nebivolol was able to improve endothelial dependent vasodilation
Pasini *et al*[108]	Nebivolol, atenolol	40 hypertensive patients with 40 controls	Randomised double-blind parallel group	FMD improved only in the group treated with nebivolol
Matsuda *et al*[109]	Carvedilol	29 patients with coronary artery disease	Randomised, placebo controlled	Carvedilol significantly improved FMD after 4 mo treatment
Agewall *et al*[116]	Atorvastatin	20 healthy smokers, 20 healthy non-smokers	Open label placebo controlled randomised cross-over	Smokers had a lower baseline FMD. Atorvastatin improved FMD in smokers but had no effect in non-smokers
Ostad *et al*[117]	Atorvastatin, ezetimibe	58 patients with coronary artery disease	Double-blind, randomised, parallel group	High-dose atorvastatin improved FMD significantly more than low dose atorvastatin + ezetimibe independently of improvement in LDL cholesterol
Gounari *et al*[118]	Rosuvastatin, ezetimibe	Patients with heart failure	Double-blind, placebo controlled, cross-over trial	Rosuvastatin caused a significant improvement of FMD compared to ezetimibe and independent of LDL cholesterol and baseline brachial artery diameter
Pitocco *et al*[121]	Metformin	42 type 1 diabetics without overt cardiovascular disease	Randomised double-blind, placebo controlled	Significant improvement in FMD by 1.32% compared to placebo
Lamendola *et al*[122]	Ranolazine	30 type 2 (non-insulin dependent) diabetics without overt cardiovascular disease	Randomised double-blind, placebo controlled	Significant improvement in FMD compared to placebo after 2 wk of ranolazine therapy
Kao *et al*[123]	Allopurinol	67 patients with CKD stage 3 and LV hypertrophy	Randomized, double-blind, parallel-group	Significant improvement in FMD compared to placebo after 9 mo of allopurinol therapy

FMD: Flow-mediated dilation.

DOES REVERSAL OF ENDOTHELIAL DYSFUNCTION HAVE ANY PROGNOSTIC IMPACT?

Given that several classes of drugs do seem to lead to an improvement in endothelial function, the next step is to consider whether these effects are translated into a prognostic benefit. There are however only a few studies which address this issue. Modena *et al*[124] evaluated 400 post-menopausal women with hypertension and endothelial dysfunction in an attempt to assess whether

an improvement in FMD using antihypertensive drugs would predict a better prognosis. The authors found that improvement in endothelial function after 6 mo of therapy was associated with a much reduced event rate (6% *vs* 21.3% in those patients with persistently impaired endothelial dysfunction). One problem might perhaps be the fact that therapeutic options which improve endothelial function also have other beneficial effects on the cardiovascular system independent of their vasodilatory contribution. A recent study in patients with heart failure showed that patients in whom endothelial function improved following institution of optimal medical therapy had a much better prognosis than those in whom there was no improvement (hazard ratio 3.0 for those with persistently impaired endothelial function)[78].

Furthermore, confounding effects of medications also need to be considered-for example, hormone replacement therapy with estrogens in post-menopausal women does cause vasodilatation, however this beneficial effect is negated by their pro-thrombotic tendency. Another potential role for identification of endothelial dysfunction is that of screening. Given that there is abundant evidence to suggest that endothelial dysfunction is present before the development of clinically significant cardiovascular disease it might be beneficial to identify patients at potential risk of future events and offer disease modifying therapy. Again however this question has not yet been answered.

While numerous drugs that improve endothelial dysfunction have been shown to improve mortality, very few studies have specifically looked at the beneficial prognostic effects of endothelial dysfunction. This is presumably because when designing studies investigating these drugs it is very difficult to isolate the effect of endothelial dysfunction reversal given the multi-site action of drugs such as ACE inhibitors and statins. Of course, as the beneficial effects of these drugs are now well established, trials specifically looking at the prognostic benefit of endothelial dysfunction are perhaps less of a priority.

CONCLUSION

In this review we have demonstrated the methods of endothelial function assessment, the significance of endothelial dysfunction (particularly as a precursor) to cardiovascular disease and its prognostic significance. Several aspects need further exploration. First, despite the widespread use of FMD in clinical trials, is it the best way of assessing endothelial dysfunction? Certainly, the failure of the technique to obtain widespread use in a clinical setting despite many years of use in clinical trials and a reasonable amount of prognostic evidence behind it would suggest that it may never be adopted in the cardiology community. However, the failing of FMD seems to be more due to technical issues (such as the time taken to measure it and operator variability) rather than a disbelief in its results or the importance of endothelial function. The development of PAT and interest in other aspects of endothelial function such as circulating biomarkers relating to thrombosis and inflammation may prove to be easier methods of assessing endothelial function. If an easier method could be found then (presuming it showed similar prognostic value as FMD in large-scale studies) perhaps this would have more widespread clinical applicability. Indeed, in our unit, FMD is only used in research studies and is not used at all clinically. The standardization of the method is of key importance with regards to whether FMD can truly penetrate the clinical arena. Secondly, should endothelial dysfunction be used as an end-point to guide therapy or should it be simply thought of as another risk factor? And if so, are there any other potential therapies which might independently modulate endothelial function? Finally, does improving endothelial function lead to improved clinical outcomes in both primary and secondary prevention?

In summary, and in answer to the question posed by the title of this review, there is evidence to suggest that reversal of endothelial dysfunction might still be a target which might improve cardiovascular outcomes in the modern era, however, we do not yet have convincing evidence that it does as yet. We know that reversal is possible, but whether it is beneficial in identifying a higher risk group in primary prevention (in addition to traditional risk factors) or as a target in secondary prevention remains a question with an as yet elusive answer. It may be that FMD (and other measures of endothelial dysfunction) is more of a marker of overall cardiovascular health (predicting adverse outcome similarly to biomarkers such as B-type natriuretic peptide and troponin), rather than a therapeutic target itself. Nevertheless, there is ample evidence that therapies that improve cardiovascular outcome (by various pathways), also seem to improve endothelial function. Given the prognostic value of FMD, it would seem logical that at least some of these beneficial effects may be mediated by an improvement in endothelial function. However, as long the most validated measurement of endothelial function (FMD) cannot reach widespread use clinically, it will remain difficult to promote the idea that reversal of endothelial dysfunction should be a primary target of treatment in its own right. Indeed, to answer the question posed in the title of this review, we believe that while reversal of endothelial dysfunction is an attractive target in modern cardiology, we still require further studies to ascertain whether directly targeting reversal of endothelial dysfunction is a worthwhile target in modern cardiology.

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REVIEW

Percutaneous management of vascular access in transfemoral transcatheter aortic valve implantation

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Abstract

Transcatheter aortic valve implantation (TAVI) using stent-based bioprostheses has recently emerged as a promising alternative to surgical valve replacement in selected patients. The main route for TAVI is retrograde access from the femoral artery using large sheaths (16-24 F). Vascular access complications are a clinically relevant issue in TAVI procedures since they are reported to occur in up to one fourth of patients and are strongly associated with adverse outcomes. In the present paper, we review the different types of vascular access site complications associated with transfemoral TAVI. Moreover, we discuss the possible optimal management strategies with particular attention to the relevance of early diagnosis and prompt treatment using endovascular techniques.

Key words: Transfemoral transcatheter aortic valve implantation; Vascular access complication; Percutaneous management

Core tip: Vascular complications are not rare in transcatheter aortic valve implantation (TAVI) by the transfemoral approach and can significantly affect the overall clinical outcome. After diagnosis, the application of simple vascular interventional techniques allows efficient complication management, thus avoiding high risk vascular surgery. We discuss the available percutaneous vascular access preparation by dedicated devices, the principal diagnostic tools for prevention and detection of vascular complications and their percutaneous management in the transfemoral TAVI setting.

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INTRODUCTION

Transcatheter aortic valve implantation (TAVI) using stent-based bioprostheses has recently emerged as a promising alternative to surgical valve replacement in selected patients[1,2]. At present, for transfemoral TAVI the most studied valves are a balloon-expandable prosthesis, the Edwards SAPIEN XT™ valve (Edwards Lifesciences, Irvine, California, United States), that has recently added to the first generation Edwards valve, the Edwards SAPIEN (and in Europe has replaced it), and a self-expandable prosthesis, the CoreValve ReValving System® (Medtronic Inc., Minneapolis, MN, United States). Percutaneous implantation is generally performed using retrograde access from the femoral artery[3]. In spite of the increasing diffusion of TAVI across the world, with a high rate of procedural success and significant clinical and hemodynamic benefits[4,5], procedural challenges remain relevant. Among the different procedural technical issues, femoral access management is emerging as a factor with paramount clinical relevance. Indeed, major vascular complications during TAVI may range between 5%

and 25% of patients[6], and are associated with a striking increase in early mortality risk[7-10].

PREDICTORS OF VASCULAR COMPLICATIONS AND SELECTION OF VASCULAR ACCESS

The rate of vascular access site complications is probably influenced by several factors, which include the size of the devices (with favorable impact expected from the reduction in sheath size required by the latest generation valves), patient anatomy and the operator's experience/technique in deploying the closure devices[11]. Periprocedural bleeding after TAVI is frequent and principally related to renal function and sheath diameters, as reported in a recent Italian multicenter study[12]. Life-threatening and major bleeding, along with severe kidney failure, are independent predictors of increased mortality after 30 d[12].

While the first introduced bioprosthetic valve (Edwards SAPIEN) was characterized by a larger diameter (internal diameter 22-24 F and external diameter 8-9 mm) and required a minimal external arterial diameter of 7-8 mm, the Edwards SAPIEN XT™ valve and the Medtronic CoreValve System® valve which are characterized by an external diameter of about 7 mm (internal diameter 16-20 F and 18 F, respectively) necessitate a minimal external arterial diameter of about 6-7 mm (6 mm for 16 F e-Sheath and standard 18 F sheath, if iliofemoral arteries are not severely calcified). Calcific and obstructive atherosclerosis of iliac-femoral arteries, which is common in the elderly population treated by TAVI, and small vessel diameter and tortuosity may often hinder safe positioning of large delivery catheters (16-24 F). In particular, the sheath to femoral artery ratio, independently predicts the Valve Academic Research Consortium (VARC) major vascular complications and 30-d mortality, with an identified cut-off of 1.05[13]. Furthermore, intravascular manipulation of these large catheters increases the risk of vascular injury, even in arteries with more friendly characteristics. Therefore, an accurate, pre-interventional screening of vascular anatomy using angiography or multidetector computed tomography (MDCT) of iliac-femoral arteries is mandatory for TAVI, to assess the presence and severity of atherosclerotic disease and determine the feasibility of an arterial approach[14]. Ideally, iliac-femoral arteries should be free of heavily calcified plaques and significant tortuosity, and with a diameter large enough to accommodate a large femoral sheath[13,15]. In comparison with standard angiography, the multiplanar capabilities of MDCT allow a detailed and complete three-dimensional assessment of the iliac-femoral system[16]. In addition to the accurate measurement of minimal lumen diameters, MDCT can assess vessel tortuosity, burden and pattern of calcification, extent of atherosclerosis, and identify other high-risk features including dissections and complex atheroma. During the procedure, fluoroscopic guidance while advancing the large diameter sheaths and delivery catheters is mandatory in order to check their navigation through complex vessel features. Ultrasound (US) guidance during positioning of these devices can help in identifying the optimal common femoral artery (CFA) puncture site and has been suggested to reduce access site complications[17]. In a multicenter randomized controlled trial, routine real-time US guidance compared with standard fluoroscopic guidance improved CFA cannulation only in patients with high CFA bifurcations, but improved first-pass success rate and reduced the number of attempts, time to access, risk of venipuncture, and vascular complications in all cases[18].

HEMOSTASIS TECHNIQUES USED IN TAVI

After an initial phase of surgical access site preparation and closure of vascular access, which is still to be considered in particular cases of alternative access (*e.g.*, transubclavian access), operators have become confident with percutaneous puncture and access site closure through commercially available suture-mediated closure devices, such as the Prostar XL10F and Perclose ProGlide (Abbott Vascular Devices, Redwood City, CA, United States) devices[19,20]. Classical surgical preparation of vascular access can be quite difficult and time-consuming, especially in patients with heavily calcified vessels and/or previous groin interventions. It is characterized by a circumferential vessel dissection, arteriotomy, clamping, and wall closure. In all these phases vascular access complications such as plaque disruption, local dissection, aneurysm formation, stenosis/occlusion, and even acute thrombosis, with consequent acute limb ischemia, can occur[21,22]. Moreover, the lesser invasive percutaneous method in an experienced center is associated with similar rates of major and minor vascular complications[23] and with lower access site infection and bleeding, and shorter hospital stay compared to the surgical approach[24].

While the Edwards SAPIEN valve is implanted through a 22 or 24 F arterial sheath (about 8 and 9 mm external diameter), the CoreValve and the Edwards SAPIEN XT valve are delivered through a 16-20 F sheath (about 7 mm external diameter). These bulky sheaths are above the "on label" use of both suture-based hemostatic devices like the Prostar XL and Perclose ProGlide. So the "preclosure" technique has been developed to allow achievement of a full percutaneous hemostasis using such devices. The "preclosure" technique is based on the application of these devices to deploy sutures before the introduction of the large arterial sheath needed for valve implantation, then the sutures are tied at the end of the procedure by pushing down knot(s) in order to achieve hemostasis percutaneously. The sequence of steps necessary for successful "preclosure" technique is depicted in Figure 1. Recently, Kahlert *et al*[25] reported that "preclosure" with a single ProGlide™ device, followed by manual compression, could provide a more efficient and safe hemostasis compared to multiple ProGlide™ and Prostar

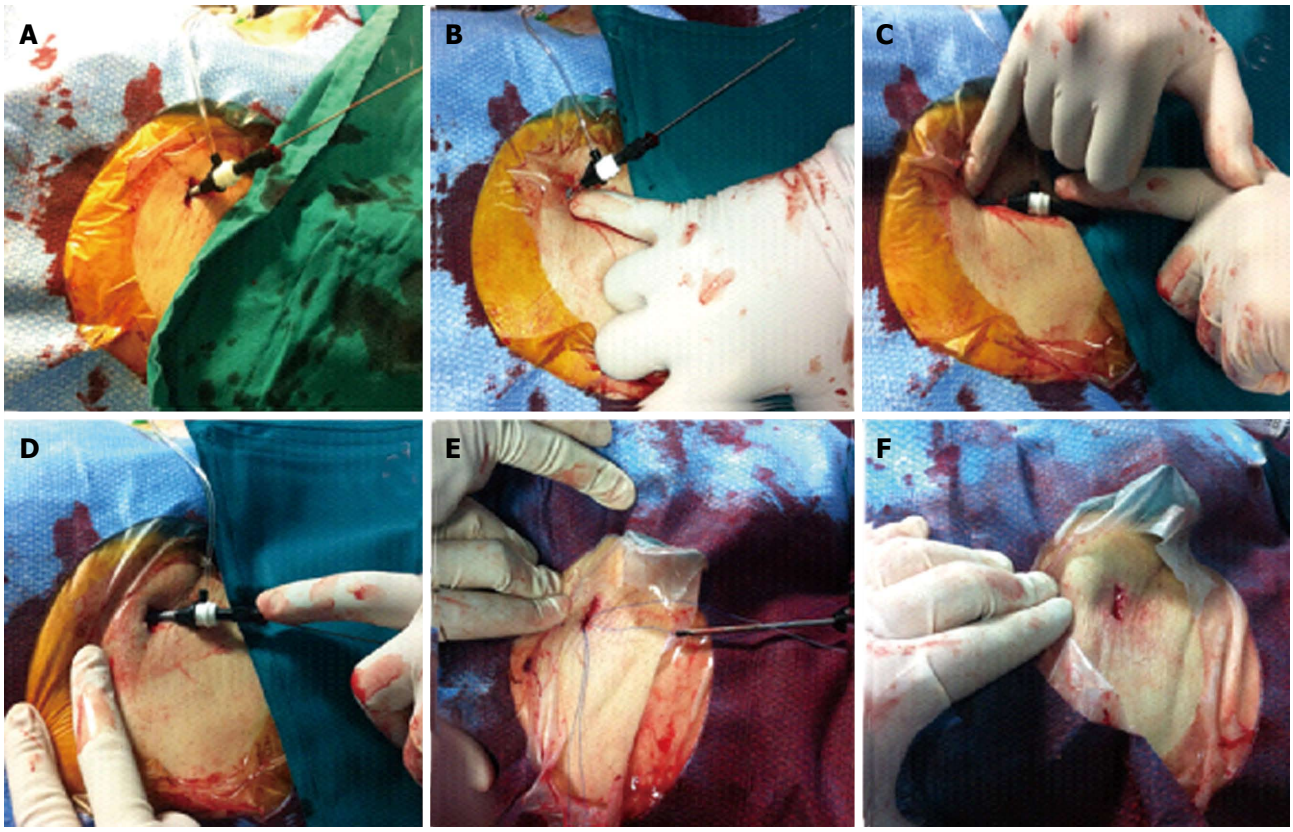

Figure 1 Pre-closure technique for hemostasis in transcatheter aortic valve implantation procedures. After angiography-guided puncture of the anterior wall of the common femoral artery (CFA) and the insertion of a 6 F sheath, the preparation of vascular access for large sheath insertion (≥ 18 F) consists of the enlargement of the access site by the insertion of a 9 F sheath (A) and dilation of the subcutaneous tissue anteriorly (B) and posteriorly to the sheath (C), using one finger. Such a maneuver should achieve a less traumatic flaring of cutaneous and subcutaneous tissues at the vascular access site and create appropriate space for both large sheath introduction at the beginning of the procedure and optimal fastening of knots over the arterial wall at procedure end (D). After 9 F sheath removal, the suture-mediated vascular closure device is inserted in the correct position, the needles are unlocked and pulled through the arterial wall (E). At the end of transcatheter aortic valve implantation, the sheath and the guide wire are removed, the sutures are fastened individually with a sliding knot and a knot pusher is used to ensure approximation of the knot to the surface of the vessel wall. Vascular suture ends are cut well beneath the surface of the skin and an optimal closure of vascular access is obtained by a single cutaneous suture without residual bleeding (F).

XL techniques.

The Prostar XL device was originally designed for a suture-based 10 F arteriotomy closure. However, it is commonly used for closing arterial access sites up to 18 F using the preclosure technique[26]. The device is a suture-mediated vascular closure system and is composed of a 10 F, 0.038-inch guidewire-compatible, hydrophilic sheath with a J-tip and a monorail design, based on two sutures (USP 3-0 braided polyester) and two pairs of nitinol needles, a needle guide, and a rotating barrel precisely controlling the needles during device deployment. After angiography-guided puncture of the anterior wall of the common femoral artery at an angle of approximately 45°, the Prostar XL is advanced over a 0.035-inch guidewire. When the device is in the correct position, indicated by pulsatile blood return from the dedicated marker lumen, the needles are unlocked and pulled through the arterial wall. After deployment of the device, the sutures are secured with mosquito clamps. At the end of the TAVI procedure, the sheath and the guide wire are removed while proximal pressure is maintained, and sutures are fastened individually with a (manually performed) sliding knot. A knot pusher is used to ensure approximation of the knot to the surface of the vessel wall. Manual pressure is then released and suture ends are cut well beneath the surface of the skin. A single Prostar XL is generally used to close arteriotomies for 18 to 19 F sheaths and two devices for 22- and 24 F sheaths at a 45° angle. It has been demonstrated to be a safe and effective method of achieving hemostasis, and to reduce times to ambulation and discharge after interventional procedures in a multicenter, non-randomized registry[26].

The Perclose ProGlide is a 6 F suture-based hemostatic device consisting of a monofilament suture and a pre-formed knot. To obtain hemostasis after removal of large sheaths, two Perclose devices are used according to the "double preclosure technique". This consists of the sequential insertion of the two Perclose devices rotated in opposite sides at 30°-45°, to create an interrupted X-figure and then closure of the arteriotomy is achieved at the end of the procedure by tying down the two knots using the two node pushers sequentially[27]. According to recent data, this technique has been suggested to be associated with a low incidence of early and late closure site complications[28-30]. Furthermore, the use of three Perclose devices has recently been reported[19].

Finally, a potentially useful adjunctive technique (which may eventually be used in conjunction with the above-

Table 1 Valve academic research consortium-2 classification of vascular access site and access-related complications

Major vascular complications
Any aortic dissection, aortic rupture, annulus rupture, left ventricle perforation, or new apical aneurysm/pseudoaneurysm OR
Access site or access-related vascular injury (dissection, stenosis, perforation, rupture, arterio-venous fistula, pseudoaneurysm, hematoma, irreversible nerve injury, compartment syndrome, percutaneous closure device failure) leading to death, life-threatening or major bleeding[1], visceral ischemia or neurological impairment OR
Distal embolization (non-cerebral) from a vascular source requiring surgery or resulting in amputation or irreversible end-organ damage OR
The use of unplanned endovascular or surgical intervention associated with death, major bleeding, visceral ischemia or neurological impairment OR
Any new ipsilateral lower extremity ischemia documented by patient symptoms, physical exam, and/or decreased or absent blood flow on lower ex tremity angiogram OR
Surgery for access site-related nerve injury OR
Permanent access site-related nerve injury
Minor vascular complications
Access site or access-related vascular injury (dissection, stenosis, perforation, rupture, arterio-venous fistula, pseudoaneurysms , hematomas, percutaneous closure device failure) not leading to death, life-threatening or major bleeding[1], visceral ischemia or neurological impairment OR
Distal embolization treated with embolectomy and/or thrombectomy and not resulting in amputation or irreversible end-organ damage OR
Any unplanned endovascular stenting or unplanned surgical intervention not meeting the criteria for a major vascular complication OR
Vascular repair or the need for vascular repair (*via* surgery, ultrasound-guided compression, transcatheter embolization, or stent-graft)
Percutaneous closure device failure
Failure of a closure device to achieve hemostasis at the arteriotomy site leading to alternative treatment (other than manual compression or adjunctive endovascular ballooning)

[1]Refers to valve academic research consortium bleeding definitions[37].

mentioned closure device-based techniques) to improve efficacy of hemostasis, is the crossover balloon occlusion technique (CBOT). This consists of the reduction of local blood pressure at the entry level of the large sheath through flow blockage obtained by inflation of a peripheral angioplasty balloon in the iliac artery using the crossover technique. The CBOT has been reported to allow safe and successful percutaneous closure in patients undergoing TAVI *via* a retrograde femoral artery approach using the 22 or 24 F sheath systems[31].

NOVEL VASCULAR SHEATHS FOR TRANSFEMORAL TAVI

More recently, a novel type of sheath has been developed to reduce the rate of vascular complications related to TAVI. The SoloPath™ (Onset Medical, Terumo Medical Corporation, Irvine, CA, United States) is a balloon expandable transfemoral introducer; it has an inner diameter of 14-21 F (outer diameter 17-24 F) and is compatible with the 18 F Medtronic/CoreValve and the 23- and 26-mm Edward SAPIEN XT delivery system. Its peculiarity is represented by a 13.5 F distal part to facilitate vessel entry, that can be expanded by the integrated balloon inflation reaching its nominal diameter, after sheath insertion, and can be deflated at the end of the procedure, enabling low-resistance removal[32,33]. The SoloPath sheath is a feasible alternative to conventional sheaths for transfemoral TAVR patients with advanced atherosclerotic disease or an arterial diameter ≤ 7 mm[34]. The available expandable sheath for Edwards Sapien XT valve is the e-Sheath™ (Edwards Lifesciences, Irvine, California, United States), a 16-18 F sheath, with a "dynamic expansion mechanism" to facilitate the valve passage, which returns to a reduced profile once the valve has passed, limiting vascular trauma. Nevertheless, this device is contraindicated for tortuous or calcified vessels, which would prevent safe entry of the sheath, and currently does not show an advantage over the 18/19 F fixed size sheath in reducing vascular and bleeding complications[35].

VASCULAR ACCESS SITE COMPLICATIONS AFTER TAVI AND THEIR MANAGEMENT

A series of vascular complications are commonly reported to be associated with TAVI, including arterial perforation, dissection, pseudoaneurysm, stenosis/occlusion and arterio-venous fistula[7-10]. The VARC, a collaboration between academic research organizations in the United States and Europe, has elaborated a consensus document on TAVI related endpoint definitions[36] and a more recent updated document[37], in which a classification of major and minor vascular access complications has been proposed (Table 1). This position paper has also provided a clear definition for the "access-related" complications, which were defined as any adverse clinical event possibly associated with any of the access sites used during the procedure[38].

Vascular access site complication rates reported in the literature are extremely variable probably because of different valve delivery systems[39], closure techniques and learning curves. To provide an overview of vascular complication frequency and type, a summary of the main published studies on TAVI-related vascular access site complications is provided in Table 2.

Optimization of hemostasis techniques and management strategies are probably pivotal. The optimal management of vascular access site complications includes a prompt diagnosis and appropriate timely treatment. At the end of the procedure, digital subtraction angiography of the iliac-femoral arteries obtained using a non-selec-

Table 2 Incidence of major vascular access site complications and specific vascular access site types across transfemoral transcatheter aortic valve implantation studies

Ref.	Bioprostheses	Population	Major vascular complications	Stenosis/ occlusion	Perforation/ rupture	Dissection	Pseudoaneurysm
Webb *et al*[40], *Circulation* 2009	ESV	113	9/113 (8%)	NA	NA	NA	NA
Ducrocq *et al*[8], *Eurointervention* 2010	ESV	54	9/54 (16.7%)	0/9	5/9 (55.5%)	4/9 (45.5%)	0/9
Tchetche *et al*[9], *Eurointervention* 2010	ESV + MCV	45 24 ESV 21 MCV	4/45 (8.9%) 2/24 (8.3%) ESV 2/21 (9.5%) MCV[1]	NA	NA	NA	NA
Piazza *et al*[41], *Eurointervention* 2008	MCV	646	12/646 (1.9%)	NA	NA	NA	NA
Himbert *et al*[42], *JACC* 2009	ESV	51	6/51 (12%)	0/6	2/6 (33%)	4/6 (66%)	0/6
Webb *et al*[1], *Circulation* 2007	ESV	50	4/50 (8%)	0/4	4/4 (100%)	0/4	0/4
SOURCE registry[43], *Circulation* 2009	ESV	463	57/463 (12.3%)	NA	NA	NA	NA
Lefèvre *et al*[44], *Eur Heart J* 2011	ESV	61	17/61 (28%)	0/61	3/61 (5%)	6/61 (10%)	1/161 (2%)
Canadian experience[45], *JACC* 2010	ESV	168	22/168 (13.1%)	NA	NA	NA	NA
Bleiziffer *et al*[46], *J Thorac Cardiovasc Surg* 2009	MCV	153	24/153 (16%)	NA	NA	NA	NA
The Milan experience *JACC Cardiovasc Interv* 2010[47]	ESV + MCV	107 61 ESV 46 MCV	22 /107 (20.6%) 13/61 (21.3%) ESV 9/46 (19.5%) MCV[1]	1/22 (4.5%) ESV	7/22 (32%) 5/13 (38%) ESV 2/9 (22%) MCV[1]	6/22 (27%) 4/13 (31%) ESV 2/9 (22%) MCV[1]	4/22 (18%) MCV
The Rotterdam experience[7], *Eurointervention* 2010	MCV	99	13/99 (13%)	NA	NA	NA	NA
The France Registry[48], *Eur Heart J* 2011	ESV + MCV	160 94 ESV 66 MCV	11/160 (7%) 6/94 (6.4%) ESV 5/66 (7.6%) MCV[1]	0/11	2/11 (18%) ESV	7/11 (64%) 4/6 (67%) ESV 3/5 (60 %) MCV[1]	0/11
Petronio *et al*[49], *Circ Cardiovasc Interv* 2010	MCV	460	9/460 (2%)	NA	NA	NA	NA
Spanish experience[50], *Rev Espan Cardiol* 2010	MCV	108	6/108 (5.6%)	1/6 (16.6%)	1/6 (16.6%)	0/6	1/6 (6.60%)
United Kingdom Registry[51], *JACC* 2011	ESV + MCV	599 193 ESV 406 MCV	50/599 (8.4%)	NA	NA	NA	NA
Toggweiler *et al*[15], *JACC* 2012	ESV+ MCV	137 126 ESV 11 MCV	24/137 (18%)[2]	16/24 (66.6%)	2/24 (8.3%)	2/24 (8.3%)	2/24 (8.3%)
Partner trial[52], *JACC* 2012	ESV	419	64/419 (15.3%)	NA	20/64 (31.3%)	40/64 (62.8%)	2/64 (3.4%)
The France II Registry[53], *NEJM* 2013	ESV + MCV	3195 2107 ESV 1043 MCV	150/3195 (4.7%) 57/2107 (2.7%) 47/1043 (4.5%)	NA	NA	NA	NA
European Sentinel Registry of TAVI[54], *Eurointervention* 2013	ESV + MCV	4571 2604 ESV[3] 1943 MCV	40/4571 (3.1%) 20/2604 (3.3%) 20/1943 (2.8%)	NA	NA	NA	NA
Sawa *et al*[55], *Circulation Journal* 2014	MCV	44	5/44 (11.54%)	NA	NA	NA	NA
Spanish National Registry of TAVI[56], *Rev Esp Cardiol* 2013	ESV + MCV	1159 504 ESV 610 MCV	42/1159 (3.6%) 25/504 (5%) 17/610 (2.8%)	NA	NA	NA	NA
Total		12862	640/12862 (5%)	18/143 (12.6%)	44/207 (21.2%)	69/207 (33.3%)	10/207 (4.8%)

[1]*P* value not significant between ESV and MCV subgroups; [2]Major plus minor complications; [3]Including transapical (29% of total ESV). ESV: Edwards SAPIEN valve; MCV: Medtronic Core Valve.

tive (*via* a pigtail catheter introduced in the aorta through the contralateral femoral artery) or a selective (*via* a diagnostic right Judkins or internal mammary artery catheter placed from the contralateral femoral artery according to the "crossover" technique) contrast injection is advisable to assess the vascular integrity and promptly manage possible complications. Percutaneous management of vascular complications after TAVI as a bailout procedure is feasible and safe, with a high rate of technical success, and long-term clinical outcomes are comparable to patients without vascular complications[57].

A wide range of vascular damage (from minor vessel complications such as localized femoral artery dissection to major complications such as vessel occlusion or perforation) has been described. Localized vascular damage without any impairment of lower limb perfusion should

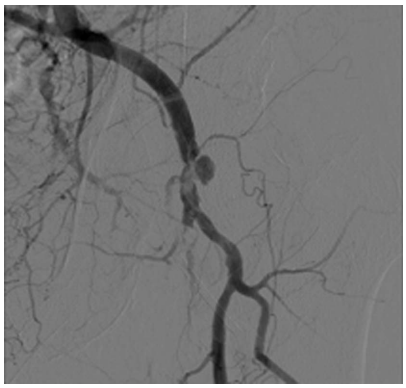

Figure 2 Post-transcatheter aortic valve implantation pseudoaneurysm. After the transcatheter aortic valve implantation procedure, digital subtraction angiography of the left iliac-femoral artery by contralateral medium contrast injection showing a pseudoaneurysm of the left common femoral artery.

be treated conservatively, with careful clinical and ultrasonographic monitoring during the following hours. The main vascular access site complications reported in TAVI studies are: pseudoaneurysm, arterial perforation, arterial dissection, occlusion and avulsion. The specific management strategies are herein discussed for each of these complications.

Pseudoaneurysm

Pseudoaneurysm consists of a pulsatile hematoma which communicates with an artery through a disruption in the arterial wall. At the end of the procedure, standard or digital subtraction angiography of the iliac-femoral arteries can reveal an arterial leak as a precursor of the pseudoaneurysm or a true pseudoaneurysm (as shown in Figure 2), depending on the time of formation. If angiographic diagnosis has not been made after the end of the procedure, close clinical surveillance can detect the increase in a new thrill or bruit, pulsatile hematoma, or marked pain or tenderness, and pseudoaneurysm can be confirmed by ultrasound. Possible complications of pseudoaneurysm are rupture, distal embolization, infection, neuropathy and local skin ischemia. However, it generally does not impair lower limb perfusion and can be treated by ultrasound-guided compression, which is a safe and cost-effective method of achieving pseudoaneurysm thrombosis[58]. However, it carries considerable drawbacks including long procedure times, patient discomfort and high recurrence rates, especially in cases requiring anticoagulant therapy. If probe compression fails, treatment options include ultrasound-guided thrombin injection, which is associated with a high success rate and is more comfortable for patients[59], coil embolization, stent graft and surgical repair.

Another vascular complication of TAVI is iliac-femoral *stenosis*, which is sometimes associated with closure device release. Mild stenosis detected by angiography in the absence of lower limb ischemia may be managed conservatively, while a significant stenosis may be treated by percutaneous transluminal angioplasty (PTA) (Figure 3), with the aim of preventing further flow deterioration in the limb by superimposition of thrombosis or development of severe post-procedural claudication. When hemostatic device-induced tight stenosis is detected immediately after large sheath removal and urgent PTA is needed at procedure end, the selection of undersized peripheral balloons is advisable in order to avoid arterial wall laceration by suture knots.

Perforation

Perforation leading to retroperitoneal hematoma is a dramatic complication of TAVI. It can be identified by angiography performed before removal of the large sheath or can appear only after sheath removal (since the sheath is usually occlusive at the level of the external iliac and femoral arteries), as well as after tying the closure device knots. After arterial perforation visualization by angiography, timely bleeding control may be obtained by the positioning of an occlusive balloon proximal to the vascular lesion site or insertion of a large sheath across the lacerated segment. To facilitate bleeding control, operators can use protamine to neutralize heparin action. If arterial laceration persists after balloon or sheath removal, percutaneous implantation of a covered stent can be performed in order to avoid the risks related with urgent vascular surgery (Figure 4). Moreover, post-procedure digital subtraction angiography of the iliac-femoral arteries can also allow detection of rarer complications with insidious diagnosis such as lateral circumflex femoral artery perforation. While femoral artery perforation is most often related to closure device failure and can cause a visible leg hematoma, iliac artery perforation may cause a retroperitoneal hematoma in the hours after the procedure, which may be suggested by low back pain and can be confirmed by CT, and can be managed by prolonged balloon inflation or coil embolization.

Dissection

Dissection of the iliac-femoral arteries can occur as a consequence of excessively traumatic sheath insertion through fragile/diseased arterial vessels. Limited, non-occlusive and retrograde arterial dissections may generally be managed conservatively, since the antegrade flow generally maintains the artery patency, pushing the dissection flap to the vessel wall. More extensive arterial dissection can be associated with vessel occlusion (due to superimposed acute thrombosis or obstructive flaps), and may cause acute limb ischemia, so prompt management is needed to restore antegrade flow. Percutaneous angioplasty and self- or balloon-expandable stent implantation can allow successful management by the crossover technique through the contralateral femoral artery (Figures 5 and 6). A valuable tip to reduce the incidence of vascular wall lacerations is to pay particular attention to vascular calcification movement during a large sheath insertion. If the operator notes a certain resistance during this maneuver, it is advisable to insert the sheath slowly stopping every two centimeters, and to use a substance to reduce friction such as sterile Vaseline. At the end of TAVI, extraction of the introducer after dilator insertion is prefer-

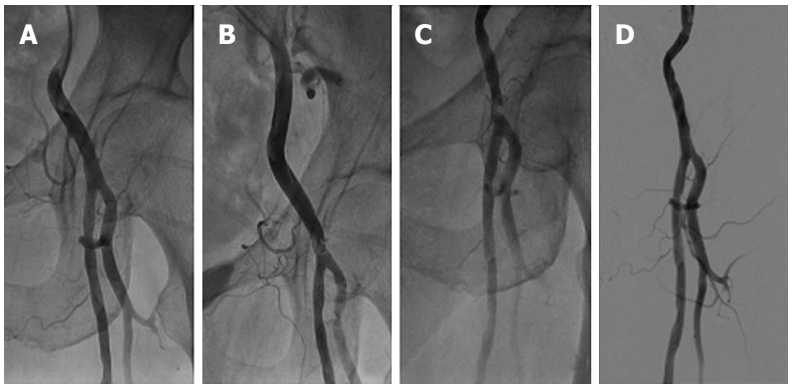

Figure 3 Post-transcatheter aortic valve implantation common femoral artery stenosis. Standard angiography obtained before 18 F sheath insertion for transcatheter aortic valve implantation showed the absence of significant stenosis, tortuosity and calcification of left iliac-femoral artery (A); after vascular access closure by Prostar XL, angiography documented the presence of an intimal flap in the right common femoral artery (CFA) wall, not determining a significant flow limitation (B); 4-mo follow-up angiography showed progression of arterial damage and the development of significant stenosis of CFA, determining claudication (Fontaine-Leriche class IIb) (C); angioplasty of left CFA was performed by right transradial access, using a 125 cm 6 F Multipurpose guiding catheter and a 300 cm BMW Universal wire; a 4.0 mm x 15 mm non-compliant coronary balloon (NC Sprinter, Medtronic, North Carolina, United States) and a 6.0 mm x 20 mm peripheral balloon (Avion Plus, Invatec, Roncadelle, Italy) were inflated to 24 atm, obtaining an optimal final result (D).

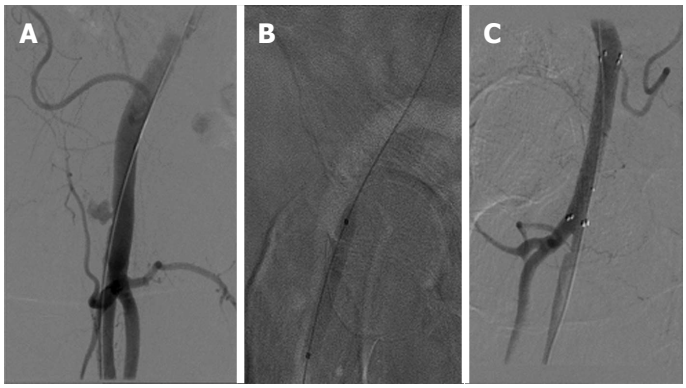

Figure 4 Post-transcatheter aortic valve implantation arterial perforation. At the end of the transcatheter aortic valve implantation procedure, digital subtraction angiography of the right iliac-femoral artery showed a perforation of the right common femoral artery (CFA) (A); angioplasty of the right CFA was performed by the crossover approach *via* the contralateral iliac-femoral artery; a 7.0 mm x 40 mm peripheral balloon (Admiral Xtreme, Invatec, Roncadelle, Italy) was inflated to 10 atm at the perforation site (B); because of the persistence of hematic extravasation, a 8.0 mm x 60 mm covered stent (Fluency Stent-Graft, BARD Peripheral Vascular, AZ, United States) was implanted, followed by dilation of 7.0 mm x 40 mm and 8.0 mm x 20 mm balloons (Admiral Xtreme, Invatec, Roncadelle, Italy) to 12 atm. At final angiography, optimal sealing of the arterial breach without residual hematic extravasation was documented (C).

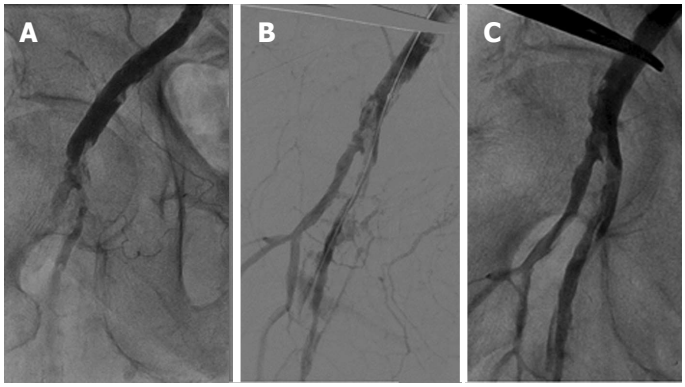

Figure 5 Post-transcatheter aortic valve implantation arterial dissection. Post- transcatheter aortic valve implantation procedure, angiography of the right iliac-femoral axis *via* the contralateral groin showing a dissection of the right common femoral artery extending proximally to the external iliac artery and determining distally an occlusion of the superficial femoral artery (A); digital subtraction angiography after reaching true lumen by a .035" wire by the retrograde approach and peripheral balloon dilation (6.0 mm x 120 mm Admiral Xtreme, Invatec, Roncadelle, Italy) to 6 atm (B); final angiography after stenting (6.0 mm x 80 mm and 9.0 mm x 60 mm Lifestent Vascular Stent, BARD Peripheral Vascular, AZ, United States) and post-dilation (5.0 mm x 80 mm and 6.0 mm x 120 mm Admiral Xtreme; Invatec, Roncadelle, Italy) showing an optimal antegrade flow in the right iliac-femoral artery (C).

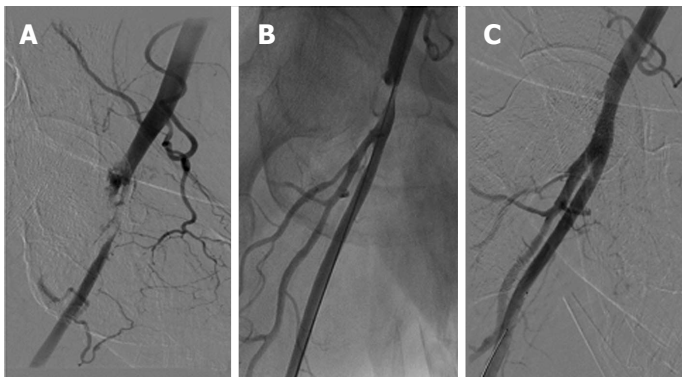

Figure 6 Post-transcatheter aortic valve implantation arterial thrombosis. Post-transcatheter aortic valve implantation procedure, digital subtraction angiography showing acute thrombotic occlusion of the right common femoral artery (A); emergency percutaneous transluminal angioplasty was performed by the crossover approach *via* the contralateral femoral artery, and consisted of initial thromboaspiration using a 6 F Multipurpose guiding catheter (Vista Brite Tip, Cordis Inc., Miami Lakes, FL, United States), obtaining restoration of antegrade blood flow (B); after prolonged dilations by 5.0 mm × 40 mm and 6.0 mm × 40 mm balloons (Pacific Xtreme and Admiral Xtreme, Invatec, Roncadelle, Italy), a 7.0 mm × 20 mm stent (Cristallo Ideale, Invatec, Roncadelle, Italy) was implanted, dilated by a 7.0 mm × 30 mm balloon (Avion Plus, Invatec, Roncadelle, Italy) to 10 atm. Final angiography showed the absence of residual stenosis (C).

Table 3 Materials for bailout endovascular interventions to manage vascular access complications (through contralateral femoral access using the "crossover" technique)

Complication	Type of bailout endovascular intervention	Devices needed
Any type	Immediate angiography and prompt access to the affected iliac-femoral axis[1]	6-9 F long (45 cm) sheaths
Iliac-femoral arteries rupture/ perforation	Immediate hemostasis to avoid shock	Large peripheral balloons in iliac arteries (diameter: 7-10 mm) or elastomeric balloon in the distal aorta
	Vascular sealing in case of persistent blood extravasation after prolonged balloon inflation	Covered stent (diameter: 7-10 mm)
Failure of hemostasis at the entry site	Prolonged balloon inflation proximal to the entry site during external manual compression	Mid-sized peripheral balloons (diameter: 6-8 mm)
Iliac-femoral arteries flow-limiting dissection	Immediate restoration of antegrade flow to avoid acute limb ischemia	Large peripheral balloons (diameter: 7-10 mm)
	Vascular sealing in case of significant stenosis/ dissection after balloon inflation	Peripheral self-expandable nitinol stents (diameter: 7-10 mm)
Iliac-femoral arteries acute thrombotic occlusion	Immediate restoration of antegrade flow to avoid acute limb ischemia	Thrombus aspiration with thrombus-extraction devices (angiojet, thrombus-aspirating catheters) or with coronary guiding catheters (multipurpose curve) Peripheral balloons (diameter: 5-10 mm) Consider distal filter protection to avoid embolization and avoid aggressive dilations since dethrombosis is usually facilitated by antegrade flow restoration

[1]Provisional delivery of a sentinel wire (*i.e.*, a 0.014"-0.018" wire placed in cross-over in distal femoral artery and jailed under the 18 F sheath) allows continuous control of the entry site and quick access to contralateral iliac-femoral axis if needed.

able to avoid traumatic action of the introducer's tip on arterial walls, especially in sharp arterial turns.

A rare complication of large artery sheath use is arterial avulsion followed by massive hemorrhage. This event is related to the tendency of the large femoral sheath to adhere to endothelium. If there is a suspicion of this dreadful complication due to resistance in sheath withdrawal, the placement of an occlusive balloon in the abdominal aorta under the renal arteries and preparation for possible surgical repair is the only option to save the patient's life[60].

A particular category of vascular access complications is represented by closure device failure, which is considered separately in the new VARC-2 classification[37]. Vascular closure device failure is not uncommon and can cause arterial dissection, perforation and occlusion. For example in a study by Van Mieghem *et al*[7], in the setting of transfemoral TAVI using the Medtronic CoreValve prosthesis, Prostar XL™ failure was responsible for about 54% of the observed major vascular events. Patient characteristics such as excessive femoral artery calcification, female gender and obesity[61], and the operator's learning curve[62] in deploying the closure devices can contribute to these events. As for the other vascular complications, closure-related complications can be managed conservatively by manual compression if there is no impairment of blood flow and leg perfusion, vice versa if there is continuous access site bleeding or significant artery stenosis or occlusion, they can be treated interventionally by PTA.

As discussed above, the prompt adoption of simple endovascular techniques may help to manage the majority of vascular complications, thus avoiding the risks of urgent vascular surgery. In Table 3 an "operative" list of the endovascular materials which may be used for bailout endovascular interventions (through contralateral femoral access using "crossover" technique) is provided.

CONCLUSION

Vascular complications are not rare in TAVI by the transfemoral approach and can significantly affect the overall clinical outcome[8-10]. At the end of the TAVI procedure, a control angiography obtained from the contralateral femoral access site allows early identification of vascular access site complications. After diagnosis, the application of simple vascular interventional techniques allows efficient complication management, thus avoiding high risk vascular surgery.

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REVIEW

Pseudoexfoliation syndrome and cardiovascular diseases

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Abstract

Pseudoexfoliation (PEX) syndrome is a well-recognized late-onset disease caused by a generalized fibrillopathy. It is linked to a broad spectrum of ocular complications including glaucoma and perioperative problems during cataract surgery. Apart from the long-known intraocular manifestations, PEX deposits have been found in a variety of extraocular locations and they appear to represent a systemic process associated with increased cardiovascular and cerebrovascular morbidity. However, as published results are inconsistent, the clinical significance of the extraocular PEX deposits remains controversial. Identification of PEX deposits in the heart and the vessel wall, epidemiologic studies, as well as, similarities in pathogenetic mechanisms have led to the hypothesis of a possible relation between fibrillar material and cardiovascular disease. Recent studies suggest that PEX syndrome is frequently linked to impaired heart and blood vessels function. Systemic and ocular blood flow changes, altered parasympathetic vascular control and baroreflex sensitivity, increased vascular resistance and decreased blood flow velocity, arterial endothelial dysfunction, high levels of plasma homocysteine and arterial hypertension have all been demonstrated in PEX subjects. Common features in the pathogenesis of both atherosclerosis and PEX, like oxidative stress and inflammation and a possible higher frequency of abdominal aorta aneurysm in PEX patients, could imply that these grey-white deposits and cardiovascular disorders are related or reflect different manifestations of the same process.

Key words: Pseudoexfoliation; Cardiovascular disease; Cerebrovascular disease; Coronary artery disease; Homocysteine

Core tip: Although much remains to be clarified concerning causes, pathogenesis and systemic role of pseudoexfoliation aggregations, there is accumulating epidemiologic, clinical and laboratory evidence that this well-described clinical entity may occur as part of a systemic disorder with cardiovascular implications. The present review aims to summarize current knowledge on cardiovascular complications which have been associated with these suspicious whitish-gray deposits.

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INTRODUCTION

Pseudoexfoliation (PEX) syndrome is an age-related disorder characterized by accumulation and deposition of microfibrillar material on multiple ocular and extraocular structures (Figure 1). The definite clinical diagnosis of the syndrome is based on slit lamp observation of the whitish flake-like deposits on anterior segment structures, particularly on the anterior lens surface and the pupillary border of the iris.

PEX syndrome is the most common identifiable

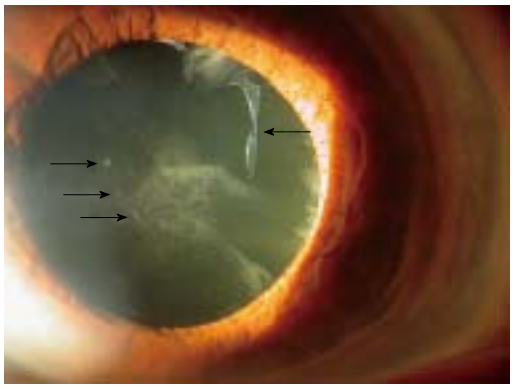

Figure 1 Pseudoexfoliation material on the anterior lens surface.

cause of open angle glaucoma, the so-called PEX glaucoma. It is also associated with cataract progression and intraoperative complications like zonular or posterior capsule rupture, poorly dilating pupil, vitreous loss, fibrinoid reaction, as well as, luxation of intraocular lens implants and corneal endothelial decompensation. In addition to the structures of the anterior segment of the eye, similar deposits have been identified in various visceral organs such as lung, heart, brain, vessels, kidney, gallbladder and meninges with unknown clinical significance.

PEX syndrome's prevalence demonstrates considerable geographic, ethnic and racial variation. Low PEX syndrome rates (< 6% in patients older than 70 years) have been reported in Greenland Eskimos, India, the eastern part of the United States, Germany, Britain, Australia, Japan, Austria, Denmark and Switzerland. In contrast, high PEX syndrome frequencies (> 15%) have been reported in Iceland, Finland, Russia, Tunisia, Saudi Arabia, Sweden, Norway, Turkey and Greece[1-3].

Although specific synthesis and pathogenesis of PEX material are still unknown, the concept of an elastotic process has recently been established. Molecular and biochemical data support the pathogenetic concept of PEX as a type of stress-induced elastic microfibrillopathy. PEX etiopathogenesis involves both genetic and non-genetic factors. Single-nucleotide polymorphisms (SNPs) in the coding region of the lysyl-oxidase-like 1 *(LOXL1)* gene, which is responsible for cross-linking of elastin, have been identified as strong genetic risk factors for PEX syndrome and PEX glaucoma[4]. Moreover, non-genetic factors including ultraviolet light exposure, dietary factors, infectious agents and trauma, as well as, oxidative stress, hypoxia and inflammation have been suggested to act as co-modulating external factors[5]. Pro-fibrotic cytokines (Interleukin-6), growth factors (GFs) and particularly transforming growth factor-β1 (TGF-β1), impaired cellular protection system with increased cellular and oxidative stress, a change in the local balance between Matrix Metalloproteinases and Tissue Inhibitor of Metalloproteinases appear to be involved in the disorder of the fibrotic matrix with accumulation of extracellular material. Ischemia/hypoxia, cross-linking mechanisms and aggregation of misfolded stressed proteins, as well as, low-grade chronic inflammatory processes have also been implicated[6-9]. PEX material seems to represent a highly cross-linked glycoprotein-proteoglycan complex which is mainly consisted of elastic microfibrillar components, such as fibrillin-1 and latent transforming growth factor binding proteins, as well as, chaperone molecules, such as clusterin, and cross-linking enzymes, such as LOXL1[10].

A variety of epithelial, endothelial and mesenchymal cells may be associated with impaired synthesis of the extracellular fibrillar material in intra- and extraocular sites. Intraocular material seems to be produced mainly in the pre-equatorial lens epithelium, the nonpigmented ciliary epithelium and the iris pigment epithelium, and secondarily in the corneal endothelium, the trabecular endothelium and by almost all cell types of the iris stroma[11]. Extraocular PEX material has been detected by electron microscope in connective tissue of visceral organs and in close proximity to fibroblasts, smooth and striated muscle cells, as well as, heart muscle cells[12,13]. These types of cells are probably involved in its production throughout the body. The fibrillar material shows ultrastructural and immunohistochemical similarities in both intra- and extraocular sites.

Although there is no clear-cut evidence that these deposits would cause degeneration of the extraocular tissues, they have been associated with cardiovascular and cerebrovascular morbidity. However, the clinical significance of the PEX-related systemic disorders remains controversial, as published results are inconsistent.

Studies implying a relationship between PEX syndrome and cardiovascular disease are mentioned below, along with others not supporting such a relationship.

HEART DISEASES

In Australia, the Blue Mountains Eye Study proposed that a history of angina, hypertension or a combined history of angina, acute myocardial infarction and stroke are significantly associated with the presence of PEX syndrome after multivariate adjustment including age, sex, glaucoma and vascular risk factors. This was attributed to the effect of elastosis in the vessel wall[14]. Citirik *et al*[15] found a significantly higher prevalence of PEX in 50 patients with coronary artery disease (CAD) proven by angiography than in healthy controls, and a higher prevalence of CAD in PEX individuals**.** PEX has been positively associated with presence of CAD among a large cohort of patients scheduled for cataract surgery[16,17]. More recently, French *et al*[18] reported significant associations of PEX and PEX glaucoma with a variety of cardiovascular disorders, including various stages of ischemic heart disease, cardiomyopathy and aortic aneurysm. Moreover, subclinical myocardial ischemia, by tissue Doppler echocardiography, has been found in PEX patients[19].

The possibility of an association between PEX and asymptomatic myocardial diastolic dysfunction (an important cause of heart failure), as assessed by two-dimensional echocardiography and pulsed Doppler echocardiography, has been suggested[20]. In addition, a higher prevalence of heart failure has been described in PEX

individuals[21].

Although there is convincing evidence that PEX syndrome is related to cardiovascular disorders, no significant relationship between PEX and CAD, aortic aneurysm or peripheral artery disease was reported by Emiroglu *et al*[22]. In the same line, arterial hypertension, ischemic heart disease, cerebrovascular disease and prevalence of diabetes mellitus did not differ between patients with or without PEX[23-27]. Of note, a higher prevalence of arrhythmia has been found in PEX individuals[23]. Also, a study by Tarkkanen *et al*[28] failed to show any significant difference in the frequency of hypertension or ischemic heart disease between patients with primary open-angle glaucoma (POAG) and PEX glaucoma, while the latter had a lower frequency of diabetes mellitus. Moreover, in the Thessaloniki Eye Study, no association was found between PEX and the history of specific or any systemic disease (self-reported history of hypertension, diabetes, cardiovascular disease, migraine, heart attack, coronary artery bypass, vascular surgery)[29]. Avsar *et al*[30] found no significant differences in time domain heart rate variability parameters (a measure of cardiac autonomic function) between patients with PEX syndrome and control subjects. Furthermore, several studies failed to demonstrate an association between PEX deposits and increased cardiovascular, cerebrovascular or total mortality[31-35].

VASCULAR AND CIRCULATION DISTURBANCES

Major manifestations of cardiovascular diseases such as a decreased blood flow and ischemia have frequently been documented in PEX syndrome. Deposition of PEX material within the vasculature with subsequent increases in vascular resistance and decreases in blood flow, vascular dysregulation and altered parasympathetic vascular control may be implicated in the pathogenesis of cardiovascular disorders in PEX subjects. Moreover, local ischemia and atherosclerosis have been correlated with elastosis in different tissues[36,37].

Increased aortic stiffness in PEX patients, which may be at least partially responsible for the increased incidence of CAD in this patient group has been described[38]. In addition, using the ultrasound wall tracking system Visontai *et al*[39] reported a lower distensibility and higher rigidity in the common carotid artery, as well as, altered parasympathetic vascular control connected to increased plasma homocysteine level in PEX/PEX glaucoma than the control group. Similar results were drawn by other studies showing lower myocardial peak systolic tissue Doppler imaging velocities and increased carotid intima-media thickness in patients with PEX syndrome when compared to controls. On the contrary, PEX and carotid plaque measurements were weakly correlated[40]. An impairment of parasympathetic cardiovascular regulation, baroreflex sensitivity and pulse wave velocity has also been described in PEX patients[41]. Arterial stiffening is an indicator of increased cardiovascular disease risk and, likewise, decreased baroreflex sensitivity has been described in hypertension, heart failure, myocardial infarction and metabolic syndrome. Lower cutaneous capillary blood flow and altered response to cold and warmth, without any change of plasma endothelin-1 concentration was also demonstrated[42]. Furthermore, Köz *et al*[43] found high levels of coronary risk markers such as lipoprotein (a), apolipoprotein A, homocysteine, as well as, impaired brachial artery dilation and increased carotid intima-media thickness in PEX patients. In a study by Praveen *et al*[25] PEX subjects had a significantly lower ankle brachial index as compared to controls, suggestive of PEX as a possible risk factor for peripheral vascular disease.

Ocular vascular and blood flow abnormalities

Dayanir *et al*[44] concluded that PEX decreases ophthalmic artery blood flow velocities and increases vascular resistance. Similar conclusions were drawn by another study where PEX patients had decreased blood flow velocities in the central retinal and the short posterior cilliary arteries and increased vascular resistance in the ophthalmic and central retinal arteries[45]. Reduced blood flow in choroid, optic nerve head and peripapillary retina of the PEX affected eye has also been found[46,47]. Moreover, Galassi *et al*[48] using color Doppler imaging found a decrease in ocular perfusion pressure and deterioration of retrobulbar haemodynamics in PEX glaucoma patients as compared to primary open-angle glaucoma patients and healthy controls. Several studies have demonstrated anterior-chamber hypoxia and iris vasculopathy (narrowing, occlusion, neovascularization) in PEX patients[49-52]. PEX as a potential risk factor for central retinal vein occlusion has also been proposed[53,54]. In support of the above, Cursiefen *et al*[55] found that PEX was significantly more common in eyes enucleated secondary to central retinal vein occlusion as compared to age-matched eyes enucleated for an intraocular tumor; however, morphological evidence of a PEX associated vasculopathy of the central retinal vessels explaining this association was not shown. Endothelin-1, a potent vasoconstrictor which could contribute to the obliterative vasculopathy seems to be increased in the aqueous humor of PEX eyes[56].

Cerebral vascular and blood flow abnormalities

A high frequency of PEX syndrome has been reported in patients with transient ischemic attacks[57-59]. A significantly higher prevalence of magnetic resonance images-defined white matter hyperintensities (ischemic changes) in patients with a clinical diagnosis of PEX with or without glaucoma *vs* control subjects, has also been documented[60]. Studies have indicated that the blood flow velocities of the middle cerebral artery were decreased in patients with PEX and PEX glaucoma[61,62] and there was a decrease in regional brain perfusion in PEX patients[63].

In addition, chronic cerebral diseases such as senile dementia, cerebral atrophy and chronic cerebral ischemia were more common in patients with PEX glaucoma than in those with POAG. The same study showed that patients with PEX glaucoma had higher probability of de-

veloping acute cerebrovascular disease than patients with POAG[51]. Alzheimer's disease has also been correlated to PEX syndrome in several, though not all studies[64-67].

Systemic arterial endothelial dysfunction

Arterial endothelial dysfunction is an independent predictor of future cardiovascular events. Vascular endothelium has a major role in the control of blood flow by releasing factors which may act either to contract the vascular smooth muscle, such as endothelin-1, or to relax it, such as nitric oxide. Atalar *et al*[68] found an impaired endothelial function in the brachial artery of patients with PEX syndrome, as assessed by vascular response to reactive hyperemia and sublingual nitroglycerin using high-resolution ultrasound. Endothelial dysfunction was attributed to the pseudoexfoliative fibrillar accumulation in the vessel wall. In the same line, endothelial dysfunction of the brachial artery was described in PEX subjects[69].

A major theory of atherosclerosis is that lesions result from an excessive fibroproliferative response to various forms of insult to the endothelium and smooth muscle of the vascular wall[70]. Endothelial exfoliation has been defined as thin, friable, mobile and translucent tissue, loosely adherent to the vascular wall[71] that may play a functional role in thrombus formation[72].

Nevertheless, other studies failed to demonstrate a correlation between PEX and endothelial damage, as biomarkers levels of endothelial injury (von Willebrand antigen, E-selectin, P-selectin and high sensitivity C-reactive protein) did not differ in blood plasma of patients with PEX *vs* controls[73,74].

Elevated homocysteine

Homocysteine is an independent risk factor for cardiovascular disease. It is associated with vascular injury and, thus, increased risk for stroke, CAD and venous thrombosis. Possible mechanisms of action include endothelial dysfunction, platelet aggregation and perturbation of clotting factors. In addition, alteration of the extracellular matrix of several tissues (mainly vessels), elastolysis and oxidative stress may be implicated.

Hyperhomocysteinemia has been suggested as a possible cause for increased vascular risk because of the potential to trigger the abnormal matrix accumulation in PEX patients. High levels of plasma homocysteine have been found in patients with PEX syndrome and PEX glaucoma[75-84]. Homocysteine concentration has been found to be elevated[83] or unaffected[77] in aqueous humor of patients with PEX glaucoma, while increased in PEX glaucoma patients' tears[84]. Vitamins B6, B12 and folate, which are involved in homocysteine metabolism and negatively correlated with total plasma homocysteine levels, have been reported to be decreased in PEX glaucoma patients[85], though not differing between PEX and control groups in another study[77]. On the contrary, Turacli *et al*[86] did not confirm the relationship between plasma homocysteine and PEX syndrome. Hyperhomocysteinemia has also been implicated in the decrease of both LOX activity and expression in vascular endothelial cells[87]. LOX downregulation has been associated with endothelial dysfunction, characteristic of earlier stages of the atherosclerotic process[88]. A possible association between SNPs in the *LOXL1* gene (which is linked with PEX syndrome) and spontaneous cervical artery dissection has also been proposed[89].

Arterial hypertension

It is known that hypertension is a major risk factor for stroke, myocardial infarction, heart failure, aneurysms of the arteries (*e.g.*, aortic aneurysm), peripheral arterial disease and chronic kidney disease. At least two studies have demonstrated a higher rate of arterial hypertension in patients with PEX[14,90]. Endothelial damage, impairment of the parasympathetic vascular regulation and elastosis have been implicated. Renal artery stenosis with subsequent arterial hypertension has also been reported[91]. However, reports are conflicting and no clear association has yet been proven, as other studies failed to demonstrate any significant relationship between PEX and arterial hypertension[15,16,17,23-26,61,92], or found arterial hypertension to be less common in PEX subjects[28,93,94].

Aortic aneurysm

Impairment in systemic macro- and microcirculation in PEX patients has been suggested. Abdominal aortic aneurysms have been attributed to atherosclerosis, though other factors are involved in their formation. An association between aneurysms of the abdominal aorta and PEX syndrome has been proposed. Histopathological examination of aortic-wall samples from patients with ocular PEX syndrome revealed accumulation of focal PEX deposits in the adventitial and subendothelial connective tissue, pronounced fibrosis, and elastosis of the tunica intima[95]. Abdominal aorta aneurysm was observed with a higher frequency in PEX patients than in control group[91,96], although, other studies failed to demonstrate any significant association[97,98].

OTHER COMMON PATHOGENETIC SIGNS

Apart from epidemiologic studies and the presence of PEX deposits on vessel wall, a possible relation between PEX material and cardiovascular disease may be supported by similar features in their pathogenesis. In addition to vascular endothelial dysfunction, hyperhomocysteinemia and blood flow changes mentioned above, disorders of the extracellular matrix by growth factors, matrix metalloproteinases, cytokines and altered enzymic action constitute part of atherosclerosis[99] and PEX fibrillopathy process. Altintas *et al*[100] demonstrated higher serum antiphospholipid antibodies (a risk factor for cardiovascular and cerebrovascular disease) in patients with PEX and PEX glaucoma than in healthy controls and in patients with POAG. In support of the above, serum asymmetric dimethyl arginine and YKL-40 levels (both independent cardiovascular risk factors) have been found higher in

PEX patients than those of the control group[101,102].

Atherosclerosis is associated with a number of oxidative events like low density lipoproteins oxidation, production of intracellular reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as, endothelial dysfunction and plaque disruption[103]. The oxidative-antioxidative balance is disturbed in patients with PEX syndrome as supported by reduced levels of antioxidants such as ascorbic acid, glutathione, trace elements, antioxidative enzymes in aqueous humor and serum and increased levels of oxidants such as hydrogen peroxide or nitric oxide, as well as, oxidative stress markers[104].

Inflammation plays a major role in all phases of atherosclerosis. Inflammatory cells like macrophages and lymphocytes both migrate from the blood and multiply within the atherosclerotic plaques. Activation of these cells leads to lytic enzymes, cytokines, chemokines and growth factors release that induce further damage[105]. Stress-induced, temporally restricted subclinical inflammation in anterior segment tissues is detected during the early stages of the fibrotic PEX process[10]. Moreover, inflammatory markers such as alpha-1 antitrypsin, Interleukin-6, high-sensitivity C-reactive protein and Tumor Necrosis factor alpha have been reported to be increased in PEX subjects[106-108].

CONCLUSION

Although more data is still required, an increased incidence of cardiovascular disorders in PEX patients and several common features in their pathogenesis suggest that PEX may be an independent risk factor for cardiovascular disease or it may occur as part of a systemic disorder with cardiovascular implications. The pathogenesis of PEX glaucoma and CAD in PEX patients may reflect different manifestations of the same process. Patients with PEX syndrome should be informed and examined frequently as cardiovascular risk may be present throughout.

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MINIREVIEWS

Management of renal artery stenosis: What does the experimental evidence tell us?

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Abstract

Optimal management of patients with renal artery stenosis (RAS) is a subject of considerable controversy. There is incontrovertible evidence that renal artery stenosis has profound effects on the heart and cardiovascular system in addition to the kidney. Recent evidence indicates that restoration of blood flow alone does not improve renal or cardiovascular outcomes in patients with renal artery stenosis. A number of human and experimental studies have documented the clinical, hemodynamic, and histopathologic features in renal artery stenosis. New approaches to the treatment of renovascular hypertension due to RAS depend on better understanding of basic mechanisms underlying the development of chronic renal disease in these patients. Several groups have employed the two kidney one clip model of renovascular hypertension to define basic signaling mechanisms responsible for the development of chronic renal disease. Recent studies have underscored the importance of inflammation in the development and progression of renal damage in renal artery stenosis. In particular, interactions between the renin-angiotensin system, oxidative stress, and inflammation appear to play a critical role in this process. In this overview, results of recent studies to define basic pathways responsible for renal disease progression will be highlighted. These studies may provide the rationale for novel therapeutic approaches to treat patients with renovascular hypertension.

Key words: Renovascular hypertension; CCL2; CCR2; Kidney; Inflammation; Atrophy

Core tip: Renovascular hypertension is a common public health problem, particularly in older patients with underlying atherosclerotic vascular disease. Recent studies have shown that restoration of blood flow in these patients fails to improve renal function or survival. Recent studies to define basic mechanisms underlying the development of chronic renal disease in renin angiotensin system (RAS) have shown that pro-inflammatory pathways may play a critical role in this process. Therapeutic approaches that target inflammatory pathways may provide the basis for novel and more effective treatments for patients with RAS.

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RENOVASCULAR HYPERTENSION IS AN IMPORTANT CAUSE OF SECONDARY HYPERTENSION

It is well recognized that hypertension is a major public health problem. The prevalence of hypertension is 29% in the United States; an additional 28% of adults have

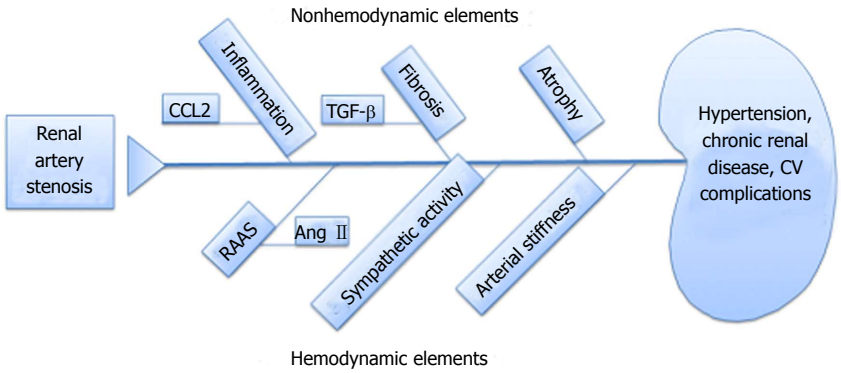

Figure 1 Summary of hemodynamic and non-hemodynamic pathways responsible for development of chronic renal damage in renal artery stenosis.

"prehypertension"[1]. Although the most common form of hypertension is "essential" hypertension, there is increasing recognition of secondary forms of hypertension that contribute to morbidity and mortality in patients with hypertension. Many of these cases have been identified through use of imaging modalities to assess patency of the coronary arteries. The prevalence of renovascular hypertension (RVH) is 7% in patients over 65 years of age[2]. In patients with coronary artery disease or aortoiliac disease, the prevalence of RVH is as high as 50%[3-5]. From 1991-1997, the annualized incidence of RVH as a cause of end stage renal disease increased by 12.4% per year, a larger increase than other causes of end stage renal disease[6]. Atherosclerosis is the most common etiology underlying RVH in this population[7-9]. In addition to chronic renal disease, atherosclerotic RVH contributes to cardiac morbidity and mortality[10]. For example, recent studies have shown that the overall 4-year survival of patients undergoing cardiac catheterization was 86% in patients without RAS but only 65% in those with RAS[11]. The extent of RAS also predicts survival, with 4-year survival of 89% in patients with RAS < 75% luminal occlusion, but only 57% in those with > 75% luminal occlusion[10,11]. Optimal treatment of these patients require the development of animal models to elucidate mechanisms of renal and cardiovascular disease progression.

ANIMAL MODELS OF RVH

The two kidney 1 clip (2K1C) model of renovascular hypertension, developed by Goldblatt, has been extensively employed to understand the pathogenesis of renovascular hypertension[12]. In his original model, dogs subjected to renal artery stenosis developed malignant hypertension, which caused extensive damage to the contralateral kidney. More recently, this model has been extended to other species, including mice, rats, and pigs[13-19]. In general, these animals do not develop malignant hypertension, and may thereby more accurately model human renal artery stenosis. In these animals, the stenotic kidney develops progressive atrophy, whereas the contralateral kidney develops hypertrophy but without major histopathologic alterations[14].

This model has allowed investigators to study the interrelationships between hemodynamic factors and non-hemodynamic factors responsible for the development of cardiovascular and renal disease (Figure 1). Hemodynamic factors include vasoactive effects mediated by activation of the renin-angiotensin-aldosterone system, increased sympathetic nervous activity, and increased arterial stiffness. Non-hemodynamic factors include the signaling pathways triggered by renal parenchymal cells and infiltrating inflammatory cells in the development and progression of renal and cardiovascular disease, and include chemokines, reactive oxygen species, and transforming growth factor β (TGFβ).

DEVELOPMENT OF CHRONIC RENAL DISEASE IN RAS: WHAT DOES THE EXPERIMENTAL EVIDENCE TELL US?

Most studies have focused on the role of renal hypoperfusion and subsequent hypoxia on the development of chronic renal damage in the stenotic kidney. It is well recognized that reduced blood flow leads to intra-renal activation of the renin-angiotensin system, leading to elevated plasma levels of angiotensin Ⅱ, a potent vasoconstrictor, and the development of systemic hypertension. However, several recent observations have called this paradigm into question. Recent imaging studies to assess renal oxygenation have suggested that the stenotic kidney is not hypoxic. It is recognized that the kidney receives far more blood than needed to support basic metabolic demands-indeed, renal tissue requires less than 10% of normal blood flow to support basic metabolic needs[20]. Furthermore, the kidney has the capacity to adapt to significant reduction in the diameter of renal artery with preservation of renal oxygenation[21]. In both human and experimental models, it appears that systemic activation of the renin-angiotensin system is transient, and that progression of renal and cardiovascular disease can occur without persistent elevation of plasma angiotensin Ⅱ levels[22]. These observations have prompted investigations into basic signaling pathways triggered by renal artery stenosis that may be responsible for maintenance of systemic hypertension and the development of chronic renal disease.

Although plasma angiotensin Ⅱ levels may not remain elevated as cardiac and renal damage progress in renal artery stenosis, there is evidence for persistent activation of the intra-renal renin-angiotensin system. The

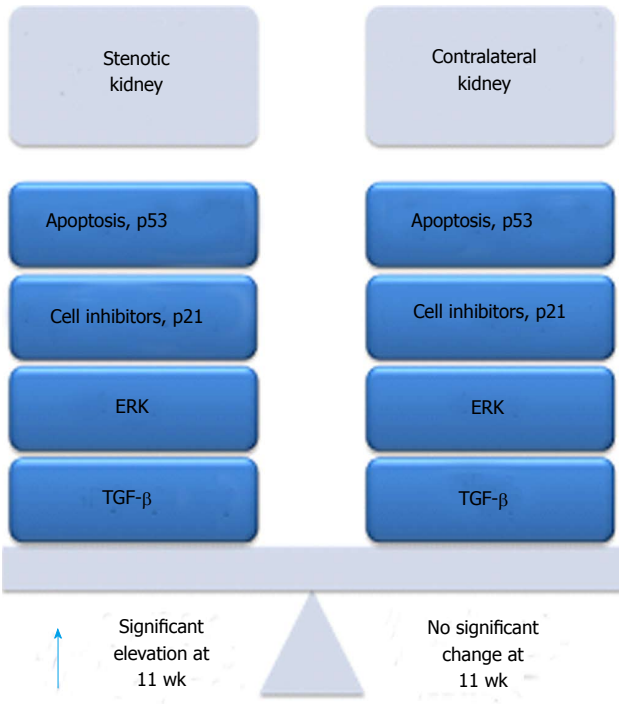

Figure 2 Mediators persistently induced in the stenotic kidney and transiently induced in the contralateral kidney in murine renal artery stenosis.

kidney can produce all elements needed to completely activate the renin-angiotensin system, including renin, angiotensinogen, angiotensin converting enzyme, and angiotensin type 1 and type 2 receptor[23-25]. In the kidney, renin is expressed primarily by the juxtaglomerular cells. Angiotensinogen is expressed in proximal tubular epithelial cells and is secreted into tubular lumens. Angiotensin I is converted to Angiotensin Ⅱ through action of ACE located on the apical brush border of tubular epithelium. We have shown that renal expression of Ren1 in the stenotic, but not contralateral, kidney persists in renal artery stenosis[26]. Based on these considerations, we embarked on a series of studies to compare signaling pathways activated in the stenotic and contralateral kidneys during the development and progression of renal damage in renal artery stenosis. A summary of our findings is highlighted in Figure 2.

In our initial studies, we correlated histopathologic alterations in the stenotic and contralateral kidneys at 2, 5, and 11 wk following renal artery stenosis surgery with signaling pathways that govern cell cycle regulation (cyclins D, E, A, and B; p21; p27), proliferation (PCNA, ERK, p38 MAPK), fibrosis (TGF-β; Smad2, Smad3, Smad4), and inflammation (MCP-1)[14]. The stenotic kidney showed progressive tubular atrophy, which was associated with interstitial fibrosis and inflammation, which closely recapitulates the histopathologic alterations observed in humans with advanced renal artery stenosis[27]. The contralateral kidney underwent compensatory enlargement, which was at least in part through hyperplasia. Compensatory enlargement in the contralateral kidney occurred in the absence of significant histopathologic alterations. We found that signaling pathways associated with cell cycle regulation, inflammation, and fibrosis were activated in both kidneys following induction of renal artery stenosis. However, these pathways were transiently activated in the contralateral kidney, returning to baseline levels by 11 wk, whereas they were progressively and persistently activated in the stenotic kidney.

A critical role for the p38 MAPK pathway in the development of renal atrophy was established in studies using the biochemical inhibitor SB203580[28]. The development of renal atrophy in the stenotic kidney was significantly decreased in mice treated with SB203580 at the time of renal artery stenosis surgery. Decreased atrophy was associated with reduced interstitial inflammatory infiltrates and decreased fibrosis. The p38 MAPK inhibitor had no significant effect on blood pressure or on plasma renin activity. Of note, treatment of mice with the ERK inhibitor U0126 did not prevent the development of renal atrophy, interstitial fibrosis, and interstitial inflammation (unpublished data).

In our previous studies, we demonstrated that TGF--β and its receptors (RI and RII) are persistently induced in the stenotic kidney of mice subjected to RAS. TGF-β has been implicated as a critical mediator of cell cycle regulation, inflammation, and fibrosis in other model systems[14,29-31]. The TGF-β signaling pathway interacts with a number of other signaling pathways, including the renin-angiotensin system and the MAPK pathways. TGF-β mediates fibrosis through interactions with Smad 2, Smad3, and Smad4. Although TGF-β knockout mice have high embryonic lethality and develop a systemic inflammatory syndrome shortly after birth, Smad3 knockout mice are viable and exhibit defects in TGF-β signaling[32].

We found that the stenotic kidneys of Smad3 knockout mice are almost completely protected from the development of interstitial fibrosis, tubular atrophy, and interstitial inflammation, despite an elevation of plasma renin activity and a reduction in blood flow of over 70%[22]. In an acute ischemia-reperfusion model, we showed that the kidneys of Smad3 knockout mice were resistant to the development of acute injury[33]. A similar protective effect has been observed in Smad3 mice subjected to unilateral ureteric obstruction.

Although we have shown that interruption of the p38 MAPK or Smad3-TGF-β signaling pathways prevent the development of renal atrophy, it is not clear how renal damage is initiated in this model. For this reason, we have conducted a series of studies to better understand the early signaling events and to correlate these with histopathologic alterations during the development of chronic renal disease in this model[26]. At 3 d following renal artery stenosis surgery, the stenotic kidney shows minimal histopathologic alterations. In particular, there is no evidence of acute injury to tubular epithelial cells, no significant interstitial fibrosis, tubular atrophy, or interstitial inflammation. Despite the normal appearance of the stenotic kidney, the tubular epithelial cells express markers of oxidative stress. It is recognized that the kidney expresses all components of the NADPH oxidase system[34] and that Ang Ⅱ promotes ROS generation through activation of

this membrane bound complex[34]. However, most studies of the interaction between Ang Ⅱ activation and ROS generation have focused on later time points, after the initiation and development of chronic renal injury.

Influx of inflammatory cells, predominantly macrophages and lymphocytes, is first seen at 7 d following RAS surgery, a time point at which the kidney begins to develop tubular atrophy[26]. Influx of inflammatory cells is associated with induction of CCL2 (MCP-1) a potent chemoattractant protein. The relevance of this observation is underscored by studies demonstrating that increased production of CCL2 is associated with the influx of inflammatory cells in human renal artery stenosis[35,36] and that the development of chronic renal disease in RAS is associated with the influx of macrophages and T cells. Recent studies have suggested that signaling through CCL2 may play a critical role in the development and progression of both renal and cardiovascular disease in renal artery stenosis and other cardiovascular and renal diseases[37-39]. Both renal parenchymal cells and infiltrating inflammatory cells express CCR2, the primary receptor for CCL2. Through activation of this pathway, infiltrating inflammatory cells are capable of generating ROS and a number of chemokines which promote renal fibrosis.

We have observed increased expression of CCL2 at 3 d, prior to the influx of inflammatory cells, suggesting that renal parenchymal cells may be the source of this chemotactic chemokine[26]. Additional studies are required to verify this observation. It is not yet known whether blockade of CCL2-CCR2 signaling will prevent the development and/or progression of chronic renal disease in RAS.

MANAGEMENT OF RENAL ARTERY STENOSIS

Until recently, management of RAS was predicated on restoration of blood flow to the stenotic kidney. It was thought that this intervention would decrease local and systemic activation of the renin-angiotensin system, thereby restoring normal blood pressure and reducing both renal and cardiovascular morbidity and mortality. Recent studies have clearly demonstrated that restoration of blood flow through stenting fails to improve renal or cardiovascular outcomes, compared to medical therapy[40]. The mainstay of medical therapy involves blood pressure control, through use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and/or other agents to reduce blood pressure[41]. There are concerns that aggressive blood pressure reduction may exacerbate damage to the stenotic kidney, although reduction of blood pressure is thought to improve overall renal function by protecting the contralateral kidney. Unfortunately, patients treated with medical therapy are still at risk for the development of progressive chronic renal disease, which in itself is a risk factor for the development of cardiovascular events. Recent studies have raised the possibility that therapies directed towards modulating the inflammatory response to chronic renal injury may have a role in management of patients with renal artery stenosis.

CONCLUSION

Optimal management of patients with RAS is limited by our lack of understanding of the events leading to the development of chronic renal damage in the stenotic kidney, and how these events contribute to cardiovascular morbidity and mortality. Inhibitors of P38 MAPK and of Smad3 signaling have been shown to prevent the development of chronic renal damage in experimental RAS. In addition to concerns regarding adverse effects of currently available compounds, there is no evidence that these agents can prevent the progression of chronic renal damage once clinical manifestations of renal artery stenosis become apparent. Similarly, human trials of antioxidant therapies to arrest the progression of systemic inflammatory conditions including atherosclerosis have been disappointing. Our recent observations, that generation of CCL2 and expression of CCR2 is an early event in RAS-an event which precedes the influx of inflammatory cells-merit additional study. In particular, it is not known whether abrogation of CCL2-CCR2 signaling with prevent the development of chronic renal disease in RAS or will arrest the progression of chronic renal disease once the disease becomes clinically apparent. Studies to address these important issues may provide the basis for changing the paradigm for treatment of renal artery stenosis from one that emphasizes restoration of renal blood flow to one that focuses on treatment of the inflammatory response to renal artery stenosis.

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MINIREVIEWS

Long term negative pressure ventilation: Rescue for the failing fontan?

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Abstract

Current treatment strategies for single ventricle patients include non-intervention strategy, surgical palliation or primary transplantation. Surgical palliation includes a staged operative course culminating in the Fontan operation. With progress in surgical techniques, the survival has been improving. However, almost all of these Fontan patients will demonstrate pathophysiologic changes that ultimately constitute "Fontan failure physiology". This article reviews the pathophysiologic changes, current approach to management of these patients and proposes a novel way of reversing some of the pathophysiologic changes by utilization of negative pressure ventilation.

Key words: Fontan; Single ventricle physiology; Negative pressure ventilation; Cardiorespiratory interactions; Congenital heart disease

Core tip: In the current surgical era for congenital heart disease, palliation of single ventricle patients has become standard of care. However, pathophysiologic failure after the thrid stage of palliation (Fontan) is commonplace, with very few therapeutic options. Failing Fontan physiology is a management challenge. Herein, we review the pathophysiology of failing Fontan, current therapies and propose a novel way of treating the failing Fontan by utilizing negative pressure ventilation to reverse some of the maladapative changes. This is a hypothesis paper. We think, the ideas central to the manuscript are worth bringing out for intellectual discussion and wider testing.

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INTRODUCTION

The Fontan pathway is a palliative pathway for single ventricle patients. This pathway allows us to utilize the single ventricle as a systemic pumping chamber and create separation between the pulmonary and systemic circuits thereby allowing sustenance of life. We have therefore dramatically altered the natural history of these congenital heart problems.

Over the last two decades, with significant improvement in the surgical and perioperative technologies, the mortality of complicated cardiac surgeries such as the Fontan procedure has been reduced[1-3]. However, as the current Fontan population becomes older, we are facing a new challenge of managing failing Fontan circulations. Currently we have very limited options for management of the failing Fontan physiology[4,5]. This paper proposes new modality for management of these complex patients and the clinico-pathologic evidence for its use.

THE FAILING FONTAN

Fontan or total cavo-pulmonary connection is a staged surgical palliation of functional single ventricle. It allows

us to designate the single ventricle (or the dominant ventricle) as the systemic ventricle. The other essential part of this pathway, then, is to establish source of pulmonary blood flow without a designated "pulmonary" ventricle. At completion, this constitutes a staged connection of the superior vena cava to the pulmonary artery (Glenn procedure) followed by connection of the inferior vena cava to the pulmonary artery (Fontan procedure).

In the current era, this inferior vena cava to the pulmonary artery connection is made by using either an intra-atrial baffle (lateral tunnel) or by using an extracardiac conduit. After completion of this stage of repair, the systemic venous return is channeled appropriately to the pulmonary artery for oxygenation, while the pulmonary veins return to the common atrium, to be ejected out of the single systemic ventricle. Thus, circulation in series is established. This allows, in theory, for fully saturated blood to be pumped out to the systemic circulation. In practice, saturations are around 92% to 94% early postoperatively, with small arteriovenous malformations and coronary sinus blood flow contributing to the lower saturation[6]. However, as the patients get older, there is a gradual decline in the oxygen saturations due to various factors. Progressive desaturation is only one of the problems of Fontan in later years. Lack of the pulmonary ventricle eventually leads to multiple problems related to the hemodynamics of failing Fontan circuit. Main reasons for late mortality are related to arrhythmias, thromboembolism and protein losing enteropathy[7] .Other manifestations of the failing Fontan circuit include systemic venous congestion, hepatic dysfunction, coagulopathy, plastic bronchitis, progressive cardiac failure and cardiac cachexia. These are major causes of morbidity and mortality in Fontan patients[4,5,8].

Along with the above, there is progressive decrease in the forward flow of blood to the pulmonary vascular bed, leading to progressive hypoxemia and cyanosis. Development of systemic to pulmonary venous collaterals further contributes to the development of cyanosis[6].

There are limited medical and surgical options for management of these patients[4,9,10]. For some patients who meet the eligibility criteria including a low pulmonary vascular resistance, heart transplantation is an option. The early outcomes of heart transplantations in patients with failed Fontan are slightly worse compared to patients with cardiomyopathies or other congenital heart diseases[11,12]. Heart transplantation is therefore a reasonable option in selected group of patients, with organ supply being a significant limiting factor. Patients with classic atriopulmonary connection and incessant arrhythmias or flow obstruction may need conversion to an extracardiac cavo-pulmonary connection[9]. Other surgical interventions focus on relieving obstructive causes of Fontan failure (*e.g.*, conduit obstruction) or systemic atrioventricular valve replacement for significant regurgitation. As a palliation for high Fontan pressures, creation of a fenestration from the Fontan to the atrium is considered[13].

Medical management of failing Fontan focuses on treating individual issues[4,5]. Systemic venous congestion and volume overload is treated with diuretics. Aggressive diuresis however, can be counterproductive. Anticoagulation, either with anti-platelet agents or coumadin is used in the presence of thrombosis. Myocardial dysfunction manifests itself as both systolic and diastolic dysfunction. Severe myocardial dysfunction may warrant intravenous milrinone therapy. There is limited data to suggest significant benefits occur from using ACE inhibitors or beta-blockers in failing Fontan[14,15]. Similarly, newer agents such as endothelin receptors antagonists have failed to show impact in Fontan patients. Medical therapy for other complications such as protein losing enteropathy has only had modest success[16].

As mentioned above, all of these constitute piecemeal approach and none of these strategies address the one of the primary problems, which is, decreased antegrade flow across the Fontan circuit to the pulmonary vascular bed causing the pathophysiology of failure.

HEMODYNAMIC EFFECTS OF NEGATIVE PRESSURE VENTILATION

Negative pressure ventilators were one of the first ventilators developed and served a vital role during the Polio epidemics in the twentieth century. Overtime, positive pressure ventilators have completely replaced them as conventional modes of ventilation. As a result, there are very limited circumstances in contemporary medicine under which negative pressure ventilation negative pressure ventilation (NPV) is being considered[17,18]. An example would be patients with neuromuscular disorders for long term respiratory support[17].

Currently, there are some commercially available devices for delivering NPV. Porta- Lung ™ is a modern version of the iron lung. It is a closed chamber system that delivers effective negative pressure ventilation and has been used for long term ventilatory support. Cuiras® ventilator is a shell that is applied over the chest and delivers NPV. This mode applies negative pressure locally over the thorax only and allows for better patient mobility and ease of access. The ventilators that drive these units have also undergone significant improvements over the years, including ability to synchronize breaths with patient initiated breaths as well as with cardiac cycle[19].

From cardiac and hemodynamic standpoint, NPV has significantly different effects as compared to positive pressure ventilation (PPV). These cardiopulmonary interactions are much more physiologic than those of PPV.

In a normal heart, NPV and by extension, negative intrathoracic pressure leads to reduction in the right ventricular afterload thus augmenting right ventricular function and right ventricular cardiac output. NPV helps maintain lung volumes close to functional residual capacity, which reduces the pulmonary vascular resistance and improves pulmonary blood flow[20]. In physiologic states as well as in patients after simple cardiac surgery, NPV has been shown to augment cardiac output[21]. In patients with Glenn or Fontan physiology, where there is depen-

dence on passive diastolic blood flow, NPV directly augments passive blood flow to the lungs by creating a negative thoracic gradient[22,23]. As a downstream consequence, there is an increase in the pulmonary venous return and cardiac output.

In experimental models and small studies, benefits of NPV in immediate post-operative period have been documented[24]. Shekerdemian *et al*[25,26] have shown hemodynamic benefits of NPV in patients with right ventricular dysfunction in post-operative period[25,26]. Similarly, augmentation of cardiac output by using NPV, in the immediate post operative period for patients undergoing Fontan procedure has also been documented[23].

We have recently documented the dramatic application of a NPV in the rescue of a failing Kawashima patient, resulting in successful recovery after failure of all conventional therapies[27].

All of these applications of NPV in Fontan patients have been for a very short term ; either in the immediate post-operative state or during hemodynamic studies. There has not been an application for long-term use of NPV in cardiac patients as a rescue measure or mode of palliation for these single ventricle patients. We propose such a novel application, based on strong hemodynamic reasoning as outlined above as well as the aforementioned short term application studies.

HYPOTHESIS

Our hypothesis is that long-term use of negative pressure ventilation is an effective mode of rescue for patients with failing Fontan physiology. Our hypothesis extends to suggest that long term use of NPV will: (1) improve antegrade flow to the pulmonary bed across the Fontan circuit (by creating intrathoracic negative pressure). This in turn would lead to: decrease Fontan pressures; decrease hepatic vein wedge pressure thereby decreasing hepatic congestion and improving hepatic function; decrease formation of ascites; and decrease peripheral edema; (2) stabilize and even improve oxygen saturation (better Fontan flow and improved oxygenation); (3) improve cardiac output (based on 21-23); and (4) provide symptomatic improvement as measured by exercise capacity and patient self-assessment scores.

CLINICAL APPLICATION

The proposed method of practical application of this management strategy is as follows. The initial step is appropriate patient selection. Patients who have undergone Fontan procedure and have been classified as failing Fontan patients will be candidates for this therapy. Patients with fixed obstruction that is reversible (such as stenosis of branch pulmonary artery) should be intervened on prior to selection. All patients should get a comprehensive imaging workup, either with echocardiography or an MRI where echocardiography is inadequate.

The recommended method of delivery of NPV is by using a synchronized biphasic cuirass ventilator. Initiation of NPV should be in hospital setting. This will provide closer monitoring during initiation as well as allow adjustments on ventilator setting, assessment of patient comfort and patient education. A baseline complete metabolic assessment including electrolytes, liver function tests and brain natriuretic peptide (BNP) should be obtained. More invasive monitoring including blood gases (arterial and mixed venous) as well as pulmonary artery pressure should not be mandated, but may be beneficial during initial experience.

Settings on the NPV to be optimized as tolerated. After this short stay, patients should be able to use the NPV at home. Home NPV therapy may be designed with various levels of intensity. The proposed level is about 10 to 12 h of NPV during evening and night hours , thus allowing patients to continue with there daily activities during the day time. For younger patients as much as 16 h of NPV time would be recommended. Recommendations for follow-up include telephone call follow-up every week to address any concerns as well as maintain compliance. Patients will be asked to check weights at home every week.

Follow-up as outpatient should be in two weeks initially, followed by monthly until the care-giver deems appropriate. A repeat complete metabolic panel and BNP should be obtained in 3 mo. Functional status assessment as well as exercise capacity testing should be performed at 6 mo. Continued follow-up to assess improvement in hemodynamics and symptomatology as deemed appropriate by the primary cardiologists should be maintained.

Possible problems related to long-term use of NPV are very minimal and have been described in other settings. Main issues are related to obtaining a good comfortable fit so as to minimize skin contact injury. Patients with upper airway obstruction or significant tracheomalacia are not suitable candidates for NPV and should be excluded[17,18].

CONCLUSION

Palliation of single ventricle patients has led to increase in long term survival for these complex patients. Current staged surgical palliation concludes with Fontan surgery. However, there are multitudes of problems related to the Fontan circulation that result in significant morbidity and mortality, ultimately resulting in a state of Fontan failure.

As described above, there are limited options for management of a failed Fontan. Here in we propose an innovative use of NPV to augment the Fontan flow and improve the underpinnings of the pathophysiology of Fontan failure.

There is strong experimental and clinical data to suggest that NPV augments the hemodynamics in patients with single ventricle physiology[21-25]. The ability of the modern negative pressure ventilators to be portable, accessible and effective has provided the opportunity of unique application of these ventilators as a long term therapy for failing Fontan patients.

The authors propose that this strategy will provide a

novel therapy to address a growing problem and provide improved quality of life to this group of patients.

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MINIREVIEWS

Risk stratification for ST segment elevation myocardial infarction in the era of primary percutaneous coronary intervention

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Abstract

Acute coronary syndromes presenting with ST elevation are usually treated with emergency reperfusion/revascularisation therapy. In contrast current evidence and national guidelines recommend risk stratification for non ST segment elevation myocardial infarction (NSTEMI) with the decision on revascularisation dependent on perceived clinical risk. Risk stratification for STEMI has no recommendation. Statistical risk scoring techniques in NSTEMI have been demonstrated to improve outcomes however their uptake has been poor perhaps due to questions over their discrimination and concern for application to individuals who may not have been adequately represented in clinical trials. STEMI is perceived to carry sufficient risk to warrant emergency coronary intervention [by primary percutaneous coronary intervention (PPCI)] even if this results in a delay to reperfusion with immediate thrombolysis. Immediate thrombolysis may be as effective in patients presenting early, or at low risk, but physicians are poor at assessing clinical and procedural risks and currently are not required to consider this. Inadequate data on risk stratification in STEMI inhibits the option of immediate fibrinolysis, which may be cost-effective. Currently the mode of reperfusion for STEMI defaults to emergency angiography and percutaneous coronary intervention ignoring alternative strategies. This review article examines the current risk scores and evidence base for risk stratification for STEMI patients. The requirements for an ideal STEMI risk score are discussed.

Key words: ST segment elevation myocardial infarction; Risk stratification; Primary percutaneous coronary intervention; Harm; Risk scores

Core tip: Risk stratification is recommended in non ST segment elevation myocardial infarction (NSTEMI) by multiple international cardiology agencies however there is no such recommendation for STEMI. The short term risk of STEMI is perceived to be high and warrant emergency percutaneous coronary intervention rather than pharmacological fibrinolysis. The risk spectrum is wide therefore consideration should be given to developing an optimal reperfusion strategy based on risk of adverse outcome and probability of reperfusion regard-

less of mode of reperfusion.

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INTRODUCTION

Acute coronary syndromes in contemporary cardiology practice

The initial management of acute coronary syndromes (ACS) depends on the presence of ST elevation on the electrocardiogram. In the United Kingdom Primary Percutaneous Coronary Intervention (PPCI) is the recommended treatment for ST segment elevation MI (STEMI). International guidelines recommend formal risk stratification using a validated risk score for all patients presenting with non ST elevation MI (NSTEMI) but not for STEMI.

In this article we review the established risk scores and their limitations. We also examine the need for a risk score for those patients presenting with STEMI.

Risk stratification and risk scores

Risk stratification is defined as "a statistical process to determine detectable characteristics associated with an increased chance of experiencing unwanted outcomes"[1]. When applied to ACS risk stratification has helped target healthcare resources and guide clinicians as to revascularisation requirement, urgency and method. Risk scores such as the Global Registry of Acute Coronary Events (GRACE) score have shown that of the spectrum of patients with ACS those who presented with STEMI had the highest short-term risk of death. This group also benefitted from rapid reperfusion therapy, an effect confirmed in the GISSI-1 and ISIS-2 trials[2,3]. Reperfusion treatment was initially limited to systemic thrombolysis (fibrinolysis). However, thrombolysis is associated with a "failure rate" of incomplete coronary reperfusion, which led to the development of mechanical reperfusion methods and the introduction of PPCI programmes[4].

Within the STEMI population, there is a spectrum of higher and lower risk patients. For example, STEMI presenting with haemodynamic instability or cardiac arrest is associated with a higher risk of mortality[5,6]. Stratification of risk in STEMI has been more difficult because PPCI has been offered and incorporated into national and international guidelines to all patients without contraindication who present with clinical and electrocardiographic criteria[7,8]. In contemporary practice it is, therefore, unlikely that a STEMI risk score would impact on decision making, since the pathway is algorithmic once a diagnosis is made. Risk scoring is therefore only used to evaluate hospital and individual operator performance. An alternative approach would be to use risk scoring in STEMI to target healthcare and refine decision-making such as by offering immediate thorombolysis to low risk patients presenting early and PPCI to other higher risk patients.

Despite progress in pre-hospital care, ambulance logistics, pharmacotherapy and PPCI techniques, STEMI continues to confer a substantial burden of morbidity and mortality and consumes significant healthcare budget. Consequently, optimal reperfusion strategy is a subject of ongoing research interest[9,10]. When compared to the NSTEMI population there has been little effort to quantify patient risk in STEMI since all randomised controlled trials studying PPCI efficacy offer PPCI as default[7,8,11].

PPCI when available or immediate fibrinolysis?

Reperfusion is most effective when delivered early. Any delay to reperfusion is associated with an increase in mortality[12-14]. In the real world patients may experience considerable delays that may negate the benefit of PPCI over immediate fibrinolysis[15,16]. The National Institute of Health and Care Excellence (NICE) has highlighted the need for further research into very early presentation of STEMI but acknowledges the current evidence in favour of PPCI[17]. The question of whether early pre-hospital thrombolysis with subsequent coronary angiography and intervention (PCI or CABG) is non-inferior to expert and timely PPCI has been evaluated recently. The Strategic Reperfusion Early after Myocardial Infarction (STREAM) study investigated early fibrinolysis *vs* PPCI. For those with early fibrinolysis with Tenecteplase (TNK) there was a suggestion of outcome equivalence albeit with an increase in intracranial bleeding[18].

PPCI RISK MODELS FOR DEATH AND BLEEDING IN CONTEMPORARY PRACTICE

The Myocardial Ischaemia National Audit Project (MINAP) is a United Kingdom national registry database of all acute coronary syndromes. The MINAP database was established in 1999 to examine the quality of management of acute myocardial infarction (AMI) in England and Wales and to meet the audit requirements of the national service framework for coronary heart disease[19,20]. Risk scores have been constructed based on trial data and statistical modelling using databases such as MINAP as bench markers for validity. The other major risk scores are summarised in the table below (Table 1).

The risk scores outlined have demonstrated some ability to predict survival. However, whilst their use has been recommended by international guidelines, their uptake by the clinical community has been poor. There are several reasons for this: The GRACE score is the most widely used but lacks point of care convenience whilst the TIMI score has this functionality but is less discrimi-

Table 1 Summary of major risk scores utilised in percutaneous coronary intervention

Risk score	Type	Population	No of patients	Outcomes	No of variables	Validation	c- statistic	Ref.
GRACE	Clinical	NSTEMI, STEMI	85771	In hospital and 6 mo mortality (8.6% and 12.9%)	7	FAST-AMI	0.8 and 0.8	[21]
GRACE – 2	Clinical	NSTEMI, STEMI	32037	1 and 3 yr mortality	8	FAST AMI	0.82 and 0.82	[22]
GUSTO -1	Clinical	STEMI	41021	30 d to 1 yr mortality (2.9%)	7	MINAP	0.8 at 30 d 0.75 at 1 yr	[21,23]
SRI	Clinical	STEMI	100686	30 d mortality	3	In time II/MINAP	0.79	[21,24]
TIMI	Clinical	STEMI	14114	30 d mortality	10	External with TIMI-9 trial	0.746	[25]
CADILLAC	Clinical	STEMI	2082	1 yr mortality	7	Stent– PAMI (900 patients, internal)	0.78	[26]
APEX - AMI	Clinical	STEMI	5745	90 d mortality	7	Internal (no external)	0.81	[27]
EMMACE	Clinical	All MI	100686	30 d mortality	3	Internal	0.78	[28]
SYNTAX	Angiographic	NSTEMI CSA		5 yr mortality	n/a	LEADERS trial	0.62	[29-33]
Clinical SYNTAX	Clinical and angiographic	NSTEMI CSA	512	5 yr mortality	Syntax score and modified ACEF score	LEADERS trial	0.69	[29]
EURO Heart	Clinical and angiographic	ACS and STEMI	23032	In-hospital mortality	16	Internal	0.89	[34]
MINAP (reference)				30 d to 1 yr mortality (5.0%)				

GRACE: Global registry of acute coronary events; FAST-AMI: French registry of Acute ST-elevation and non-ST elevation MI; GUSTO: Global utilisation of streptokinase and tissue plasminogen activator (TPA) for Occluded coronary arteries; SRI: Simple risk index; TIMI: Thrombolysis in acute myocardial Infarction; CADILLAC: Controlled abciximab and device investigation to lower late angioplasty complications trial; APEX: Ami assessment of pexelizumab in acute myocardial infarction trial; EMMACE: Evaluation of methods and management of acute coronary events; SYNTAX: Synergy between pci with taxus and cardiac surgery trial; CSA: Chronic stable angina; ACEF: Age, creatinine, ejection fraction score; LEADERS: Limus eluted from a durable versus erodable stent coating trial.

natory. The SRI, GUSTO and CADILLAC scores are seldom used in clinical practice and external validation is limited. Perhaps the major limitation of all these scores is that myocardial infarction is not always sub-divided into NSTEMI or STEMI. Finally some of the scores (including the TIMI risk score) are based on data derived from a pre-PPCI era or are based on angiographic findings that can not be known at the time of patient presentation.

However, the single dominant reason risk scores are rarely used for STEMI patients is the assumption that all patients presenting with STEMI are at high risk. Furthermore current evidence and international guidelines encourage the rapid diagnosis and treatment with no requirement for risk stratification. The fallibility of risk scores for STEMI is compounded by the issue of timing of data availability for data for a risk score calculation the emergency management of STEMI should not be delayed for the purpose of completing a range of risk parameters which may not be immediately available. For example some scores use parameters such as blood pressure measured on admission and troponin (GRACE) whilst others do not specify.

There are several other risk models which have been developed with varying degrees of validation across a variety of patient cohorts, *e.g.*, All ACS or all PCI. Others have been developed in an era which do not reflect contemporary practice, *e.g.*, The Primary Angioplasty in Myocardial Infarction score (PAMI)[35]. These will not be reviewed in detail in this manuscript as they are of limited clinical applicability, and have often excluded the highest risk patients such as the National Cardiovascular Data Registry (NCDR) PCI risk score[36].

BLEEDING RISK SCORES

Bleeding is an important outcome of ACS. The majority of patients with ACS will receive anti-coagulants and dual anti-platelet therapy and some patients will receive fibrinolysis or PCI that increase bleeding risk. There are limited data on bleeding risk scores in the setting of PPCI. The CRUSADE bleeding risk score (CBRS, Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA Guidelines) has been utilised and validated in a NSTEMI population but not in the STEMI cohort[37]. A prospective study from Spain has suggested that the bleeding risk in patients with PPCI in their cohort was less than that of the NSTEMI group. The lower rate of bleeding observed in this group may be due to the cohort having a lower baseline risk (younger, predominantly male) there was also a lower incidence of cardiovascular disease. A radial approach for PCI was associated with a decreased risk of major bleeding although the exact cause for this is unclear. This study lacked data on contemporary practice as patients on newer antiplatelet agents such

as Ticagrelor were excluded[38].

PPCI outcome-survival

In contemporary practice, survival rates following PPCI are high and approach 95% to 97% at 3 years[13,19,40]. However, within this group there is a wide range of individuals with varying levels of underlying risk. The elderly have worse absolute outcomes compared to their younger counterparts. In the APEX-AMI study the 90-d mortality was 13.1% in the elderly (> 75 years) and 2.3% in the < 65 years cohort. In this study age was the strongest predictor of mortality (hazard ratio 2.07 per 10 year increase (95%CI: 1.84-2.33)[41,42].

Mitigating against this absolute higher mortality is the fact that the elderly have a higher baseline risk and their relative risk is reduced by PPCI more effectively than by fibrinolysis. In the elderly STEMI population this has been demonstrated in the TACTICS-TIMI 18 trial in which there was a greater absolute risk benefit in favour of revascularisation[43]. Registry data support this finding, in the Australian ACACIA registry decreased referral rate and rate of revascularisation was noted in the elderly population. The exact reason for this is not clear; however it may be due to a perceived increase in risk by referring physicians or judgements based on frailty. In the same registry there was increased absolute benefit to early revascularisation in the elderly compared to the young following adjustment for baseline risk[44].

A final limitation of studies that report all-cause mortality is a failure to consider that longer-term survival may be affected by non-cardiac pathology. These factors may influence outcome beyond the index STEMI event. The elderly population are exposed to increased mortality attributable to non-cardiovascular factors than compared with their younger counterparts whether they have recovered from STEMI or not[45].

PPCI outcome-absolute risk reduction

The impact of any treatment is dependent on the baseline risk. The relative risk reduction of treatment in a low risk group is small and the number needed to treat (NNT) is high, this was illustrated in the In the PCAT-2 collaboration (Primary Coronary Angioplasty Trialist versus Thrombolysis) where the NNT with PPCI for a lowest quartile was 516 compared with 17 in the highest risk quartile. A patient with a risk score of 5 would decrease their absolute risk by 10% whereas the patient with a risk score of 1 would decrease their absolute risk by less than 1%[46]. Yet the potential benefit of PPCI must also be considered in context of the risk of harm. In a young age group the risk of bleeding from fibrinolysis is low whereas the elderly have a higher incidence of intracranial bleeding[47].

The challenge of optimally treating high-risk patients is exacerbated by the increased prevalence of an atypical presentation. A failure or delay to make a diagnosis prevents risk evaluation and reduces the benefit of treatment, up to 90% of patients under the age of 65 present with chest pain *vs* 57% over 85 years[40]. Elderly patients are more likely to present with atypical features such as left bundle branch block (34%), acute heart failure without significant chest pain (45%) all of which may delay diagnosis. In the real world delays in diagnosis and access to treatment are common and contribute to harm. Some authors advocate tailoring trials and treatment specifically to include the elderly high risk cases[45,47,48].

PPCI-important secondary outcomes

"Other than mortality are important factors in determining overall efficacy". Gharacholou *et al*[27] showed that compared to their younger counterparts the elderly have a higher baseline risk and a higher rate of post infarct/PPCI complications, in particular stroke (1.5% *vs* 0.4%), CCF (11.5% *vs* 2.7%) and shock (6.9% *vs* 2.1%). After correction for baseline characteristics age was a predictor of death (HR = 2.07; 95%CI: 1.84-2.33, $P < 0.001$)[41]. For high risk elderly patients there are no randomised trials to guide optimal management. Inferences about management have been drawn from analysis of sub-groups from PPCI trials[51].

Hospital length of stay is less following PPCI than with fibrinolysis (3 d *vs* 5 d)[50]. But there is relatively little data on quality of life in STEMI patients beyond 1 year and no data on the relative quality of life between high risk patients (often the elderly) and lower risk patients. Recent data from the GRACE registry suggests favourable 5 year survival but there are no long term data for quality of life following PPCI in either the younger or elderly group[49].

Recently the United Kingdom National Health Servicehas begun to focus attention on this by introducing measures of patient report experiences and outcomes. There is some evidence (outwith PPCI) that while it may provide more information it does not necessarily alter clinicians management strategies[52]. Data from the FREEDOM study (Future Revascularization Evaluation in Patients With Diabetes Mellitus: Optimal Management of Multivessel Disease) has suggested quality of life benefits for PCI at 2 years however these were in chronic stable angina patients[53].

THE IDEAL PPCI RISK SCORE

A discriminatory risk score is required when the effectiveness of treatment depends on baseline risk. An optimal risk score for PPCI would predict which patient would benefit maximally from an intervention and predict who would come to harm and what weighting should be ascribed to that. The risk of death and morbidity in the context of an anterior STEMI is high and reperfusion treatment with thrombolysis or PPCI outweighs the risk of bleeding in most patients. Conversely the risk of harm in a late presenting or limited inferior STEMI may outweigh the perceived benefits of reperfusion treatment and conservative treatment could be advocated.

Currently there is no risk scoring system within the context of STEMI and physicians are encouraged to rapidly activate a treatment pathway with little or no as-

sessment of perceived risks and benefits. The reasons for this practice have been discussed and are summarised by a lack of guideline recommendation, impractical or non-discriminatory scoring systems and a perception that all STEMI patients are high risk. A further limitation is that the clinical trials on which evidence is based are highly selective samples. Typically these trials recruit less than 10% of patients screened and often the very highest risk patients are excluded. This has the effect of excluding 'real world' patients from evaluation of interventions. Any scoring system derived from a clinical trial by default is not applicable to a real world population. A lack of applicability of trial data to the real world is often cited as a reason to not offer therapies. Trials performed in highly selected patients that show efficacy of treatment may drive the widespread delivery of this treatment to an "all-comers" population. This may be effective but may not be cost effective. The same treatment (PPCI) may be offered for example, to a 40-year-old male presenting within 60 min of onset of STEMI. Currently PPCI would be offered, with an number needed to treat of > 500 to save one life. Thrombolysis delivered immediately may be as effective with little chance of harm. Conversely a late presenting elderly female who has a much higher risk of death, lower likelihood of reperfusion with fibrinolysis, higher rate of significant bleeding and therefore is much more likely to benefit from mechanical reperfusion, number needed to treat = 17[46].

Opportunities and missed opportunities of care

Is the current philosophy of STEMI treatment correct? PPCI has been calculated to cost the NHS in England £5176 per patient during office hours versus fibrinolysis at £3509[54,55]. This represents a significant burden of healthcare resource devoted to a treatment that in some patients is probably life saving in many others not. There is little licence or encouragement for physicians to discriminate between these very different patient groups and the mandate is to treat rapidly. However, there is no doubt that this approach has been effective and real world survival following STEMI treated by PPCI is remarkably high.

Can refinement with risk adjustment improve the pathways further? Clearly the determination of absolute risk and absolute benefit in high-risk populations is difficult, as is proving that the elderly benefit in the long term from intervention and aggressive secondary prevention. One of the challenges confronting front line clinicians is lack of clear prognostic data that takes into account the patient as a whole and not simply their acute STEMI presentation. The idea of assessing potential harm as well as possible/likely benefit has recently been given increased attention.

In the United States the wide disparity of care has in recent years been highlighted. There is considerable variation in practice both in geographical terms and in differing financial arrangements. Since the introduction of The Affordable Care Act (ACA, Obamacare) a substantial amount (United States $1.1 billion) of the United States health budget has been appropriated to funding towards Comparative Effectiveness Research. The intention being to improve quality, streamline care and demonstrate not only medical efficacy but medical effectiveness. This alteration in the funding landscape has profound implications for physician choice and may influence clinical decision making. Some authors have suggested that it may lead to creeping government control of medical practice by influencing reimbursement[56]. This is analogous to the system in the United Kingdom where NICE delivers guidelines based both on treatment efficacy and overall clinical effectiveness. While this system has its merits in trying to alleviate some of the problems associated with the so called "postcode lottery" NICE is not empowered to make funding allocations although patients have a right to NHS approved treatments NICE recognises that further research is recommended into optimal reperfusion strategies for those presenting early.

In contrast to the front loading of healthcare provision at the time of presentation with STEMI there remains a significant failure in prescribing simple evidence based treatments following the initial treatment. Provision of secondary prevention pharmacotherapy has been described using a missed opportunities for care model. A study using a large United Kingdom national database (MINAP) which demonstrated that outcome (death) was related to not prescribing clinically indicated and evidence based treatments, *e.g.*, statins[57]. In another study of elderly patients the authors found that following PCI healthcare inequalities expressed as missed opportunities for care in the short term (30 d) correlated with mortality[58].

Efficacy of treatment

The MINAP based study result above illustrates the importance of proof of benefit and not simply reduction of risk[57,58]. The efficacy of secondary preventative medication in a population has been established. What is less clear is the prognostic benefit in high risk individuals. We have already seen above that missed opportunities equate to outcome.

As pressure on healthcare budgets have come under increased scrutiny, research methodology, *e.g.*, high cost of Randomised Control Trials (RCTs) have come under review. This has reinvigorated interest in research methods that provide prognostic information. Comparative effectiveness research has been suggested as a possible route towards improving outcomes and reducing costs whilst providing policy makers and clinicians with clinically useful and evidence based tools to achieve optimal care. An example of this would be the use of electronic medical records to generate evidence from different areas and compare outcomes based on geographical locations[59]. Altesrnatively a design similar to the recent STREAM study when ethical approval was granted for a centre to conduct a trial of early fibrinolysis *vs* PPCI for early presenters[18].

CONCLUSION

Clinicians are generally poor at judging risk and predicting the absolute benefit and harm of their interventions.

The evidence in NSTEMI care has clearly shown the importance of calculating these metrics. This has led to a plethora of risk scores and recommendation to use these in international guidelines.

Provision of STEMI care in the United Kingdom is currently algorithmic and not risk adjusted yet we have seen that the same treatment pathway (PPCI) may deliver treatment that is very beneficial in some but not in others. One reason to risk stratify is to target healthcare resource; many patients should continue to be treated by emergency PCI, others may be treated with immediate fibrinolysis and others without reperfusion treatment at all.

The STREAM and GRACIA-2 data have suggested that some patients can be treated as effectively and certainly more cost effectively with rapid thrombolysis avoiding emergency angiography[14,17]. These data come from trials and consequently have all the limitations of selection and applicability but have generated an important hypothesis. If discriminatory STEMI risk scores were available, applicable to real world patients and widely used could the current algorithm of emergency angiography be adapted to include fibrinolysis? If this change were incorporated would the outcomes be non-inferior or the cost benefit calculation superior. There are huge challenges to proving this hypothesis. Some clinicians will feel that such a change is retrograde step and there is a risk of generating a complicated pathway that may harm the very patients it is intending to improve outcomes for. The trials involved to mark such a paradigm shift in the current guidelines may be costly, difficult to recruit to and may not provide a definitive answer. Thus the question "Would this change be non-inferior to PPCI overall and would it be cost beneficial" may be difficult to answer. The first step is to generate a practical discriminatory risk score that is based on real world data in a STEMI population. Ideally the score should account for potential harm associated with PCI or thrombolysis, should generate baseline risk and calculate treatment effects. Such a risk score does not yet exist although registry data are available on which these could be derived. A validated score has ability to predict the impact of healthcare on treatment and evaluate cost-benefit.

If substantial health care resource is being driven towards treatments that are only minimally effective in some patients then refinement of the STEMI pathway by risk adjustment should be formally evaluated. There are merits to keeping treatment pathways simple and providing algorithmic care if this is globally effective. However stratifying patients by risk and calculating treatment effects with thrombolysis or PCI may be as effective. Such a pathway could be delivered with reduced overall cost and no less efficacy[60].

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CASE REPORT

Late intervention in an asymptomatic pediatric patient with anomalous left coronary artery

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Abstract

Anomalous left coronary artery from the pulmonary artery (ALCAPA) is most commonly diagnosed within the first year of life with congestive heart failure symptomatology reflecting left ventricle (LV) dysfunction. The late diagnosis of ALCAPA is presented in a 5-year-old without significant LV dysfunction, mild LV dilatation and only mild mitral regurgitation that did not change significantly after surgery. The timing of surgical intervention in the late diagnosis of ALCAPA remains unclear despite risks of significant ongoing myocardial injury secondary to coronary artery hypoperfusion and progressive mitral valve dysfunction. Intervention in this case allows for revascularization which may reverse ventricular and valvular dysfunction.

Key words: Congenital heart disease; Congenital heart surgery; Coronary artery imaging; Coronary artery surgery; Reperfusion

Core tip: This case report presents the rare case of a late diagnosis of anomalous left coronary artery from the pulmonary artery and the associated challenge with determining the need for surgical revascularization in the absence of symptomatology and definitive literature. Late surgical intervention undertaken in this patient may reverse ongoing myocardial dysfunction and prevent permanent left ventricular damage.

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INTRODUCTION

This case report describes a late diagnosis of anomalous left coronary artery from the pulmonary artery (ALCAPA) in an asymptomatic patient with only mild left ventricular dilatation. Because of a lack of literature to guide management in our patient, the benefits of surgical revascularization were weighed against risks of surgery on a hemodynamically stable patient with relatively preserved ventricular function. Ultimately, the patient underwent surgery in hopes of preventing further suboptimal coronary perfusion.

CASE REPORT

The patient was initially seen with an asymptomatic 2/6 apical systolic murmur at age two. The electrocardiogram was normal and the initial transthoracic echocardiogram demonstrated good ventricular function, no atrial or ventricular septal defect, and mild mitral regurgitation (MR). There was abnormal flow in the region of the left coronary artery (LCA) suspicious for a LCA fistula.

To further visualize the LCA, a transesophageal echocardiogram performed at age two under general anesthetic confirmed normal left and right ventricular function, a

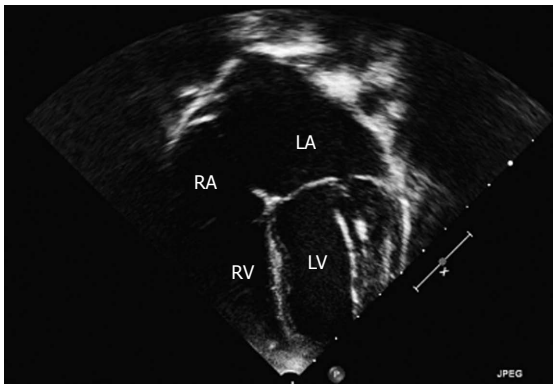

Figure 1 Four-chamber echocardiographic images illustrating echogenic left papillary muscle and chordae apparatus. LV: Left ventricle; LA: Left atrium; RA: Right atrium; RV: Right ventricle.

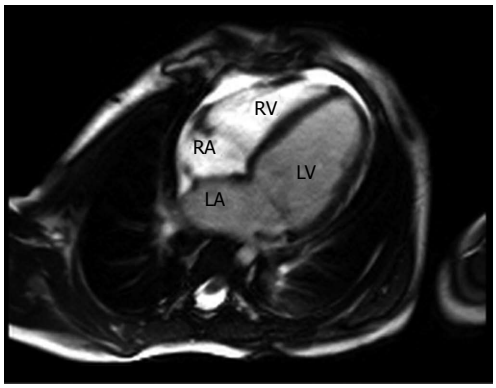

Figure 2 Four-chamber cardiac magnetic resonance cine imaging demonstrating a dilated left atrium and left ventricle. LV: Left ventricle; LA: Left atrium; RA: Right atrium; RV: Right ventricle.

small LCA fistula and mild MR with normal ventricular chamber dimensions. Repeated echocardiograms showed evidence of mild dilatation of the left atrium (LA) and left ventricle (LV) with mild MR. Increased echogenicity of the LV myocardium was noted but there was no evidence of endocardial fibroelastosis on these pre-operative echocardiograms (Figure 1). At age 5 further investigation with cardiac magnetic resonance imaging (MRI) saw findings consistent with the previously noted dilated LA and LV, accompanied by normal LV systolic function and mild MR (Figure 2). The LCA system was suboptimally visualized by cardiac MRI but raised the question of an anomalous LCA. Cardiac computed tomography was then performed with angiography demonstrating ALCAPA. Numerous collateral vessels from the right coronary artery to the left coronary system, along with a dilated LA and LV were visualized (Figure 3).

Surgical repair was undertaken with a direct aortic reimplantation performed under cardiopulmonary bypass after arresting the heart with cold blood cardioplegia. The left coronary button was excised and anastomosed with a flap of ascending aorta. The pulmonary root was reconstructed with bovine pericardium before anastomosis to the main pulmonary artery (PA). The patient was weaned from cardiopulmonary bypass uneventfully after a total bypass time of 78 min.

Post-operatively, the patient was hemodynamically stable spending one night in the pediatric intensive care unit. Brief episodes of hypotension were managed routinely with inotropes and intravenous fluid. She was discharged ten days after surgery, weaned off oxygen and prescribed enalapril and aldactazide. Her post-operative course was complicated by mild postpericardiotomy syndrome that responded to acetylsalicylic acid therapy.

The immediate post-operative echocardiograms demonstrated mild MR along with mild dilatation of the LA and LV, and a small pericardial effusion. The patient's LV function remained preserved with the shortening fraction improved from 35% to 40%. At her two-month post-operative assessment, she was asymptomatic, remaining on low dose enalapril. Her echocardiogram showed mild LA dilatation, mild LV dilatation, and two separate jets of mild MR. Ventricular function was relatively unchanged with a shortening fraction of 38% and ejection fraction of 69%.

Structural assessment of the LV demonstrated a quantitative reduction in LV dilatation following the procedure with the left ventricle internal diameter-diastole reduced from 4.4 cm pre-operatively to 3.9 cm one month after surgery. The degree of MR pre and post-operatively had not changed, with repeat assessment scheduled at six months post-operatively.

DISCUSSION

ALCAPA is rare congenital anomaly in which the LCA receives deoxygenated blood via the PA. It does not present as a hemodynamic problem in utero as high pulmonary vascular resistance allows for antegrade blood flow from the PA into the LCA. As the pulmonary vascular resistance falls, retrograde flow into the PA develops producing coronary steal or hypoperfusion.

ALCAPA has an incidence of 1/300000 live births[1] and its symptoms of failure to thrive, dyspnea, diaphoresis and findings of left sided heart failure usually present within the first two months of life[2]. Such early diagnoses of ALCAPA usually require prompt surgical revascularization. Without surgery, 90% of infants with ALCAPA will often die in the first year of life due to myocardial ischemia secondary to coronary vessel hypoxia[3].

In order to survive, collateral vessels are necessary to supply the LV myocardium. However, the ALCAPA continues to place stress on the LV, resulting in steadily deteriorating systolic function and LV scarring. Our patient's MR secondary to subclinical decline of her LV function indicated that the structure of her LV was indeed a concern despite her collateral vasculature. The mildly increased echogenicity of the LV endocardium raised the concern of endocardial fibroelastosis eventually developing with resultant myocardial scarring. This led to the decision for surgery rather than careful cardiac follow-up.

Surgical revascularization is the treatment of choice for ALCAPA. The surgery is most often scheduled immediately after diagnosis of ALCAPA in order to prevent

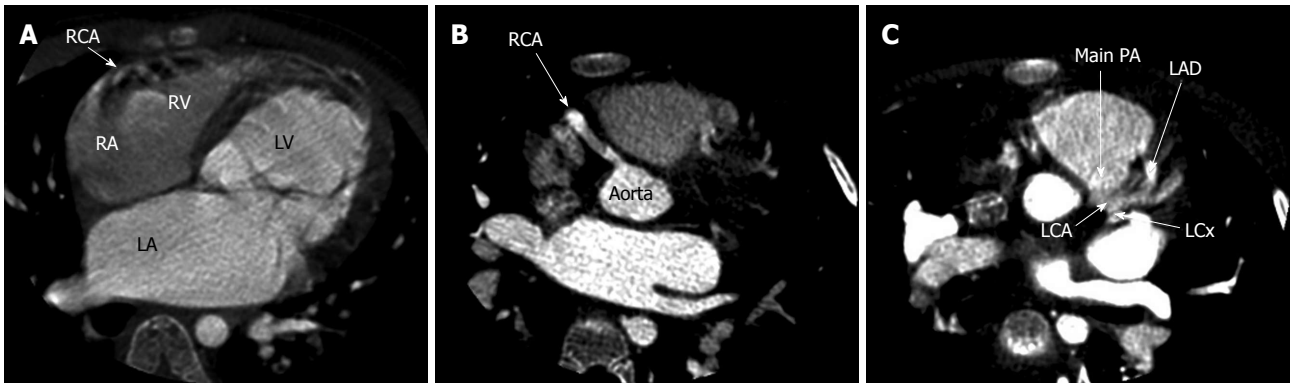

Figure 3 Computed tomographic angiography. A: Computed tomographic angiography demonstrating extensive collateral vessels from the right coronary artery as well as a dilated left ventricle; B: Computed tomographic angiography showing dilated right coronary artery; C: Computed tomographic angiography illustrating the left anterior descending artery communicating with the main pulmonary artery. LV: Left ventricle; LA: Left atrium; RA: Right atrium; RV: Right ventricle; RCA: Right coronary artery; LAD: Left anterior descending artery; LCA: Left coronary artery; LCx: Left circumflex artery; PA: Pulmonary artery.

further ischemia and necrosis of cardiac muscle. Most surgeries involve either a Takeuchi operation or direct reimplantation of the LCA[3].

The role of revascularization in an asymptomatic patient with functional collateral coronary vessels and years of subclinical myocardial ischemia remains somewhat contentious. Literature in the adult population suggests that myocardium supplied by longstanding ischemic vasculature may be permanently scarred and unable to regain function despite intervention[4]. This did not appear to be the case with our 5-year-old patient. Surgical intervention places patients at risk of arrhythmias, acute cardiac failure and long-term complications such as intrapulmonary tunnel baffle leaks and suprapulmonary stenosis[3]; however, these did not occur in the case presented, but may develop over more time.

Recovery of LV function is well reported in pediatric patients following revascularization. However, our patient maintained normal ventricular function. Early diagnosis and intervention is correlated with a decreased need of mitral valve repair or replacement in the future[5]. Asymptomatic patients with unrepaired ALCAPA have chronic ischemia despite the production of collateral coronary vessels. The LV continues to undergo adverse remodeling with deterioration in systolic and diastolic function over time. Several studies have demonstrated successful recovery of LV function in patients following surgical intervention for ALCAPA[6,7]. However, patients with poor LV function pre-operatively have higher mortality rates and increased need for mitral valve intervention after ALCAPA surgery.

In the setting of decreased LV function, there is hypothesis regarding the possible existence of hibernating myocardium in ALCAPA. The hypothesis suggests myocytes supplied by the ALCAPA undergo an adaptive change and become hypokinetic rather than necrotic despite being hypoperfused[8]. The function and viability of the cardiomyocytes is preserved because of the successful formation and utilization of a compensatory but inadequate collateral supply. Shivalkar *et al*[2] demonstrated that although chronically hypoperfused, histology of myocardium from some patients with ALPACA is comprised of viable myocytes with minor morphological changes likely from cellular adaptation to longstanding ischemia.

This case illustrates the considerations in revascularizing an asymptomatic ALCAPA patient with longstanding hypoperfused myocardium but preserved ventricular function in the absence of definitive literature. Surgical intervention was recommended to prevent the potential of further LV dysfunction and mitral valve dysfunction manifesting from further suboptimal myocardial perfusion. The late diagnosis of ALCAPA in an asymptomatic 5-year-old with preserved LV function, mild LV dilatation and mild MR that did not change after surgery further illustrates the clinical dilemma of the timing of surgical intervention.

In conclusion, this case describes the late presentation of a child with subclinical myocardial ischemia secondary to ALCAPA. The patient compensated well hemodynamically for five years with only mild LV dilatation and mild MR. The need for surgical intervention considered the risks of cardiopulmonary bypass on scarred LV myocardium versus the benefits of repair and potential reversal of ongoing myocardial dysfunction and LV myocardial damage, but the timing of such surgery remains somewhat controversial.

COMMENTS

Case characteristics

A late diagnosis and operative revascularization procedure was performed in a 5-year-old female.

Clinical diagnosis

The patient presented with an asymptomatic apical systolic murmur.

Differential diagnosis

Left coronary artery fistula, anomalous left coronary artery arising from the pulmonary artery

Imaging diagnosis

Cardiac computed tomography with angiography demonstrated an anomalous left coronary artery from the pulmonary artery.

Treatment

Surgical revascularization with a direct aortic reimplantation was performed under cardiopulmonary bypass.

Related reports

The role of revascularization in an older asymptomatic patient with anomalous left coronary artery from the pulmonary artery (ALCAPA) is unclear as there lacks strong clinical literature to guide timing of treatment.

Experiences and lessons

Late surgical intervention ALCAPA may reverse ongoing myocardial dysfunction and prevent permanent left ventricular damage, even in hemodynamically stable patients.

Peer review

The authors present a rare case report of late diagnosed ALCAPA. Surgical intervention was undertaken and left ventricular function was relatively preserved. The authors emphasized the importance of the timing of surgical intervention from the viewpoint of risks and benefits. The manuscript is clearly written and well organized.

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee.

Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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