An Exploratory Look at Bicuspid Aortic Valve (Bav) Aortopathy: Focus on Molecular and Cellular Mechanisms

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Abstract: Bicuspid aortic valve (BAV) is the most common congenital heart malformation. BAV patients are at increased risk for aortic valve disease (stenosis/regurgitation), infective endocarditis, thrombi formation and, in particular, aortic dilatation, aneurysm and dissection. This review aims at exploring the possible interplay among genetics, extracellular matrix remodeling, abnormal signaling pathways, oxidative stress and inflammation in contributing to BAV-associated aortopathy (BAV-A-A). Novel circulating biomarkers have been proposed as diagnostic tools able to improve risk stratification in BAV-A-A. However, to date, the precise molecular and cellular mechanisms that lead to BAV-A-A remain unknown. Genetic, hemodynamic and cardiovascular risk factors have been implicated in the development and progression of BAV-A-A. Oxidative stress may also play a role, similarly to what observed in atherosclerosis and vulnerable plaque formation. The identification of common pathways between these 2 conditions may provide a platform for future therapeutic solutions. (Curr Probl Cardiol 2021;46:100425.)

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Introduction

Epidemiology, Embryology, Anatomical Classification and Aim of the Review

Bicuspid aortic valve (BAV) is the most common congenital malformation of the human heart. Its prevalence is 0.5%-2% in the general population.[1,2]

The incidence in male Caucasians is 1.5%, while a lower incidence has been reported in females and in non-Caucasians individuals.[3] BAV-associated aortopathy (BAV-A-A) is a common finding in BAV patients.[3]

BAV-A-A has been shown to be associated to the traditional cardiovascular risk factors, as such as hypercholesterolemia and hypertension, but data are not conclusive.[4]

BAV-A-A is characterized at an early stage by asymptomatic dilation of the different portions of the aorta, particularly of the ascending tract. BAV-A-A is a markedly heterogeneous entity and it is most frequently diagnosed in adulthood, when complications like aortic valvular dysfunction or endocarditis may arise.[1-5]

Dilation may occur in the aortic root, the tubular ascending aorta, the proximal aortic arch, or any contiguous combination of them.[5] Of note, BAV accounts for more morbidity and mortality than all other congenital heart lesions combined.[6] The increased mortality may occur via multiple mechanisms, like valvular disease (aortic stenosis or insufficiency), ascending aortic aneurysm, and aortic dissection.

BAV morphology is commonly defined through the Sievers' classification,[7] which is based on the number and location of the raphe(s). The main category is based on the number of raphes, while the first subcategory is based on spatial position, and the second subcategory reflects the valve function (normal, insufficiency, stenosis).

The tubular ascending aorta is most commonly involved, although all segments (the aortic root and the aortic arch) can be affected. The "root phenotype," see below, of BAV-A-A, (where the dilatation is predominant at the level of the sinuses of Valsalva,) represents the potentially most severe and rapidly progressive aortopathy.[8] By contrast, degenerative aneurysms of the aorta tend to start in the midascending portion and then progress both distally and proximally, while those associated with connective tissue disease (eg, Marfan disease) are usually confined to the aortic root.

In BAV-A-A aortic dissection can occur without aortic valve dysfunction.

The most common clinical presentation of BAV disease is calcific aortic valve stenosis, usually presenting between the fifth and seventh decades of life. On the other hand, aortic insufficiency more frequently occurs at a younger age, particularly in males.[9,10] Calcific aortic stenosis is strongly associated with an asymmetrical dilatation of the tubular ascending aorta, while insufficiency is mainly accompanied by aortic root dilatation (the so-called root phenotype). However, according to Della Corte et al,[10] variable combinations can occur. These authors proposed a classification based on the segment of the proximal aorta involved, distinguishing the "root," and the "ascending" phenotype.[10] Echocardiography-based classifications have been also proposed.[11] The symmetry of aortic dilatation has been considered relevant for the pathogenesis. Indeed, "asymmetrical dilatation" refers to the predominant enlargement of the greater, outer curvature (usually designed as "convexity," by contrast to the lesser, inner curvature or "concavity").[12]

Of note, there is a common embryogenic origin between the aortic valve and the ascending aorta. In fact, the aortic root, the ascending aorta and the aortic arch all derive from neural crest cells, (while the descending aorta derives from paraxial mesoderm).[2,13]

Multicenter registries, including large cohorts of BAV patients, have the potential of improving the knowledge on the natural history of the disease, as well as of giving insights into better management.[14,15]

Recent guidelines[16] cover all major aspects of BAV management in terms of imaging, medical therapy, indications for surgery, and follow-up.

Nevertheless, to date, the precise molecular and cellular mechanisms that lead to BAV-A-A remain largely unknown.[17]

This review aims at exploring the possible interplay of genetics, extracellular matrix (ECM) remodeling, abnormal signaling pathways, oxidative stress, and inflammation as contributing factors to the development of BAV-A-A.

The identification of common pathways shared with atherosclerosis and vulnerable plaque formation may provide a platform for future studies and search on common therapeutic solutions.

Focus on: Histopatology of the BAV-A-A

SMCs and ECM

The aorta and large vessels are made of the intima (a layer of endothelial cells directly upon the internal elastic lamina), the media (concentric

layers of smooth muscle cells—SMCs and their extracellular matrix—ECM), and the adventitia (mainly made up of collagen-producing myofibroblasts).[18]

The main mechanical function of the media is to provide elastic recoil for pulsatile aortic pressure, enabled by its organized composition of SMCs and the ECM.

SMCs are the prevalent cells within the aortic wall. They are not terminally differentiated, but express proteins involved in contraction and ECM synthesis, both during development and in response to mechanical and chemical stimuli. Quiescent contractile SMCs produce low levels of ECM proteins, but express contractile proteins including smooth muscle alpha-actin and myosin heavy chains.[19]

SMCs are bound to elastic fibers, fibrillin-1 (Fbn-1) and collagen type VI, with basal lamina connections linking them to each other and providing a template structure for laminar structure.[20]

In general, the media of arteries is made of multiple lamellae, the number of which is likely determined during embryogenesis and is related to the diameter and the mechanical stress of the vessel. The aorta has the greatest number of lamellae. When activated, SMCs proliferate, migrate and produce large amounts of ECM proteins, thereby regulating the aorta's mechanical properties in response to physiological wall stresses. Tyrosine kinases, integrin, and G-protein receptor-mediated factors (basic fibroblast, platelet-derived, epidermal, and insulin-like growth factors) expressed on the cell surface stimulate proliferative SMC phenotype. Angiotensin (AT) II modulates both contractile and proliferative phenotypes through type I and type II receptors (ATR-I and ATR-II), respectively.

Elastin, collagen types I, III, IV, V, and VI, fibronectin, Fbn-1, fibulin-4, proteoglycans of dermatan, chondroitin, and heparin, along with other proteins constitute the ECM.[21]

Elastic microfibrils are linked to SMCs of adjacent lamellae through integrins. Each lamella is oriented obliquely to adjacent lamellae, regulating the stress distribution across the aortic wall. Microfibrils are composed of polymeric fibrillin wrapped around an amorphous elastin core. Fbn-1 has many protein- and integrin-binding sites and can sequester growth factors, such as the transforming growth factor β (TGF-β). In addition, to provide a compliant structure, the microfibril has a cell adhesion function for SMCs, the intima, and the adventitia.[21]

Tricuspid Aortic Valve vs Bicuspid Aortic Valve (TAV vs BAV)

Ascending aortic dilation unrelated to BAV is characterized by severe elastin degeneration with fibrosis and cystic degeneration of the media, as well as by inflammatory infiltrate with adventitial and intimal thickening.[22]

BAV-A-A has distinct characteristics.

The ascending aorta of patients with BAV generally shows noninflammatory loss of SMCs, with multifocal apoptosis and medial degeneration.[22] Nevertheless, the fiber architecture is similar to that of the normal aorta.[23]

Grewal et al[24] compared the histopathology of aortic aneurysms related to BAV, TAV, and Marfan's syndrome. Some degree of heterogeneity was found in the 3 groups with respect to parameters of matrix remodeling and vascular smooth muscle markers. Both Marfan's syndrome and BAV showed an altered distribution and decreased Fbn-1, as well as lower level of differentiated SMC markers. On the other hand, aortic tissue in TAV is made of well-differentiated SMCs, which are susceptible to age-related changes (increased expression of progerin, increased inflammation, and apoptosis) leading to degeneration and cytolytic necrosis. This progressively weakens the aortic media, with possible complications like aortic dilation and dissection. By contrast, in BAV and Marfan's syndrome the weakness of the aorta is mainly due to deficient differentiation of SMCs and quantitative decrease of Fbn-1. Indeed, immature SMCs are unable to produce sufficient amount of Fbn-1, which in turn plays a pivotal role for the structural stability of the vessel wall. In Marfan's syndrome, Fbn-1 mutations further favor apoptosis of SMCs, partly related to increasing signaling of AT II receptors.

Proteins alterations can be also influenced by the shear stress of the aorta. In BAV patients the aortic dilatation often occurs along the convexity (outer curve), where an increased change often ascribed to greater shear stress is thought to arise from asymmetrical flow across the valve. This has been reported to favor elastic fiber fragmentation, reduced collagen types I and III expression, and SMCs apoptosis.[25]

Finally, Billaud et al[26] analyzed precise differences in TAV vs BAV in relation with vasa vasorum density and remodeling. They found that aneurysms in TAV patients displayed lower vasa vasorum density when compared to BAV. The density of vasa vasorum was inversely related to increasing aortic diameter.

The Role of the Matrix Metalloproteinases (MMPs)

Fragmented elastin fibers with reduced Fbn-1 content and decreased types I and III collagen suggest the presence of a high proteolytic activity.[27] Matrix metalloproteinases (MMPs) and their specific tissue inhibitors (tissue inhibitors of metalloproteinases—TIMPs) are key players in the regulation of ECM remodeling.[28]

MMPs are members of a family of enzymes produced by inflammatory, endothelial, and vascular cells. They are involved in the degradation of ECM components, including fibronectin, collagen, elastin, and proteoglycans.[29]

Increased activity of MMPs, (MMP-1, MMP-2, MMP-9, MMP-12, and MMP-14) has been found in BAV patients as compared to TAV subjects. In particular, increased MMP-2 is considered a circulating biomarker of BAV patients, due to its significant correlation with the proximal aortic diameter.[30-32]

The expression of TIMPs is also regulated during tissue remodeling to maintain a balanced ECM metabolism. Studies have demonstrated increased TIMP-1, TIMP-2, and TIMP-4 levels in patients with BAV aortopathy.[30] This has been further supported by data showing elevated proteolytic indices (ie, MMP/TIMP ratio) for MMP-1, MMP-2, MMP-9, and MMP-12.[33]

In addition, increased signaling by the soluble cytokine TGF-β through SMC membrane receptor has been shown to play a role in BAV-A-A, favoring the progression of the aortopathy.[34-36] In fact, deficiency of Fbn-1 leads to an excess of free and active TGF-β due to a decrease in microfibrils and a failure in matrix sequestration of the large latent complex. Defective activity of TGF-β receptors and dysregulation of MMP/TIMP ratio can facilitate aneurysm formation.

Focus on: The Role of Oxidative Stress in BAV-A-A

A number of studies suggest the contribution of oxidative stress in BAV-A-A.

Billaud et al showed compelling evidence of elevated oxidative damage in ascending aortic tissue from BAV patients.[37]

Indeed, increased superoxide-anion levels were found in ascending aortic tissue from BAV patients without aneurysm, as compared to tissue from BAV patients with aneurysm, or subjects with a normal TAV with or without aortopathy. Notably, BAV tissues did not express sufficient superoxide-dismutase (SOD) activity. SOD is a protective enzyme that acts by scavenging superoxide-anions through conversion into the less

reactive intermediate, hydrogen peroxide. Increased oxidative damage in BAV aortic specimens was demonstrated also by an increase in 8-iso-prostaglandin-F2-alpha.

8-iso-prostaglandin-F2-alpha is the result of nonenzymatic peroxidation of arachidonic acid in membrane phospholipids. Increased lipid peroxidation is known to perturb the homeostasis of the plasma membrane, leading to cell death in several cell types, including SMCs.[38]

Overall, these results point out insufficient antioxidant defenses as a major contributing factor to BAV-A-A through Reactive Oxygen Species (ROS)-related alterations in the integrity of the ECM.

According to Jones,[39] the decreased antioxidant defenses may be a consequence of elevated wall stress secondary to BAV-induced alterations in blood flow over the valve.

Della Corte and Cotrufo, have shown changes in regional ECM protein production and deposition in the convexity of the ascending aortic wall in BAV patients, as compared to the concavity.[40,41]

Phillippi et al[42] hypothesized that antioxidant stress defense in the ascending aorta of BAV patients may vary depending on the BAV morphotype. They evaluated gene expression of 3 SOD isoforms in 3 different regions of the proximal ascending aorta on the basis of their proximity to the aortic valve cusps. The expression of SOD in different circumferential regions of the proximal ascending aorta varied according to valve morphotypes.

The authors also examined the contribution of metallothioneins in BAV.[43] Metallothioneins are metal-binding proteins that regulate MMPs and act as antioxidant due to their redox capabilities. They are generally upregulated in conditions of increased oxidative stress. However, metallothioneins gene expression and protein levels have been found significantly lower in aortic tissue and cultured aortic SMCs from BAV patients as compared to control subjects. Such dysregulation of metallothionein in ascending aortic SMCs may contribute to inadequate response to oxidative stress and to aneurysm formation in BAV patients.

Finally, the role of the vascular endothelial growth factor (VEGF) in BAV has been also explored.[42,44] SMCs from BAV patients showed a blunted VEGF induction during exposure to ROS. Thus, the lack of metallothioneins/VEGF activation in response to ROS may play a role in ECM homeostasis of the ascending aorta in BAV.

Focus on: Genetic Mechanisms in BaV-A-A

The relatively low incidence of BAV in the general population has hampered adequate genome-wide association (GWA) studies. On the other hand, a genome-wide association study focused on aortic valve stenosis[45] gave some insights also about BAV-A-A. In particular, 2 new aortic stenosis loci, on chromosome 1p21 and on chromosome 2q22 have been associated with BAV.

Mutations in several genes have been associated to human BAV, particularly regarding deleterious loss-of-function mutations in GATA4, GATA6, NOTCH1, and in the homeodomain-containing transcription factor NKX2.5 genes. Such mutations represent useful basis for gaining insights into the molecular mechanisms underlying the pathogenesis of BAV.[46-49] On the other hand, none of them correlated with any specific BAV aortic phenotype, thus questioning their impact on the BAV-A-A.

In particular, NOTCH promotes the endothelial to mesenchyimal transition, and acts as a key signaling during cardiac valve formation. NOTCH pathway is critical for the maintenance of vascular integrity and repair. Kostina et al[50] demonstrated that downregulation of NOTCH signaling in aortic endothelial cells from BAV patients with aortic aneurysm.[50] Balistreri et al investigated the interplay of NOTCH signaling pathway activity with endothelial progenitor cells (EPCs) number in BAV patients. Authors found that NOTCH signaling activity and EPCs number was significantly reduced in BAV patients, either in presence or in absence of ascending aortic aneurysm. These data may suggest that NOTCH deregulation and EPCs may be implicated in the development of BAV and the associated vascular complications. It may be a potential mechanism through which BAV condition is associated with an increased predisposition to develop BAV-A-A due to vascular dysfunction and remodeling.

GATA family members (1-6) contain 2 zinc-finger domains that bind to consensus site. Mutations in GATA have been related to various human congenital heart diseases. GATA 4 has been more extensively studied, since it plays a dominant role in heart development.[49-52] GATA 6 is also pivotal for proper cardiac and valvular morphogenesis in vertebrates, and is a target gene for NKX2.5.[52]

Compound heterozygosity of GATA4 and 6 have been associated to embryonic lethality. GATA6 mutations have been associated to dysfunction of the semaphoring-plexin pathway in cardiac neural crest cells, which are key for appropriate maturation of the aortic valve.[53]

A novel heterozygous GATA6 mutation, p.E386X, has been recently identified[47] in a family with BAV inherited as autosomial dominant trait. Functional studies have revealed that the mutant GATA6 protein have no activity compared to its wild-type counterpart.

GATA4 and 6 are also implicated in regulating the expression of hepcidin, the liver hormone that controls iron bioavailability and it is also involved in inflammation.[54,55]

Focus on: Searching for Biomarkers

The search for circulating biomarkers for BAV-A-A able to improve risk stratification is of great potential interest in the clinical setting.

Up to now, the routine use of such biomarkers is limited, due to the lack of validation in multicenter trials.[56] Besides the above mentioned molecules (MMPs, TIMPs, and TGFβ), possible role has been proposed for the soluble form of the receptor for advanced glycation end products (sRAGE),which is a circulating ligand member of the immunoglobulin super family. Advanced glycation end products are have been associated with valvular and vascular remodeling, and trigger the release of sRAGE. Branchetti et al[57] found that elevated levels of circulating sRAGE were associated with the presence of BAV and BAV-A-A, independently of aortic diameter.

TGF β-1/endoglin ratio has been also suggested as a possible early biomarker for BAV-A-A.[58] Endoglin is a TGF β-1receptor able to modulate TGFβ-1 binding and signaling.

High TGF β-1/endoglin ratio is has been associated with a deleterious pattern of gene expression in the aorta of BAV patients, possibly reflecting an unfavorable TGF β-1 related gene expression profile.

This ratio may be a useful biomarker for the rate of progression of aortic dilation, and it may have a potential for BAV-A-A stratification risk.

Increased levels of asymmetrical dimethylarginine have been also reported in patients with BAV-A-A.[59] Whether or not this could actually be helpful in detecting BAV patients at increased risk of complications remains to be determined.

Noncoding RNA

Noncoding RNAs (ncRNA) are functional RNA molecules that are transcribed from DNA but not translated into proteins. They regulate gene expression at the transcriptional and post-transcriptional level.[60] ncRNAs can be subdivided into microRNAs (miRNAs), long noncoding

RNAs, and small nuclear RNAs. miRNAs exert a wide spectrum of regulatory activities at the molecular and cellular level by binding to a specific target messenger RNA with a complementary sequence. This induces messenger RNA cleavage/degradation or blocks translation. Some candidate miRNAs for BAV have been selected based on previously reported association with aortopathies.

In particular, miR-1 has been reported to regulate the proliferation of vascular SMCs by targeting insulin-like growth factor 1, and thereby influencing the development of vascular diseases.[61] Similar signaling pathways have been also reported for miR-143/miR-145[62] and miR-21.[63]

MiR-17 gene cluster (miR-17, miR-18a, and miR-19a) and miR-17-related miRNAs (miR-20a and miR-106a) have been shown to influence TIMP-MMP balance by inhibiting TIMP expression. This in turn increases MMP2 activity, likely involved in ECM breakdown and aortopathy progression.[64]

MiR-29b and miR-133a also exhibit regulatory effects on the ECM remodeling by interacting with MMP-2 and MMP-9 activity.[65]

MiR-21, miR-26, miR-29, miR-122, miR-130a, miR-133a, and miR-143/145 have been linked to the pathogenesis of proximal aortic aneurysms and acute aortic syndrome.[66,67]

Martinez-Micaelo et al[68] applied a miRNome-wide microarray approach to identify the circulating miRNAs that are specifically associated with BAV and aortic dilation. The authors found that the expression of the circulating miR-122, miR-130a, and miR-486 correlated significantly with the morphology of the aortic valve (BAV vs TAV), while the expression of plasma miR-718 was strongly influenced by the dilation of the ascending aorta.

Borghini et al[67] have studied the miRNAs expression in the aneurysmal aortic tissue. Analysis of the entire miRNome expression in the aortic tissue of 7 BAV patients vs 6 TAV patients with aortopathy found a total of 12 differentially expressed miRNAs. In a surgical cohort of BAV-A-A patients,[69] the expression of circulating miR-17 and miR-106a in the BAV root phenotype patients correlated with the severity of aortopathy and the risk of adverse aortic events. Similar findings were reported by Wu et al.[64] These authors analyzed the impact of miR-17-associated miRNAs on the severity of bicuspid aortopathy in the aortic tissue. They found a significantly increased expression of miR-17-associated miRNAs (miR-17, miR-18a, miR-19a/b, miR-20a/b, miR-106a/b, and miR-93) in less dilated aortic tissue as compared to severely dilated aortic tissue. To evaluate the role of miR-17/TIMP/MMP signaling cascade in BAV-A-A progression, the authors also assessed, TIMP12 and 3 expression and

MMP-2 activity in the aortic tissue. They found that miR-17-related miRNAs expression gradually decreased after development of severe aortic dilation, with corresponding reduction of circulating miR-17/miR-106a levels. Overall, these studies can be viewed as preliminary proof-of-principle analysis, and further investigations are needed to define the value of circulating miRNAs as potential biomarkers of BAV-A-A (Fig 1).

Focus on: Possible Shared Mechanisms Between Atherosclerotic Plaque Vulnerability and BAV-A-A

The pathophysiological mechanisms underlying the possible association between the atherosclerotic plaque and BAV-A-A are controversial and constitute an attractive working hypothesis. The hypothesis is based in particular on the common features shared between BAV-A-A and atherosclerotic plaque vulnerability, considering the role of SMCs, ECM, MMPs, and oxidative stress in the pathophysiology of both diseases.

The precise mechanisms underlying plaque destabilization are still unknown. Disruption of the plaque and luminal thrombosis are mostly determined by the expansion of the necrotic core (NC) driven by various mechanisms, including accelerated macrophage apoptosis, and defective phagocytic clearance (defective efferocytosis). Oxidative stress is implicated in the expansion of the NC, and many oxidized compounds contribute to the macrophage apoptosis. In addition, oxidized derivatives of polyunsaturated fatty acids promote defective efferocytosis.[70]

Plaque rupture is the most frequent etiology of sudden coronary death (55%-60%), followed by erosion in about one-third and thrombi attributed to calcified nodules in only less than 10%.[71]

Rupture-prone or vulnerable plaques are mostly characterized by the existence of high inflammatory cell infiltrate and a large NC covered by a thin fibrous cap, with reduced SMCs and ECM content.[72]

The term thin-cap fibroatheroma generally indicates plaques with average cap thickness of 65 μm, characterized by expansive remodeling, neovascularization, inflammation, large plaque size, plaque hemorrhage, and calcifications.[73]

SMCs are predominant in the fibrous cap of stable atherosclerotic plaques,[73,74] while increased apoptosis of the fibrous cap characterizes the plaque destabilization resulting in reduced ECM content.[74]

SMC apoptosis is favored by the infiltration of inflammatory macrophages, lymphocytes and mast cells, through the release of proapoptotic substances. Cell-to-cell interactions after MMPs degradation of ECM can also contribute to SMCs apoptosis.[75]

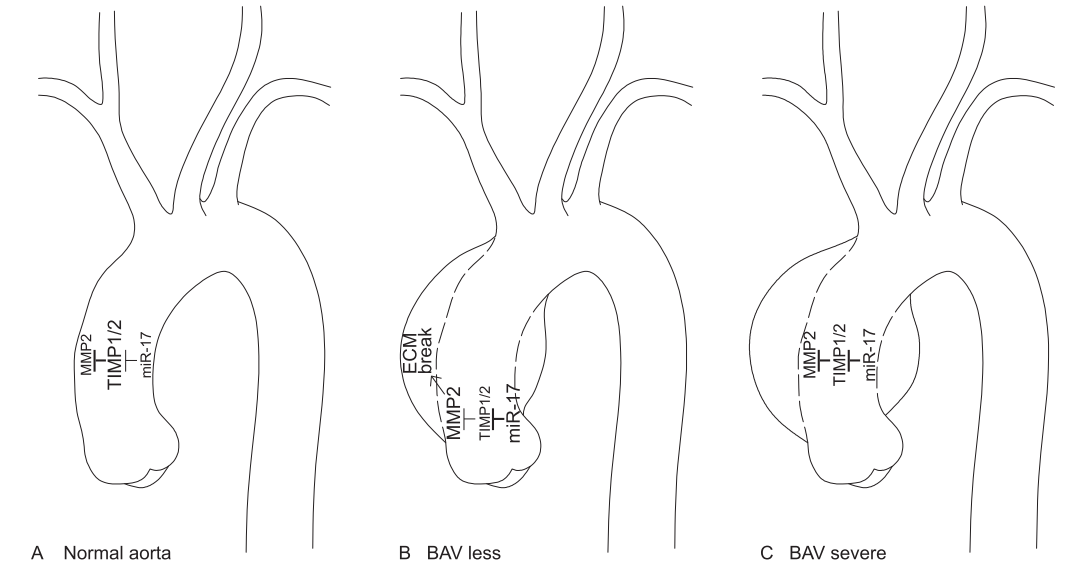

FIG 1. (A) Normal aorta: expression of the miR-17 this determines a high activity of TIMP1/2 that blocks MMP2; (B) Mildly-dilated BAV aortopathy: significant increased expression of miR-17 inhibits TIMP1/2, with a consequent strong activation of MMP which causing ECM disruption; (C) Severely dilated BAV-A-A: miR17 is reduced, TIMP1/2 is strongly activated, and MPP2 returns to normal level (adapted from Wu et al[64]). BAV, bicuspid aortic valve; ECM, extracellular matrix; MMP2, metalloprotein 2; miR17, microRNA; TIMP 1/2, tissue inhibitors of metalloproteinase 1/2.

MMPs are considered as powerful ECM degradation enzymes that cause plaque instability. MMP-2 enhances the plaque stability by promoting cap formation, while the activation of MMP-9 and MMP-12 promotes inflammation and plaque vulnerability.[75]

MMPs activity can be increased by oxidative stress and particularly in the presence of ROS.[75] A further mechanism leading to SMC apoptosis is triggered by ECM degraded products and accumulation of apoptotic macrophages in the advanced atherosclerotic plaques. These apoptotic cells eventually become necrotic and converge into the NC, particularly when phagocytes are unable to adequately remove the apoptotic cells. This process is called defective efferocytosis and it is now considered a key feature of the vulnerable plaque.[76]

Recently, the role of the endoplasmic reticulum (ER) stress on NC expansion has been investigated, possibly through the unfolded protein response or/and the nuclear erytroid-related factor 2, (for a recent review see[70]).

ER stress is considered an important event during atherosclerosis initiation, development, and clinical progression.[77] Myoishi[78] studied human atherosclerotic coronary artery lesions obtained at autopsy or after directional coronary atherectomy. Both SMCs and macrophages exhibited a markedly augmented expression of ER stress markers in the thin-cap atheroma and in the ruptured plaques compared with intimal thickening, fibrous plaques, and thick-cap atheroma. Only advanced, "vulnerable" plaques showed evidence of increased expression of ER-related apoptosis markers. In agreement with Myoishi,[78] our group has recently reported[79] that the "tissue around the NC" (TANC), but not the periphery of the same carotid plaques, is characterized by an abnormal amount of macrophage-derived apoptotic cells. This phenomenon may be related to the sustained ER stress, since TANC is characterized by an abundance of ER apoptosis-related gene expression, while ER survival genes prevail in carotid plaque periphery. These results suggested that ER stress may promote macrophage apoptosis in TANC and favor the NC expansion.

Up to now, no studies have investigated the possible links between ER stress and BAV-A-A. Nevertheless, the major stressors that are likely to be involved in ER stress activation are compounds or processes that are present in advanced lesions. They are markers of oxidative stress: oxysterols, oxLDL, oxidized phospholipids, hypoxia, and peroxynitrite[72] and, as recently demonstrated by our group,[80] oxidized derivatives of polyunsaturated fatty acids.

As discussed above, increased oxidative stress has been reported in BAV-A-A. Thus, it is tempting to speculate about a role of ER stress also

in BAV-A-A pathophysiology. Up to now, no experimental data are available about ER activation on BAV-A-A, so this fascinating hypothesis needs further explorative studies.

In general, common mechanisms shared between BAV and atherosclerosis are under investigation. Further studies are needed to clarify the precise relation among BAV, BAV-A-A, and atherosclerosis, as recently reviewed by Magni.[81]

Nevertheless, dyslipidemia and the activation of proinflammatory pathways (nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 inflammasome and Toll-like receptor 4) appear to play a consistent role in BAV-A-A progression. Nevertheless, the correlation between markers of lipid metabolism and the extent of BAV-A-A has not been univocally accepted, as examined.[82,83]

In the area of lipids, a role has been shown for lipoprotein (a) a low-density lipoprotein-like particle with a proatherogenic role. It is considered a risk factor for coronary artery disease (CAD).[84] Higher lipoprotein (a) levels have been found in BAV patients.[85]

Other analogies that may be considered between atherosclerosis and BAV concern the endothelial dysfunction, as shown by reduced flow-mediated dilation in BAV patients.[86]

A large meta-analysis[87] assessed whether aortic valve morphology has a different association with CAD. The conclusion was that BAV patients did not exhibit a lower risk of CAD compared to TAV patients, but differences in age and diabetes comorbidity had a consistent impact. This topic requires further studies.

Another possible link between BAV and atherosclerosis is related to the role of nitric oxide (NO), that is a key regulator of normal endothelial function in the vessels.[88] Endothelial NO synthase (eNOS) is expressed in the endothelium. It has been demonstrated that eNOS protein expression is decreased in BAV if compared to TAV.[89] Moreover, abnormalities in eNOS enzymatic activity can induce calcification in the aortic valve, if associate with hypercholesterolemia.[90]

The decrease of available NO causes the stimulation of valvular myofibroblast proliferation and ECM production which leads to the development of atherosclerosis in the aortic valve.[90]

Other studies support the concept that the valve calcification process may have a similar pathophysiological process as that of vascular atherosclerosis, as reviewed.[91]

A particular relationship among lipid metabolism, low-density lipoprotein-related receptor-5 (Lrp5) / beta-catenin and calcification pathways has been reported.[91] In particular the transcription factor Cbfa1 (core-

binding factor 1), the main transcription factor involved in osteogenesis, in animal models, increases in relation to hypercholesterolemia, while atorvastatin can reduce its gene expression. Moreover, Cbfa1 has been shown to be expressed in human degenerative valves derived from surgical valve replacement.[92,93] This process involves Notch1 and Lrp5 / beta-catenin.[91] A reduction of Notch1 and an increase in Lrp5 induce Cbfa1 activation resulting in atherosclerosis and endothelial osteogenesis.[91]

Finally, a role has been recognized to the wall shear stress[94] which acts as endothelial cells trigger and modulator, with analogies to the atherosclerotic process favoring adaptation, inflammation, and remodeling of the vessel wall and lumen bone matrix synthesis and calcification.[90,94,95]

Conclusions

To date, the precise molecular and cellular mechanisms that lead to BAV-A-A remain unknown.

A number of clues, particularly an increased oxidative stress, suggest similarities with the pathophysiology of atherosclerosis and plaque formation (Fig 2). The identification of common pathways between these 2

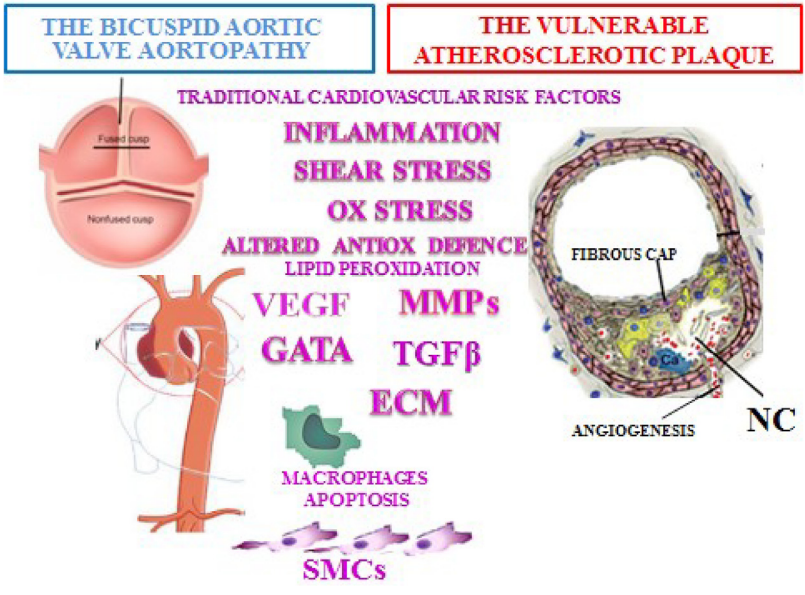

FIG 2. Common features of BAV-A-A and the vulnerable atherosclerotic plaque on the basis of the current knowledge. Ca, calcifications; ECM, extracellular matrix; MMPs, metalloproteins; NC, necrotic core; ox, oxidative; SMCs, smooth muscle cells; TGF- β, transforming growth factor β; VEGF, vascular endothelial growth factor.

conditions may provide a platform for future studies searching for common therapeutic solutions.

Authors'Contribution

CM, DG, LC, and MS conceived and wrote the paper.

Compliance With Ethical Standards and Statement of Human and Animal Rights

The study was conducted in accordance with the ethical standards laid down in the Helsinki Declaration of 1975 and its late amendments.

Informed Consent

Not applicable (review article).

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