

Review Article

Pathogenesis of aortic stenosis: not just a matter of wear and tear

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Abstract: Aortic valve stenosis (AS) is the commonest form of valvular heart disease in the Western world. Its prevalence increases exponentially with age and it is present in 2-7% of all patients over 65 years of age. In view of the considerable cardiovascular morbidity and mortality associated not only with AS, but even its earlier stage, aortic sclerosis, many investigations have been directed towards better understanding of its pathogenesis, with the ultimate objective of developing strategies to retard its progression. Although risk factors and downstream mediators appear similar for AS and atherosclerosis (older age, male sex, hypertension, smoking, hypercholesterolemia, and diabetes, as many as 50% of patients with AS do not have clinically significant atherosclerosis. On the basis both of recent experimental evidence and clinical trials, it appears that atherogenesis is not pivotal to the pathogenesis of AS. On the other hand, there is increasing evidence of active involvement of aortic valve fibroblasts with resultant increased production of reactive oxygen species, active pro-inflammatory and pro-fibrotic processes culminating in calcification. We also discuss the evidence of involvement of the nitric oxide system in the pathogenesis of AS. The renin-angiotensin system has also emerged as a major player in the pathogenesis of AS. Histologically, there is increased ACE expression and elevated angiotensin II levels in stenotic valves, while we have just demonstrated amelioration of AS with the use of ACE inhibitors in an animal model. We further discuss intervention studies aimed at retarding AS progression, including recent failures of statins to retard progression of AS in large randomized clinical studies. Finally, we discuss the special case of bicuspid aortic valve, including its genetics and unique associated features.

Keywords: Aortic valve stenosis, bicuspid aortic valve, nitric oxide, oxidative stress, inflammation, fibrosis, calcification, intervention studies

Introduction

Aortic stenosis (AS) may be defined as narrowing of the aortic valve, due primarily to a combination of progressive fibrosis and calcification of the matrix, with consequent increase in valve stiffness, progressive reductions in valve area and concomitant increases in left ventricular afterload and work. The earliest stages of AS have been designated aortic valve sclerosis (ASc), implying disordered valve morphology (including potential calcification as well as fibrosis) in the absence of marked obstruction to left ventricular outflow.

AS is currently the most common form of valvular heart disease in the Western world [1], in large part because the most frequently occurring form of AS develops predominantly in indi-

viduals of advancing age. For example, in the Helsinki Ageing Study [2], the proportion of individuals with detectable valve calcification increased from approximately 40% to 75% between ages of 65 and 85 years, while approximately 3% of subjects over 75 years of age had severe AS.

Given that the only proven therapy for severe AS is aortic valve replacement, and that there is currently no definitive evidence that any treatment can retard progression of the disease, the development of severe symptomatic AS in elderly individuals presents an increasing medical and health economic dilemma. On the one hand, severe AS rarely remains asymptomatic for any great length of time: patients classically develop variable components of exertional dyspnoea, angina (irrespective of the presence or

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Table 1. Causes of AS. Wide frequency range generally reflects the age group(s) assessed by individual studies as well as population subgroups studied [79, 115-117].

Etiology	Approximate frequency (%)	Associated features
Aging/calific	50 - 70	Increased risk of coronary events.
Bicuspid aortic valve	6 - 40	Dilatation or dissection of the aorta, involving the aortic root, ascending aorta, or aortic arch.
Rheumatic	2 - 11	Mitral valve almost always affected as well
Unicuspid aortic valve	<1	Dilatation or dissection of the aorta, involving the aortic root, ascending aorta, or aortic arch.
Post-endocarditis	<1	Extra-cardiac embolic phenomena.

absence of epicardial coronary artery disease) and atrial or ventricular arrhythmias, resulting in poor quality of life, increased rates of hospitalization and increased mortality rates [3]. Although the precise natural history of severe AS in the elderly is uncertain, a number of studies suggested that mortality is high in the somewhat selected subgroup of individuals who do not undergo aortic valve replacement [4-7]. Additionally, both ASc and AS may be associated with cardiovascular problems which are apparently remote from the valve itself. For example, there is considerable evidence that ASc is an independent marker of increased risk of cardiovascular events [8, 9], while severe AS is associated with increased risk of haemorrhage, especially into the gastrointestinal tract, primarily because of the development of acquired von Willebrand's factor deficiency [10, 11].

It should also be stated at the outset that AS is a substantially heterogeneous disease, but with only two "common causes". These are AS developing in a previously normal trileaflet valve, and AS associated with congenitally bicuspid aortic valves (BAV). These and other, rarer, causes of AS are summarized in **Table 1**.

Given that AS frequently develops in otherwise normal valves in aged individuals, this has been regarded as degenerative AS, implying a relationship to the normal process of "wear and tear" within the valve [12]. The purpose of this review is to demonstrate that AS is not primarily a "degenerative" process, but rather the result of a progressive inflammatory process within the valve matrix. Furthermore, we will present evidence that the development and progression of AS, rather than occurring with the implied inevitability of a purely degenerative disease, should theoretically be amenable to pharmacotherapy.

Aortic valve anatomy and physiology: what are the homeostatic determinants?

The normal aortic valve consists of several layers of fibroblast-rich tissue, containing both collagen and elastin fibres, covered by a monolayer of endothelial cells [13]. There are also minute intravalvular blood vessels [14], consistent with a substantial oxygen demand by the other matrix cells.

The normal aortic valve interstitial cells are probably mainly fibroblasts, but some smooth muscle cells have been identified [15], raising the issue of potential variability in tone of the valve, an area which has been pursued in a number of experimental studies. For example, Pompilio et al [16] showed that intact porcine aortic valve contracted in response to phenylephrine, and also exhibited (endothelium-dependent) relaxation with acetylcholine. Furthermore, normal aortic valve interstitial cells (presumably including smooth muscle cells) have been shown to contract in response to 5-hydroxytryptamine, endothelin-1 and norepinephrine [17]. An important, but incompletely resolved issue relates to the prevalence and function of myofibroblasts, which exhibit some contractile properties, within normal aortic valves. The role of the myofibroblasts will be discussed more extensively in the context of valve pathology.

The physiological role of the aortic valve endothelium has attracted considerable attention over the past 15 years, although its role is still incompletely understood. A number of studies suggest that valve endothelium behaves in a qualitatively similar manner to vascular endothelium, for example releasing nitric oxide (NO) in response to stimuli such as acetylcholine [16], or 5-hydroxytryptamine [18].

A critical but unresolved issue is what is the physiological response of the valve endothelium to shear stress, a normal stimulus for NO release, but also potentially for activation of endothelial NAD(P)H oxidase [19, 20], which in turn would lead to release of superoxide anion (O₂⁻) and "scavenging" of NO. Despite ongoing uncertainties, the differences between normal aortic valve and vascular endothelial cells regarding responses to shear stress were reviewed by Butcher et al [21], who demonstrated that the changes in gene expression in response to shear stress varied substantially.

Finally, little work has been done regarding other endothelial autocooids within the valve. Notably, little is known about the physiology of prostacyclin release from the valve, although it has been detected [22]. No studies have properly addressed the issue of tissue penetration of autocooids released from valvular endothelium, other than the obvious implications of studies measuring valve contraction. It is therefore uncertain to what extent endothelial NO and prostacyclin might modulate the function of valve interstitial cells, a physiologically important issue given evidence that in AS valve endothelium is dysfunctional or absent, as discussed below.

Cellular histopathology of AS

Features of stenotic aortic valve lesions

Histopathologic studies have demonstrated that development and progression of calcific AS are based on an active process that shares some similarities with atherosclerosis. It has been suggested that aortic valve lesions begin with disruption of valve endothelium predominantly on the aortic side due to high shear stress [23-25].

Inflammation and lipid deposition

Aortic valve lesions typically present with areas of subendothelial thickening, which represents the early stage of aortic stenosis. Increased thickening of aortic valve leaflets is characterized by accumulation of inflammatory infiltrates of predominantly macrophages and T-lymphocytes, lipids, oxidized lipids, (summarized in **Figure 1**) [25-28] all of which potentially activate a host of pro-fibrotic and pro-inflammatory markers. Macrophages and T-lymphocytes have been detected and tend to be located near the

surface of the lesion [25-28]. Immunohistochemical studies have found co-localization of apolipoproteins (apo) B, apo (a), apoE with lipid laden foam cells and macrophages [29] as well as oxidative modification of residential low density lipoproteins (LDLs) in early stenotic aortic valve lesions [30].

Valvular matrix remodelling and fibrosis

The presence of macrophages and T-lymphocytes, along with oxidized LDL and apolipoprotein accumulation activate several pro-fibrotic and pro-inflammatory cytokines which may modulate aortic valve remodelling and subsequent calcification. Transforming growth factor β 1 (TGF- β 1) [31] and interleukin-1 β [32] have been found in valve matrix and are associated with increased local production of matrix metalloproteinases I and II (MMP-1 and MMP-2). All of these contribute to cell apoptosis, extracellular matrix formation, remodelling and consequently predispose to calcification. In addition to TGF- β 1 and interleukin-1 β , tumour necrosis factor- α (TNF α), another pro-inflammatory cytokine commonly responsible for immune regulation, inflammation and tissue remodelling, is co-localized with MMP-1 [33]. Furthermore, tenascin C, an extracellular matrix glycoprotein implicated in cell proliferation, migration, differentiation and apoptosis, which is involved in stimulation of bone formation and mineralization, is co-localized with MMP-2 in calcified aortic valve leaflets [34], and is associated with progression of AS [35].

Angiotensin II (Ang II), an important mediator of inflammation and fibrosis, could be formed by angiotensin converting enzyme (ACE) as well as the mast cell (MC)-derived neutral protease, chymase [36]. ACE has been identified in stenotic but not in normal aortic valves [37]. It has been shown that MC-derived chymase is also upregulated in stenotic valves, providing further evidence for local production of Ang II [38]. In addition, cathepsin G, another neutral protease also capable of generating Ang II, is present in increased concentrations throughout human stenotic aortic valves compared to normal valves [39]. These findings provide a potential basis for a role of angiotensin II in aortic valve remodelling along with other pro-fibrotic and pro-inflammatory mechanisms.

As summarized in **Figure 1**, current concepts of the pathogenesis of AS centre histologically on

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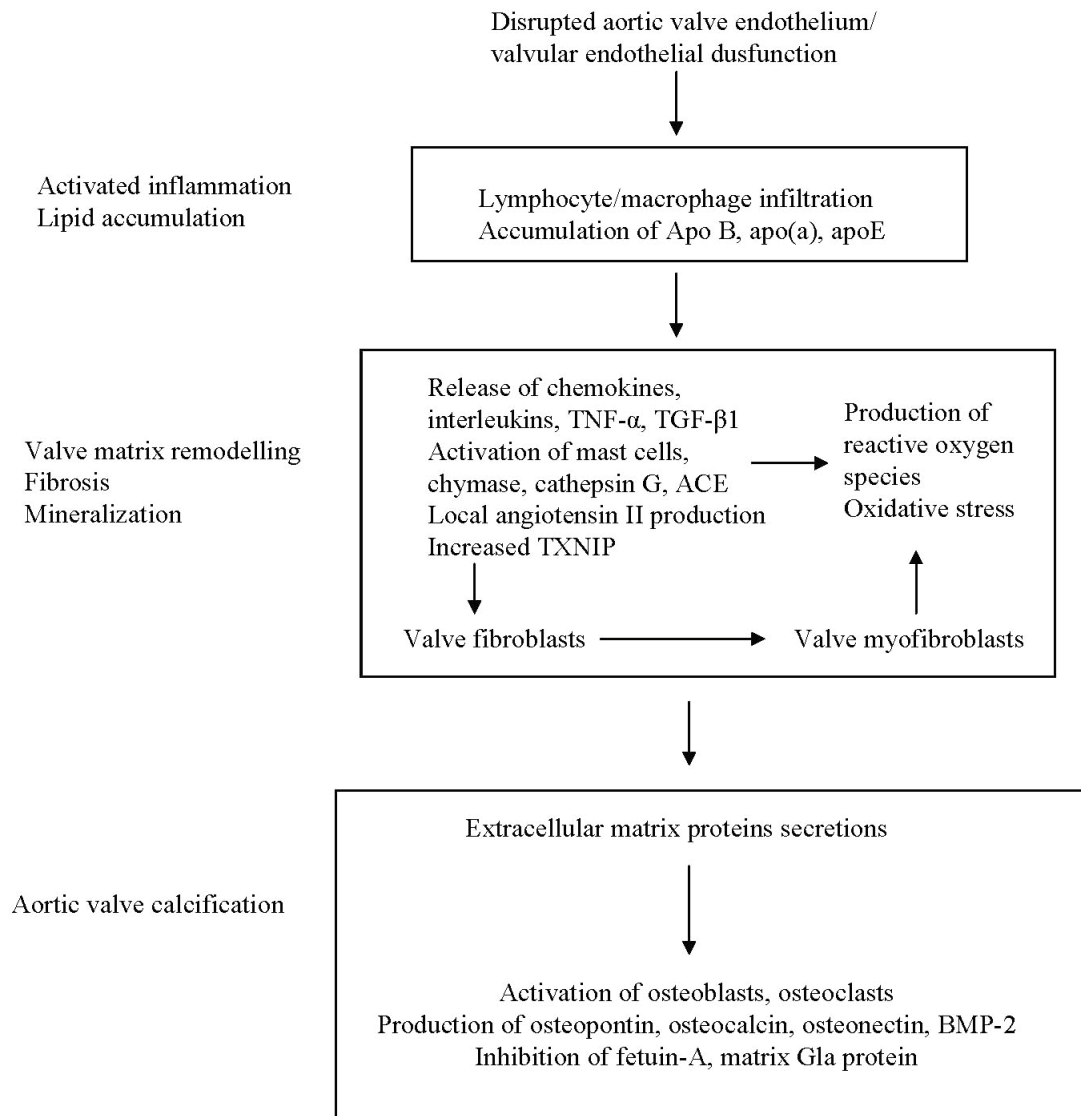


Figure 1. Schema of postulated mechanisms underlying aortic valve lesion formation. Inflammatory infiltrations of T-lymphocytes and macrophages, along with lipid accumulation, is primarily responsible for early thickening of aortic valves. Interactions between chemical stimuli and disruption of valvular homeostasis: pro- and anti-fibrotic mechanisms. Later stages of aortic stenosis: - cytokine release and angiotensin II promote extracellular matrix proteins secretion at early stages of mineralization which in turns begin the processes of bone formation. This process occurs largely at the end stage of aortic stenosis where aortic valves mobility is significantly reduced due to a build up of bone-like calcific nodules.

inflammation and lipid deposition, and biochemically on activation of cytokines and matrix metalloproteinases, together with generation of angiotensin II. These processes are postulated to induce injury of all valve components, leading to fibrosis and calcification.

Calcification

Calcification of aortic valve leaflets tends to

occur more predominantly in the later stages of AS, and is located deeper in the lesion [25]. Active calcification is a major factor in reducing valvular mobility in severe AS. Early lesions of aortic valves show fine stippled mineralization, progressing to active bone formation resulting in gross calcification at later stages of the disease (**Figure 1**).

The process of calcification (and sometimes

ossification) of aortic valve leaflets resembles that associated with atheroma formation. The presence of inflammation, fatty streak formation from lipid depositions, cytokine release, metalloproteinases, ACE, Ang II, all possibly contribute to the production of an extracellular matrix, and matrix vesicles that initiate mineralization.

Co-localization of macrophages and oxidized LDLs with osteopontin, a protein needed in bone formation, has been found and thought to be involved in extracellular matrix production in human stenotic aortic valves [40, 41]. Furthermore, Mohler et al [42] described an active process of calcification using immunohistochemistry in an extensive study of 347 human stenotic aortic valves. In addition to active osteoblasts and osteoclasts, this and other investigators [43] detected bone morphogenic proteins 2- and 4- (BMP-2, BMP-4). BMPs stimulate osteoblastic differentiation with subsequent calcification. In agreement with previous studies [25, 41], Mohler et al [42] also found that macrophages and lymphocytes accumulate in areas of calcification.

There is also evidence of angiogenesis, which is essential for longitudinal bone growth in stenotic valves. T-lymphocytes aggregates tend to co-localize with sites of neoangiogenesis within ossified valves [42]. This and the presence of heat shock protein 60 (*hsp60*) [44], commonly expressed by cells under stress conditions, together indicate a highly active, and chronic immunomediated process from stress to inflammation to calcification.

It has also been shown that the calcification process of aortic valves may also be regulated by receptor activator of nuclear factor κ B, its ligand (RANK, and RANKL), and the soluble receptor osteoprotegerin (OPG) [45]. The study detected increased concentrations of RANKL in calcified compared to control valves. Furthermore, there was a significant reduction of OPG positive cells in aortic stenotic valves compared to controls. It has been shown that in mice deficient for OPG, vascular calcification was associated with increased expression of RANKL [46]. Additionally, in Kaden et al [45] shown that long-term cell culture of stenotic aortic valves in the presence of RANKL induced significant increase in matrix calcium deposition and the formation of cell nodules compared to controls. Thus, the

RANKL/OPG pathway may also be involved in the calcific process of aortic valve stenosis.

A recent study [47] also revealed an association between presence of aortic stenosis and low serum levels of the anti-calcific protein fetuin-A. Furthermore, there was evidence that fetuin-A was deposited in calcific aortic valves. Nevertheless, the relative importance of these findings to the overall pathogenesis of aortic stenosis remains uncertain.

Matrix Gla-protein (MGP), another anti-calcific protein implicated in development of AS, is synthesised in different tissues and undergoes vitamin K dependent γ -carboxylation (reviewed by [48]). The γ -carboxylated form of MGP is involved in inhibition of tissue calcification possibly by preventing differentiation of vascular smooth muscle cells into chondrocyte-like cells or by blocking BMP function [48]. Patients with aortic valve calcification were found to have lower concentrations of uncarboxylated MGP compared with normal controls [49]; additionally both renal dysfunction and oral warfarin therapy were predictive of low MGP levels. Interestingly, another study found elevated levels of both uncarboxylated and carboxylated de-phosphorylated forms of MGP in patients with severe AS [50]. The exact biological significance of these findings is still unclear.

Oxidative stress

A number of studies have documented increased intravalvular content of a variety of prooxidants in models of AS and in clinical samples [23, 51-54]. Importantly, this evidence of increased oxidative stress was not specifically associated with extent of local atherogenesis in any study.

Thioredoxin, thioredoxin reductase, NADPH oxidase and thioredoxin binding protein (TXNIP) are components of a ubiquitous system, which regulates intracellular redox stress [55]. TXNIP in particular is a fundamental mediator of increased redox stress as it binds to, and inactivates thioredoxin [56]. Increased expression of TXNIP is associated with activation of apoptosis signalling pathways [57], and is suppressed by endothelial NO release [58]. We have recently demonstrated in a rabbit model of mild AS with histological features similar to that of human disease, increased intravalvular concentration

of TXNIP compared to control animals [53]. Furthermore, co-treatment with ramipril retarded the development of AS in this model, as measured by reduction both in transvalvular velocity and valve echogenicity on echocardiography [59]. This retardation further correlated with reduction in valvular calcium and macrophage infiltration and a reduction in TXNIP accumulation within the valve matrix. This finding further underscores the importance of ACE - Ang II system in pathogenesis of AS.

Evidence of the role of nitric oxide in the pathogenesis of aortic stenosis

AS, even in its early phases is associated with the pathogenesis of acute coronary syndromes (ACS) [8, 60, 61], which are paralleled by platelet hyper-aggregability [62]. Stenotic aortic valves constitute a pro-aggregatory milieu, potentially contributing to thromboembolism [24, 63]. It has been shown that patients with AS exhibit increased platelet reactivity [64], and thrombus formation has been documented on severely stenosed valves [65]. AS has recently been linked to the phenomenon of nitric oxide (NO) resistance, even at its earliest stages [66, 67].

NO is a physiological modulator of both vasomotor tone and platelet aggregation. These effects of NO are predominantly mediated by cyclic guanosine-3',5'-monophosphate (cGMP), via activation of soluble guanylate cyclase (sGC). However, in patients with ischemic heart disease, platelets and coronary/peripheral arteries respond poorly to the anti-aggregatory and vasodilator effects of NO donors (e.g. nitroglycerin) [68]. This "NO resistance" represents a multifaceted disorder at sites of abnormal NO-driven physiology, and as such may contribute to the increased risk of ischemic events. NO resistance results from a combination of "scavenging" of NO by superoxide radical (O_2^-) and of inactivation of sGC. The haem moiety of sGC is a "receptor" for NO and a mediator of NO-dependent activation. However, reactive oxygen species, and O_2 in particular, diminish sGC sensitivity to NO because of the oxidation of the enzyme-bound haem and its subsequent loss (haem-deficient sGC).

We have investigated [66] whether AS, either

with or without concomitant coronary artery disease, is associated with impaired NO responsiveness. Two major abnormalities of platelet function were documented in patients with AS. First, platelets manifested increased aggregability in response to ADP. Second, the anti-aggregating effects of NO donor SNP were significantly reduced, thus representing NO resistance at the platelet level. However, presence of the former abnormality did not account for the occurrence of the latter.

The severity of NO resistance was independent of presence/absence of hemodynamically significant coronary artery disease. These results indicate therefore that AS represents an additional "marker" of platelet NO resistance. Interestingly, there was no correlation between AVS severity and the extent of platelet NO resistance. This suggests that platelet abnormalities and impairment of NO-related mechanisms may appear early in the clinical course of AVS.

Indeed, in our recent cross-sectional population study evaluating biochemical and physiological correlates of the presence of aortic valve sclerosis (ASc), a precursor to AS, in aging individuals [67] we have documented that ASc was also associated with impaired platelet responsiveness to NO. In that study, the extent of ASc manifestation was strongly associated with the extent of platelet resistance to NO. This finding is of potential relevance to the association between ASc and thrombotic events [8]. Furthermore, tissue resistance to NO *per se* might also contribute to the calcification process [69].

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthase (eNOS) and a marker and mediator of endothelial dysfunction [70-72]. We have also demonstrated that plasma concentrations of ADMA are elevated in patients with AS, compared with controls [73]. Thus, this finding provides further support to the suggestion that NO generation is impaired in AS.

A critical question which arises is whether the pathogenesis of AS is fundamentally related to dysfunction of the valve endothelium. NO resistance in AS may be paralleled by impairment of aortic valve endothelial function. Previous studies [63, 74, 75] have demonstrated that even

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Table 2. Subtypes of BAV by anatomical structure and associated features [118].

Subtype of BAV	Valvular anatomical features	Associated features
Type I (70-75%)	Fusion of the right and left coronary cusp resulting in anterior – posterior commissural orientation	More common in males. Aortic root dilatation and higher prevalence of aortic coarctation.
Type II (25-30%)	Fusion of the right and non-coronary cusp result in right and left commissural orientation.	More common in females. Higher association with progression to aortic stenosis and regurgitation
Type III (~1%)	Fusion of the non-and left coronary cusp.	

early aortic valve calcification is associated with some decrease in endothelial function and even loss of valvular endothelium. Narrowing of the aortic valve orifice in AS, together with deformation of leaflets and increasingly rough surface of the valve, contributes to local turbulence in blood flow which creates shear stress, affecting both valve endothelium and passing platelets [76]. Furthermore, loss of aortic valve endothelium may predispose towards calcification of the aortic valve leaflets [69, 77, 78]. Potentially, aortic valve endothelium plays a critical role in maintaining normal aortic valve function.

In isolated porcine and canine aortic valves, it was shown that the aortic valve endothelium releases NO and prostacyclin, which both exert local anti-thrombotic effects and reduce leaflet tone [16, 18, 63]. The main "luminal" implication of these studies was that the stenotic aortic valve constitutes a pro-aggregatory milieu: - the clinical consequences of a loss of valve anti-aggregatory function must be considered together with platelet hyperaggregability and platelet resistance to NO occurring in AVS [66]. However, it is equally possible that disordered valve endothelial function contributes to development of valve matrix pathology. In our recent studies [69] with porcine aortic valve interstitial cell cultures, calcific nodule formation and associated redox stress were inhibited by NO donors. This suggests that NO has a direct protective effect extending to the inhibition of the calcification processes in aortic valve cells.

Bicuspid aortic valve (BAV): a special case?

BAV represents the most common congenital cardiac abnormality, with estimates of its prevalence ranging from 0.5 to 2% of the general population [79]. Structurally, BAV is heterogeneous, depending on the pattern of valve leaflet fusion, and its association with extravalvular

disease, as summarized in **Table 2**. However, the most common pattern, accounting for about 75% of BAV cases, is fusion of the right and left (R-L: Type I) valve leaflets, while Type II BAV (fusion of the right and non-coronary cusps) accounts for virtually all other cases.

A fundamental and unique issue with BAV is the association with dilatation of the aortic root and arch, which is a particularly prominent feature of Type I BAV, and which is associated with risk of aortic dissection and rupture. On the other hand, progression of AS appears to be more rapid in Type II [79], where most patients require eventual aortic valve replacement.

From a pathophysiological point of view, BAV is unique and intriguing because it presents as a basis for a rapidly progressive form of AS and/or aortopathy, and because of the possible insight it provides regarding the pathogenesis of AS.

As regards the rapid progression, in a retrospective study of 156 adult patients with BAV, Thanassoulis et al [80] have demonstrated a mean increase in diameter of ascending aorta of 0.37mm/year and of aortic sinus of Valsava of 0.17mm/year. Tzemos et al [81] documented increase in aortic valve gradient of 0.7 mmHg per annum in a prospective cohort of 642 subjects with BAV.

As regards available data on the pathogenesis of BAV, despite a very large number of recent investigations, both basic and clinical, no coherent picture has emerged. Rather, it needs to be emphasised that there is increasing evidence of heterogeneity of pathogenesis, notably between Type I and Type II BAV, with increasing evidence of redox stress and inflammatory activation for Type I, and of predominant endothelial dysfunction for Type II [82]. Furthermore, there appears to be a substantial genetic component to BAV

pathogenesis.

As regards the issue of inheritance of BAV, although all series show a predominance of male patients, the overall thrust of genetic investigations is to suggest autosomal dominant inheritance in most cases. A number of studies reveal multiple cases within kindred [83-85]. The most extensively documented mutations underlying BAV occur in the NOTCH1 gene [86], which would also predispose towards valve calcification, given that NOTCH signalling has been linked to expression of osteogenic genes osteopontin and osteocalcin [87]. However the relevant signal transduction pathways for AS development in BAV remain uncertain [88] and there is also evidence of multiple mutations associated with BAV [85].

The issue of association of BAV with endothelial dysfunction and possibly with impaired eNOS signalling has attracted considerable attention since it was observed that mice lacking the eNOS gene frequently also had BAV (but not other congenital cardiovascular abnormalities) [89]. However, Fernandez et al [82] have more recently clarified this observation: the abnormality is actually Type II BAV (right - non-coronary cusp fusion). Interestingly, these investigators documented that Type I BAV is present in inbred Syrian hamsters, without any known association with eNOS deficiency

While no other endothelial function studies have specified BAV subtype, there is substantial additional evidence that BAV may indeed be associated with eNOS deficiency and/or endothelial dysfunction. Aicher et al [90] quantitated eNOS protein expression in the aortic wall (but not the valve) at the time of surgical valve replacement and observed lower levels of eNOS in BAV versus tricuspid ("normal") valves in the presence of AS. Furthermore, eNOS expression was negatively correlated with aortic diameter. A further study has examined brachial flow-mediated dilatation (FMD): - in a group of men with BAV, there was an association between aortic dilatation and impaired FMD [91], consistent with findings of Archer et al [90]. A number of other studies have evaluated the association between AS and NO deficiency, but not always in the context of BAV: these findings will be discussed later.

The concept of inflammatory change and oxida-

tive stress is a relevant component of the pathogenesis of BAV, and is in a sense linked to that of NO deficiency. For example, Tzemos et al [91] observed that aortic dilatation in BAV was associated not only with impaired FMD, but also with increased plasma levels of matrix metalloproteinase 2 (MMP-2), which might have contributed to the dilatation process. Furthermore, Phillippi et al [92] demonstrated that susceptibility to oxidative stress was increased, and responsive expression of metallothionein decreased in BAV aortae. While these findings implicate oxidative stress and inflammatory activation, there is also evidence that endothelial progenitor cell mobilization in response to these changes may be abnormal [93], contributing to perturbation of aortic and valvular endothelial function.

Intervention studies to retard the progression of AS

Statins

Statins were the first commercially available drugs studied in aortic stenosis given their established benefit in primary and secondary prevention of cardiovascular diseases, as well as evidence of association of lipid infiltration of aortic valve in AS [25, 29, 30]. Whilst there are many "dyslipidemic" animal models of AS (see animal model section), only one model so far has shown benefit of statin therapy [94].

There were a number of very encouraging human retrospective studies of statins in AS, suggesting significant reduction in the rate of progression of AS with statin use [95-99]. Yet, only one small prospective open-label non-randomized observational study of rosuvastatin in patients with moderate AS showed slowing of haemodynamic progression of AS [100]. Three large prospective double-blind randomized placebo control trials of statins in AS failed to show any retardation of AS progression [101-103].

ACE-inhibitors (ACEI)/Angiotensin receptor blockers (ARB)

Numerous studies have demonstrated increased ACE activity/expression and Ang II presence in stenotic valves [37-39], providing a rationale for investigation of benefits of ACEI/ARB therapy in AS. Treatment of cholesterol-fed rabbits with angiotensin receptor-1 blocker olme-

sartan was associated with decreased macrophage infiltration and reductions in osteopontin and ACE in aortic valves [104]. Unfortunately, no hemodynamic valvular measures of AS progression were performed. We have demonstrated hemodynamic retardation of AS, concomitantly with reduction in calcification, macrophage infiltration, redox stress and improvement in endothelial function, with ACEI ramipril treatment in a rabbit model of AS [59].

As regards human studies, no prospective trials of either class of agents have been published to date. Two main retrospective evaluations of population studies have provided conflicting data: - Rosenhek et al [98], utilizing echocardiographic parameters, found no significant effect of ACE-I therapy on AS progression (albeit with a trend to slower progression), while O'Brien et al [105], in a study using CT-based calcification assessment, found lower rates of AS calcification, after correction for comorbidities.

Bisphosphonates

Bisphosphonates, usually used for treatment of osteoporosis in humans, inhibit bone resorption as they cause osteoclast apoptosis [106, 107]. They also inhibit an enzyme in the cholesterol synthesis pathway, which causes abnormalities in the cytoskeleton in the osteoclast, thus reducing bone resorption [108]. Thus, bisphosphonates may directly reduce valvular calcification via their osteoblast action, as well as indirectly via inhibition of inflammation and resultant fibrosis. A study in a rat model of dialysis suggested that etidronate, now rarely used bisphosphonate, limited aortic calcification [109].

Two small retrospective human studies of bisphosphonates demonstrated reduction in the AS progression rate [110, 111]. However the small size and observational nature of these studies make the results hypothesis generating at best.

Aldosterone blockade

Aldosterone has been implicated in animal studies in vascular inflammation and myocardial fibrosis, and these were ameliorated by aldosterone blockade [112]. Eplerenone, an aldosterone-receptor antagonist, shown to improve outcomes in heart failure patients [113], was tri-

alled in a randomized double-blind placebo-controlled study in patients with moderate-severe AS [114]. This small trial failed to show reduction in rate of progression of valve stenosis.

Conclusion

The major objective of this review is to present the case that pathogenesis of AS is an active process that involves a combination of inflammatory activation, increased oxidative stress, fibrosis and calcification, which should be amenable to therapeutic intervention.

AS is the commonest form of valvular heart disease and its prevalence is rising due to increasing longevity, especially in Western world. We present evidence that AS, previously thought to be "degenerative" disorder of aging, is a complex active process, involving valvular endothelium, fibroblasts and extracellular matrix. The process is characterized by inflammatory activation and lipid deposition within valve lesions. There is extensive valvular matrix remodelling and fibrosis with increased production of MMP-1 and -2, TGF- β 1, interleukin-1 β and TNF α . There is extensive evidence for increased production of Ang II, a major pro-inflammatory and pro-fibrotic mediator, within stenotic valves. This would lead to further fibrosis and calcification. Impaired activation of anti-calcific modulators, such as fetuin-A and MGP, is also important in AS. There is concurrent increased in oxidative stress and evidence of impairment of the nitric oxide system as well as associated systemic endothelial dysfunction.

In BAV, representing the most common congenital cardiac abnormality, valvular inflammation is combined with aortopathy. BAV illustrates unique interplay of genetically driven inflammatory activation and, in some cases, deficiency of nitric oxide formation.

The most promising targets for pharmacological interventions have been thought to be lipid infiltration and atheroma formation. However, results of all randomized double-blind prospective studies of statins have been disappointing [101-103]. ACE-I/ARB therapy, on the other hand, shows promising results. Although post-hoc clinical data are limited and inconclusive, we have recently demonstrated in an animal model that ACE-I ramipril retards progression of mild

AS [59]. In a separate study, olmesartan treatment was also associated with reductions in macrophage infiltration and ACE in aortic valves in a rabbit model [104].

Therefore AS represents the result of a prolonged inflammatory process leading to valve calcification and ossification, which represents a potential target for therapeutic interventions.

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