Aortic stenosis: new pathophysiological mechanisms and their therapeutic implications

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KEY WORDS
aortic sclerosis, aortic stenosis, atherosclerosis, biomarkers

ABSTRACT
Aortic stenosis (AS) represents the most common type of acquired valvular heart disease. Its incidence increases with age; therefore, from 3% to 9% of adults over 75 years of age develop AS. The pathophysiological mechanisms and role of biomarkers in the prediction of AS have been extensively studied. Progression of AS is characterized by a number of abnormalities in calcification regulation, inflammation/adipokine dysregulation, prothrombic state, and altered von Willebrand factor function. The current understanding of the mechanisms of AS involves a complex role of the multiple cell types, in particular myofibroblasts and macrophages. The introduction of transcatheter aortic valve implantation provides invaluable opportunities for periprocedural and long-term monitoring of the changes in the biomarker profile. Effective pharmacological treatment, especially in the early stage of AS, is largely unknown. The current review discusses not only the pathophysiology of AS but also attempts at pharmacological treatment.
by promoting destructive, inflammatory, and (auto)immune processes. Both the development and progression of AS are actively mediated by chronic inflammatory processes with contributing inflammatory-cell infiltrates, lipoproteins, lipids, and factors responsible for calcification. The development of AS involves the up-regulation of several signaling pathways including the expression of bone regulatory factors. Among the most likely signaling pathways, osteoprotegerin (OPG)/receptor activator of NF-κB ligand (RANKL)/receptor activator of NF-κB (RANK) plays an important role in the calcification process. Importantly, OPG and RANKL were expressed not only in stenotic but also in sclerotic aortic valves.

In the early stage of AS, initial AS plaque resembles the plaque of coronary artery disease (CAD). Subsequent studies also found that CAD and AS shared similar risk factors. Risk factors and mediators leading to calcific AS, such as older age, male sex, hypercholesterolemia, atherosclerotic lesions, smoking, and diabetes, are also similar to those recognized as classic risk factors for vascular atherosclerosis. Additional evidence indicates that AS, like CAD, was an active inflammatory process with inflammatory cells present in the lesions and increased blood concentrations of C-reactive protein observed in AS patients. Inflammation is actively implicated in aortic valve calcification and can also precede rapid progression to severe disease. However, there are significant differences between vascular atherosclerosis (more unstable process) and aortic valve (valvular) calcification (more stable process). In the progression of CAD, plaque rupture is the major event leading to clinically relevant events, whereas in AS, it is progressive calcification, even with lamellar bone formation that causes immobility of the valve. In addition, the significance of several biological and clinical differences between CAD and AS cannot be excluded. In fact, less than half of the patients undergoing aortic valve replacement have CAD and only a small percentage need a coronary bypass surgery. Furthermore, while smooth muscle cells are mainly

**FIGURE** Disease mechanisms and time course of calcific aortic stenosis. Shown is the relationship among disease stage, valve anatomy, clinical risk factors, mechanisms of disease, and the age of the patient. Endothelial disruption with inflammation (dashed line) and lipid infiltration are key elements in the initiation of disease. There are few data on the prevalence of disease initiation in at-risk patients, and progressive disease develops in only a subgroup of these patients. Progressive leaflet disease, which is associated with several disease pathways, develops in approximately 10 to 15% of patients with aortic sclerosis. Once these disease mechanisms are activated, leaflet calcification results in severe aortic stenosis in nearly all patients. With end-stage disease, tissue calcification (red line) is the predominant tissue change, resulting in valve obstruction. Current imaging approaches are reliable only when substantial leaflet changes are present (in patients with progressive disease or valve obstruction), which limits clinical studies of interventions to prevent or slow the progression of early disease. LRP denotes lipoprotein receptor–related protein complex, OPG, osteoprotegerin, and RANKL, receptor activator of NF-κB ligand.

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involved in atherosclerosis, these cells, in their typical form, are rather not seen in diseased aortic valve leaflets, in which fibroblasts and myofibroblasts are more prominent. Activated myofibroblasts are likely to derive from either valvular interstitial cells or a subpopulation of endothelial cells that have transformed from endothelial to mesenchymal formation.2 Finally, atherosclerotic plaques develop and frequently undergo destabilization, while in AS, a massive calcification of the aortic valve and its fibrocalcific thickening represent an advanced stage of the disease.

**Role of adipokines** From the current pathophysiological point of view, atherosclerosis, metabolic syndrome, and AS are considered to be a complex syndrome characterized by chronic inflammation. It has been postulated that adipocytokines, which participate in inflammation and cardiac hypertrophy and remodeling, might be implicated in the progression of AS. Adiponectin, lepin, and resistin play a regulatory role not only in glucose metabolism, insulin resistance, but also in inflammatory process associated with adipose tissue dysfunction.16

Adiponectin is considered an antiatherosclerotic and cardioprotective cytokine.7 Adiponectin releases nitric oxide, modulates macrophage function, and inhibits the production of tumor necrosis factor α and interleukin (IL) 6. It stimulates the synthesis of anti-inflammatory cytokines such as IL-10 and IL-1β. From the clinical perspective, hypoadiponectinemia is associated with an increased risk of CAD and higher carotid intima–media thickness.18 In summary, it predicts the severity, extent, and pattern of atherosclerosis in the coronary arteries.19

Leptin acts as a proatherogenic hormone. It stimulates platelet activity, smooth muscle cell proliferation, oxidative stress, and endothelial dysfunction. Hyperleptinemia has been reported to be associated with increased cardiovascular morbidity and a higher risk of arterial hypertension. This mediator is also involved in myocardial hypertrophy and the regulation of cardiac contraction.20,21

Resistin has proinflammatory properties and it increases the release of proinflammatory markers from endothelial cells (eg, endothelin 1) and the synthesis of vascular cell adhesion molecule 1. In animal models, the main action of resistin is to impair insulin sensitivity. Plasma resistin levels have been shown to correlate with inflammation, but not with insulin resistance, and they may predict coronary atherosclerosis.22 Monthly et al.23 demonstrated that higher plasma resistin levels are associated with the extent of valvular calcification and inflammation in elderly patients with AS.

**Disturbances in hemostasis** Patients with severe AS who were deficient in high-molecular-weight multimers of vWF are characterized by enhanced thrombin formation and platelet activation. This phenomenon may result from the ambivalent effect of high shear stress in a stenosed aortic valve on the hemostatic system and might help explain two aspects of AS, namely, Heyde syndrome and an increased tendency for thromboembolic episodes. In Heyde syndrome, a tendency for bleeding, mostly mucocutaneous and gastrointestinal episodes, is observed in up to 20% of patients with severe AS.24 A major mechanism underlying the link between AS and bleeding was described by Warkentin et al.,25 who detected a deficiency in high-molecular-weight multimers of vWF acquired type 2A von Willebrand syndrome due to increased proteolysis of vWF multimers. High shear stress as a hemodynamic force can modify the structure of the vWF molecules, leading to exposure of the bond between Tyr842 and Met843, which is sensitive to the destructive action of a specific metalloprotease (A Disintegrin And Metalloproteinase with Thrombospondin – ADAMTS13). The loss of the largest vWF multimers leads to regulatory impairment of primary hemostatic balance and the resultant bleeding risk.

Recently, the local expression of vWF has been detected in porcine aortic valves,26 which is consistent with the findings in human aortic valves of patients undergoing valve replacement due to severe AS (Natorska J, unpublished data). Moreover, histamine-stimulated porcine aortic valve endothelial cells released vWF protein and ADAMTS13 into the culture medium, and vWF significantly increased valvular interstitial cell nodule formation and calcification.26

**Role of biomarkers** The understanding of these complex abnormalities using biomarker monitoring provides an opportunity to assess the effects of treatment. The practical implication is important in the face of the dynamic development of therapeutic modalities. Several key studies have focused on biomarkers signaling atherosclerotic process, which is responsible for the degeneration of the aortic valve, promoting the development of stenosis. The next important issue concerns the prognostic value of hemodynamic biomarkers (eg, N-terminal pro B-type natriuretic peptide [NT-proBNP]) before the replacement of the stenotic aortic valve and the importance of a reduction in NT-proBNP levels after the intervention. A recent introduction of transcatheter aortic valve implantation (TAVI) has provided further attractive opportunities for several important periprocedural findings, eg, myocardial ischemic complications.

The main topics to be discussed concerning the role of biomarkers in AS are as follows:

1. the role of plasma biomarkers in the native aortic valve (analysis of progressive stages): early stage, minimal-mild stenosis/aortic sclerosis; late stage, moderate-to-severe stenosis
2. comparison of the plasma biomarker level before vs. after the intervention (effect of surgical artificial valve replacement or TAVI)
3. ex-vivo tissue assessment in the excised valve.
Role of plasma biomarker levels in the early stage: minimal-mild aortic stenosis/aortic sclerosis

Even in mild AS (prehypertrophic status of the myocardium), the inflammatory process, myocardial fibrosis, and cardiac remodeling can be detected. These destructive processes showed a positive and escalating hemodynamic-biochemical correlation with the important role of biomarker measurement. According to Park et al., the left ventricular end-diastolic volume index was independently associated with matrix metalloproteinase (MMP) 1, while the aortic transvalvular mean pressure gradient was independently associated with several biomarkers including MMP-2, transforming growth factor β, and IL-1. They further reported that myocardial fibrosis in mild AS is independently associated with 3 factors: left ventricular volume overload, aortic valve pressure overload, and inflammation.  

With regard to the detection of osteogenesis in the early stage of AS, Sainger et al. studied mild vs. severe calcific aortic valve disease and identified a correlation between the level of circulating biomarkers and various parameters of the valve as evaluated by transesophageal echocardiography (TEE). With regard to osteogenesis biomarkers, higher plasma osteopontin (OPN) levels were observed in AS patients. Parathyroid hormone levels were also increased in those subjects in comparison with controls. Of note, the plasma levels of OPN were also significantly higher in patients with mild AS (even before calcium deposition was detected on TEE). In addition, NT-proBNP levels were significantly increased in the early stage of AS. The serum levels of OPN, parathyroid hormone, and fetuin A showed a significant correlation with various stages of AS, with variations in their levels occurring before the first markers of atherosclerosis (calcium nodules) were visualized on TEE.  

With respect to therapy, an important question arises to the possibility of pharmacological protection against atherosclerotic valve degeneration in early AS (Figure) by monitoring bone metabolism biomarkers. Correspondingly, the effect of atorvastatin (20 mg/d) on the circulating levels of calcification biomarkers in patients with aortic sclerosis has also been studied. Atorvastatin at moderate doses can reduce the plasma levels of calcification biomarkers in patients with aortic sclerosis and mild AS. Similarly, a positive effect of atorvastatin, which lowers the plasma levels of the biomarkers of inflammation, has been observed in patients in the early stages of aortic valve disease.

Role of plasma biomarkers in moderate-to-severe AS

The plasma NT-proBNP level is related to an abnormally high wall tension induced by left ventricular pressure overload. In AS, the diagnostic value of the NT-proBNP level has been reported recently by Banovic et al. In more advanced stages of AS, they found the level of this hemodynamic biomarker to be markedly increased, even in asymptomatic patients with preserved left ventricular contractility. Interestingly, the value of this biomarker is significantly higher in AS patients with limited coronary flow reserve below 2.5. The NT-proBNP borderline value of 334.00 pg/ml was calculated as the best cut-off point for the diagnosis of coronary flow reserve ≤2.5.  

A prospective study by Farre et al. showed that patients with moderate-to-severe AS were followed clinically after NT-proBNP sampling. During follow-up, a clinical endpoint was defined as one of the following events: surgery, hospital admission due to angina, heart failure or syncope, or death. Using the optimum NT-proBNP cut-off point of 515 pg/ml, they reported event-free survival rates at 1 and 2 years of 93% and 57%, respectively, in patients with NT-proBNP levels of less than 515 pg/ml, compared with 50% and 31%, respectively, in those with NT-proBNP levels exceeding 515 pg/ml. The authors concluded that the measurement of NT-proBNP provides valuable prognostic information in patients with asymptomatic moderate-to-severe AS.  

In a subset of patients with severe AS, high gradient, and preserved systolic function, Piestrzeniewicz et al. found low transvalvular flow to be related to high blood levels of NT-proBNP. In addition, they found an inverse relation between procollagen III N-terminal propeptide and stroke volume index, indicating enhanced tissue fibrosis as an underlying pathology.  

Biochemical studies have clearly indicated that AS is not only associated with an inflammatory process but also predisposes to a prothrombotic state. Furthermore, transvalvular gradient as an index of turbulent flow was independently associated with the activation of coagulation and platelets. Hematological abnormalities in AS suggesting a causative role of tissue factor (TF) have been reported. TF may be involved in the mineralization of aortic valves. This destructive process occurs by enhancing the generation of the proinflammatory OPN N-half through the pathway of thrombin induction in the coagulation cascade. These authors documented that TF co-localizes with calcification at the aortic side of the leaflets (the highest amount of TF is observed in the extensively calcified regions). Both TF and α-thrombin are associated with ONP and its thrombin cleaved form (ONP N-half). Moreover, TF increases vascular endothelial growth factor production and stimulates angiogenesis with neovascular invasion in degenerated valves. TF deposit in AS was also analyzed by Natorska et al. who reported significant associations between the percentage of TF-positive areas and transvalvular gradients. Additionally, correlations between TF-positive areas and low-density lipoprotein cholesterol levels were observed. Another hematological abnormality recognized in some patients with AS is the acquired type of von Willebrand disease (type 2A). In contrast to...
this hematologic disease, spontaneous thrombosis of a native aortic valve has been observed in AS patients, despite the presence of regular sinus rhythm. This valvular thrombogenesis may be stimulated by rapid turbulent flow with increased shear stress.

In a study by Kolasa-Trela et al., the biomarker level of NT-proBNP, compared with the other subgroups with a mild leak. An increase in NT-proBNP levels after surgery exceeding 1640 ng/l in 5 days was associated with a significant increase in mortality. Therefore, in the periprocedural period, a serial measurement of NT-proBNP levels can be used for stratifying patients with a significant perivalvular leak, which is associated with a dramatical increase in mortality at 6 months. Finally, the use of NT-proBNP measurements for clinical decision making and follow-up of patients undergoing TAVI has been further supported by several recent studies.

In a study on a prognostic value of tumor marker carbohydrate antigen CA125, the serum levels of this biomarker before and after TAVI independently predicted death and major adverse cardiac events, involving all-cause death and a composite endpoint of death, admission for heart failure, myocardial infarction, and stroke. CA125 is an emerging cardiac biomarker. In heart failure, it has shown to be elevated and is released by mesothelial cells as a response to effusions and inflammation. It has been shown that CA125 is strongly related to heart failure severity and adverse outcomes in heart failure. Recently, in the setting of AS, elevated levels of CA125 have also shown to be associated with disease severity and adverse outcomes.

In another study on aortic valve replacement, OPG levels were found to be associated not only with cardiovascular mortality during follow-up but also with left ventricular and left atrial remodeling in patients with symptomatic severe AS undergoing surgery. Moreover, plasma OPG levels were associated with long-term postoperative outcome and may identify patients with poor symptomatic improvement following valve replacement surgery. Aortic valve tissue assessment. The most interesting aspect of biomarker analysis is associated with the use of the newest imaging methods for valve tissue examination. Based on the study by Dweck et al., 18F-sodium fluoride (18F-NaF) and 18F-fluorodeoxyglucose (18F-FDG) have been shown to be promising novel biomarkers of disease activity in AS. The investigators compared the 18F-NaF and 18F-FDG uptake with a histological characterization of the excised aortic valve and reported that the 18F-NaF uptake identifies active tissue calcification and predicts disease progression in calcific AS.

In another interesting study, Nagy et al. suggested that bone remodeling in calcified aortic valves may be stimulated by multifocal microfractures, with subsequent recruitment of osteoclasts and osteoblasts. They documented that the circulating mediators of bone homeostasis correlated with the severity of AS and distribution of bone turnover in a surgically explanted calcified aortic valve. The plasma levels of tartrate-resistant acid phosphatase (TRAP), RANKL, and Runx-related transcription factor 2 (Runx2/Cbfal) correlated with the severity of AS. The local transcript levels of TRAP correlated with echocardiographic parameters quantifying stenosis severity. The expression level of Runx2/Cbfal1 was a predictor of AS severity.

A key observation from the ex-vivo studies is that AS, characterized by extensive calcification of the aortic valve leaflets, is associated with the infiltration of inflammatory cells. Activated mast cells contribute to the induction of fibrosis and calcification, and these inflammatory cells were detected in the excised valves on immunostaining. The aggressor cells colonized together with macrophages, and neovessels were detected mainly in the calcified regions of the leaflets. Wypasek et al. further stressed that an increased number of these inflammatory cells, together with a significant increase in aortic valve stenosis, was associated with a worse clinical outcome.
of mast cells in a stenotic aortic valve correlates well with the severity of valve narrowing.

The role of coagulation abnormalities in the pathogenesis of AS has been extensively studied. Recently, the importance of the fibrin presence and its determinants in calcified stenotic aortic valve leaflets has been assessed.\textsuperscript{49} Immunofluorescence measurement was performed in decalcified leaflets using antibodies against human fibrin (Fn) and TF. Fn-positive (41.4%) and TF-positive (25.3%) areas were increased in AS valves. AS patients had elevated plasma D-dimer levels and prothrombin fragment 1+2, and Fn-positive areas correlated with TF-positive areas, D-dimer, prothrombin fragment 1,2, the time required for plasma fibrin clot formation, and maximum absorbance of fibrin clots, but not with clot permeability or lysis time. Thickness of the Fn layer within AS valves was associated with the maximum transvalvular gradient. Patients with high peak gradient (>75 mmHg) showed a significant association between Fn-positive areas and maximum gradients. Large fibrin amounts, mostly colocalized with TF, are present within the valve leaflets of patients with severe AS. In-vivo thrombin generation and fibrin clot formation are associated with the extent of Fn presence within the leaflets, which might be involved in the destructive progression of AS.

With regard to Lp-PLA2, the data obtained from plasma measurement\textsuperscript{50} have been confirmed by a recent pathomorphological study,\textsuperscript{51} which demonstrated that Lp-PLA2 is highly expressed in AS and plays a vital role in the mineralization of valve interstitial cells. In another study, 3 biomarkers, namely, sPLA2-lla, C-reactive protein, and C3d, were significantly more activated in athroesclerotic aortic valves compared with degenerative aortic valves.\textsuperscript{51}

Pharmacological treatment: perspectives

Anti-osteoporotic drugs

In a retrospective study,\textsuperscript{52} AS patients receiving therapy for osteoporosis were found to have a significantly smaller decrease in aortic valve area on follow-up in comparison with patients not receiving this treatment. The possible explanation is the action of antiosteoporosis drugs in delaying calcification and stenosis of the aortic valve. The exact mechanism for the prophylactic action of these drugs (mostly bisphosphonates were used) is not clear, although several possibilities can be suggested. First, inhibiting bone turnover decreases the availability of calcium phosphate crystals that would be otherwise deposited on the valvular leaflets. Second, inhibiting a critical step in lipid production (farnesyl pyrophosphate synthase) prevents early stages of AS. Third, there may exist a common mechanism (endocrine or paracrine) by which the aortic valve and skeleton exhibit rapid bone turnover.

Statins

Several retrospective and nonrandomized studies have indicated that lowering atherogenic lipoproteins may delay the hemodynamic progression of AS. However, randomized placebo-controlled, prospective studies on the effect of lowering low-density lipoprotein on AS progression: SALTIRE,\textsuperscript{53} SEAS,\textsuperscript{64} and ASTRONER\textsuperscript{55} have provided inconclusive results. In contrast, the RAAVE\textsuperscript{56} study has shown a positive effect of statins on slowing the progression of AS. All those studies included mainly patients with moderate-to-severe AS.

Conclusions

With the growing frequency of AS in the general population and the increasing availability of various surgical and nonsurgical catheter-based interventional procedures, the role of biomarker monitoring has become extremely important in the diagnosis, prognosis, and treatment of this disease. From a pathological point of view, the understanding of AS at the molecular level has vastly increased over the past decade. Various key pathways and triggering factors have been underlined, and it has been indicated that there are complex interrelationships between lipid retention, inflammation, purinergic signaling, and osteogenic pathways. Although it seems difficult to target 1 upstream factor to control the development of AS, it is likely that targeting the key mechanisms responsible for mineralization and fibrosis might lead to the development of novel pharmacological therapies. The identification of novel key molecular targets is definitely a priority to develop a treatment for AS.

REFERENCES

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ARTYKUŁ POGŁĄDOWY

Stenoza aortalna – nowe mechanizmy patofizjologiczne i powiązane z nimi implikacje terapeutyczne

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SŁOWA KLUCZOWE
biomarkery, miażdżyca, stenoza aortalna, zesztywnienie płatków aortalnych

STRESZCZENIE
Stenoza aortalna (aortic stenosis – AS) jest najczęstszym rodzajem nabytej wady zastawkowej serca. Częstość występowania tej wady rośnie z wiekiem pacjenta, w związku z czym u 3–9% dorosłych osób w wieku powyżej 75 lat rozwija się miażdżycowy typ AS. Mechanizmy patofizjologiczne oraz rola monitorowania biochemicznego w AS są przedmiotem intensywnych badań. W zjawisku progresji AS stwierdza się szereg zaburzeń w procesach regulacji zwapnienia, stanu zapalnego, deregulacji poziomu adipokon, aktywacji prozakrzepowej i dysfunkcji czynnika von Willebranda. Poznanie mechanizmów tych procesów wiąże się z przebadaniem kompleksowej roli rozlicznych komórek, przede wszystkim miofibroblastów czy makrofagów. Wprowadzenie do leczenia metody przezskórnej implantacji zastawki aortalnej dostarczyło wartościową możliwość monitorowania zmian poziomu biomarkerów w obserwacji około zabiegowej oraz długoterminowej. W zaprezentowanej pracy omówiono nie tylko procesy patofizjologiczne, ale również próby farmakoterapii AS.