Biomarkers Associated with Aortic Stenosis and Structural Bioprosthesis Dysfunction

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KEYWORDS

• Lipid • Calcium • Hemostasis • Platelets • Aortic stenosis • Biomarkers

KEY POINTS

- Lipoprotein(a) is the most promising lipidic biomarker for identifying patients at risk of developing, or with faster progression of, aortic stenosis (AS).
- Angiotensin II and calcium-phosphorus product may be interesting to identify patients with faster progression of aortic valve remodeling/calcification.
- Platelet-related markers might allow assessment of AS patient hemostatic status both before and after valve replacement and aortic valve calcification.
- Recovery of high molecular weight von Willebrand factor multimers after transcatheter aortic valve replacement may predict patient outcome.
- Cardiovascular risk/comorbid profile (i.e. diabetes, metabolic syndrome, PCSK9) are the main factors contributing to- and serving has circulating biomarkers to identify structural valve degeneration.

The timing of follow-up and intervention in aortic stenosis (AS) is not yet well established and remains controversial. Indeed, the progression of AS is highly variable from one patient to the next. After replacement of the native valve, the prosthesis may also be dysfunctional. The first line of approach for the evaluation of prosthesis is echocardiography, which may be challenging. The use of blood biomarkers to identify patients at risk of AS or faster progression of AS, as well as a dysfunctional bioprosthesis, may have important value in routine clinical practice.

LIPIDIC INFILTRATION

In the early stage of AS, endothelium disruption linked to mechanical stress allows infiltration of lipids. Lipid particles promote inflammation and permeation of inflammatory cells into the valve, which secrete proinflammatory and profibrotic cytokines. Blood levels of low-density lipoprotein (LDL), and especially small and dense LDL and oxidized LDL, have been associated with the presence and faster progression of AS (**Table 1**).[1–3] Atherogenic lipoprotein particles such as LDL contain apolipoprotein B (apoB) (**Fig. 1**) while nonatherogenic lipoprotein particles (high-density lipoprotein) contain apolipoprotein A-I (apoA-I). Recently, the apoB/apoA-I ratio has been found to be independently associated with faster hemodynamic progression of AS in younger patients (<70 years old) (see **Fig. 1**).[4] Accordingly, a strong association was found between increased apoB/apoA-I ratio and the risk

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Table 1
Circulating biomarkers and mechanisms implicated in native aortic stenosis and structural valve degeneration of aortic bioprostheses

Mechanisms implicated	Biomarkers	Native Aortic Stenosis	Bioprosthetic aortic valve SVD
Dysregulation of mineral metabolism	↑Calcium-phosphorus product	✓	✓
	↓ Creatinine clearance	✓	✓
	↑ Total desphosphorylated MGP	✓ (≤57 years)	
	↓ Fetuin-A	✓	
	↑Osteopontin	✓	
	↑Osteoprotegerin	✓	
Lipid-mediated inflammation and metabolism processes	↑ HOMA index	✓	✓
	↑ Total cholesterol		✓
	↑ Triglycerides	✓	✓
	↑ ApoB/ApoA-I ratio	✓	✓
	↑ PCSK9	✓	✓
	↑ Lp-PLA2	✓	✓
	↑ Autotaxin	✓	
	↑ small and dense LDLs	✓	
	↑ oxidized LDLs	✓	
	↑ Lp(a)	✓	
Inflammation and macrophage activation	↑ soluble CD14	✓	✓
Tissue remodeling and inflammation	↑ Angiotensin II	✓	
	↑ Angiotensin converting enzyme	✓	
Hemostasis imbalance	↑ Thrombin-antithrombin complexes	✓	
	↑ Prothrombin factor 1+2 (F1+2)	✓	
	↑ Soluble CD40 ligand	✓	
	↑ β-thromboglobulin	✓	
	↑ Plasminogen activator inhibitor-1	✓	
	↑ P-selectin and	✓	
	↑ Activated $\alpha_{IIb}\beta_3$ integrin	✓	
	↑ Serotonin	✓	

Abbreviations: HOMA, homeostatic model assessment; LDLs, low density lipoproteins; Lp(a), lipoprotein (a); Lp-PLA2, lipoprotein-associated phospholipase A2; MGP, Matrix g-carboxyglutamate protein; PCSK9, proprotein convertase subtilisin/kexin 9; SVD, structural valve degeneration.

of developing structural aortic bioprosthetic valve deterioration.[5]

Lipoprotein(a) (Lp(a)) is an LDL-like particle that contains an apo(a) and transports oxidized phospholipids. Lp(a) has been associated with both the presence of AS and faster progression of AS (see **Fig. 1**).[6,7] The blood level of Lp(a) is almost only determined genetically, and one single-nucleotide polymorphism on the Lp(a) locus, associated with elevated level of Lp(a), has been found to be associated with aortic valve calcification (odds ratio per allele, 2.05; $P = 9.0 \times 10^{-10}$).[6] Thus, among lipidic biomarkers associated with AS, Lp(a) is probably the most promising. Around 20% of the population has an increased Lp(a); however, specific thresholds identifying patients at risk of AS or faster progression of AS are as yet not established (75 versus 125 nmol/L).

Going along the Lp(a)/oxidized LDL pathway, the lipoprotein-associated phospholipase A2 (Lp-PLA2) transforms oxidized phospholipids in free fatty acids and lysophosphatidylcholine, which is transformed by autotaxin in lysophosphatidic acid, a phospholipid that promotes inflammation, fibrosis, and calcification. Activity of both Lp-PLA2 and autotaxin has been associated with the presence of AS[8]; however, only Lp-PLA2 activity has been associated with faster progression of AS (see **Table 1**).[9]

Despite inflammation playing an important role in AS initiation and progression, no robust biomarkers linked to inflammation have yet been proposed.

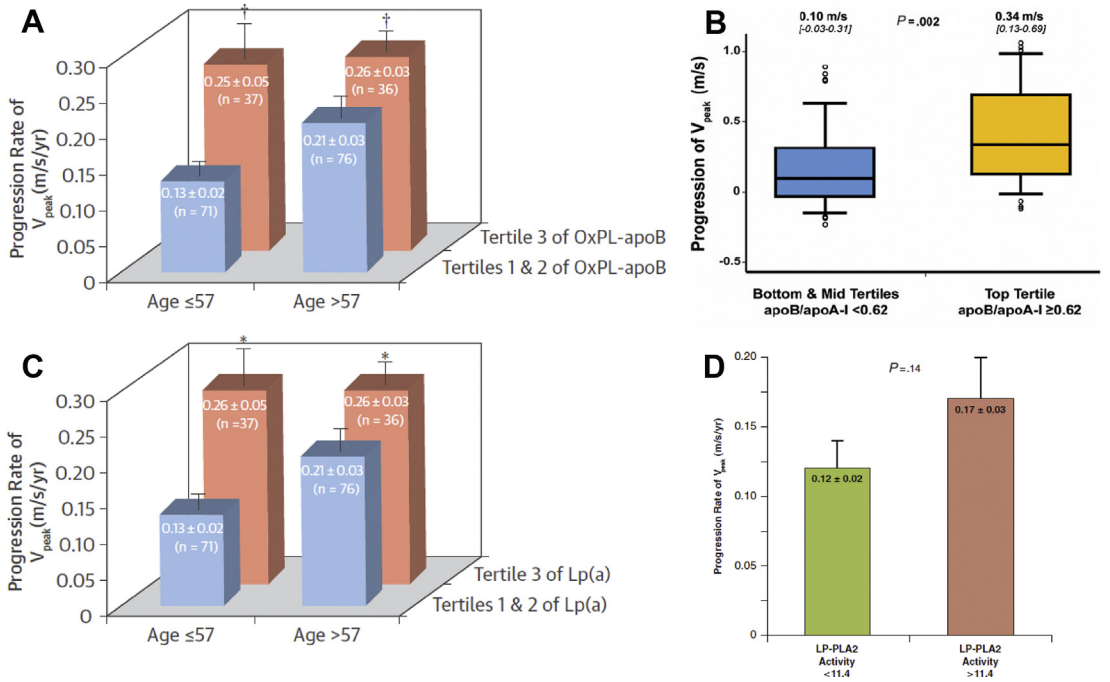

Fig. 1. Annualized progression of aortic valve stenosis according to blood level of lipidic biomarkers. (*A*) Comparison of progression of peak aortic jet velocity according to age and top tertile of oxidized phospholipids on apolipoprotein B-100. † $P < 0.05$ tertile 3 (>5.5 nM) compared with tertiles 1 and 2 (≤5.5 nM) of OxPL-apoB–age ≤57 group. Error bars = SEM. (*B*) Comparison of progression of peak aortic jet velocity in patients younger than 70 years (n = 80) according to top tertile of apoB/apoA-I ratio. (*C*) Comparison of progression of peak aortic jet velocity according to age and top tertile of Lp(a). * $P < 0.05$ tertile 3 (>58.5 mg/dl) compared with tertiles 1 and 2 (≤58.5 mg/dl) of Lp(a)–age ≤57 group. (*D*) Comparison of progression of peak aortic jet velocity according to the level of lipoprotein-associated phospholipase A2. (*From* R. Capoulade, K. L. Chan, C. Yeang, et al., Oxidized Phospholipids, Lioprotein(a), and Progression of Calcific Aortic Valve Stenosis. Journal of the American College of Cardiology 2015; 66:11; with permission.)

EXTRACELLULAR MATRIX REMODELING AND FIBROSIS OF AORTIC VALVE

Remodeling of the extracellular matrix of the aortic valve is mediated through matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). Indeed, MMPs are endopeptidases that are the most responsible for collagen and other protein degradation of the extracellular matrix. An imbalance between MMP and TIMP activity leads to a pathologic remodeling of the extracellular matrix in the aortic valve.[10,11]

Several MMPs and TIMPs have been identified within the aortic valve, such as MMP2, MMP3, MMP9, and TIMP1. However, correlation between the blood level of these molecules and faster progression of AS is yet to be proved.

In human calcified aortic valves, angiotensin-converting enzyme and chymases are expressed and colocalize with angiotensin II (see **Table 1**).[12,13] The production of angiotensin II within the aortic valve promotes fibrosis and remodeling of valvular tissues owing to the increase in transforming growth factor β, MMP2, and collagen secretion by valvular interstitial cells.[14]

In addition, angiotensin II receptor type 1, which is known to activate vasoconstriction, cell proliferation, inflammation, fibrosis, and thrombosis, is expressed by aortic valve fibroblasts in lesion areas.[12,13] In patients operated on for severe symptomatic AS, the circulating levels of angiotensin II were associated with inflammation and tissue remodeling of the aortic valve.[15] Moreover, higher circulating levels of angiotensin-converting enzyme and angiotensin II have been associated with faster development or progression of AS.[13,16–19]

AORTIC VALVE CALCIFICATION

Aortic valve calcification occurs mostly by accumulation and organization of calcium hydroxyapatite microcrystals in the collagen layer. Serum phosphorus has been proposed as a key regulator of aortic valve calcification. Indeed, the calcium-phosphorus product has been associated with

the presence of AS and is correlated with AS severity in patients with and without renal disease (see **Table 1**).[20,21] Moreover, higher calcium-phosphorus product has also been correlated with aortic bioprosthesis calcification.[22] Furthermore, calcium supplements, which are extensively prescribed in older adult patients, are independently associated with higher calcium-phosphorus product.

Matrix γ-carboxyglutamate protein (MGP) is well known to be an inhibitor of cardiovascular calcification. To inhibit ectopic calcification, MGP requires carboxylation and phosphorylation.[23] In the ASTRONOMER (Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin) trial, increased circulating levels of total desphosphorylated MGP were associated with a faster progression rate of AS in younger individuals (≤57 years old), whereas older patients experienced a rapid stenosis progression rate of AS, regardless of the total desphosphorylated MGP levels (**Fig. 2**).[24]

Paradoxically, higher levels of total desphosphorylated MGP were observed in the patients with faster AS progression, probably linked to a feedback mechanism increasing the production of uncarboxylated desphosphorylated MGP in response to the ongoing ectopic calcification processes.

Many calcium-binding proteins have been found in stenosed aortic valves; however, the circulating level of these proteins has not always been linked to the presence and/or faster progression rate of AS. Fetuin-A is known to protect against cardiovascular calcification. In AS, a recent meta-analysis confirmed that plasma fetuin-A levels are lower in patients with AS than in those without AS.[25] However, fetuin-A was not associated with slower progression of AS.[26] Accordingly, levels of plasma osteopontin or osteoprotegerin are associated with the presence of aortic valve calcification and/or stenosis,[27,28] although association with AS progression was never demonstrated (see **Table 1**).

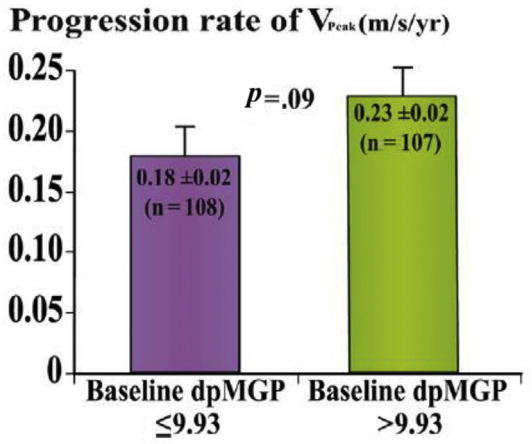

Fig. 2. Annualized progression rate of peak aortic jet velocity according to baseline desphosphorylated matrix γ-carboxyglutamate protein level. (*From* R. Capoulade, N. Cote, P. Mathieu, et al., Circulating Levels of Matrix Gla Protein and Progression of Aortic Stenosis: A Substudy of the Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) Trial. Canadian Journal of Cardiology 2014; 1088-1095; with permission.)

A ROLE FOR PLATELETS IN AORTIC STENOSIS PATHOPHYSIOLOGY?

Platelet interaction with vascular endothelial cells is the initial step of hemostasis, which leads to repair of vascular breaches and limits blood loss.[29] Injured endothelial cells express procoagulant and platelet-activating molecules, and components of the subendothelial matrix, mainly collagen, are exposed to flowing blood, which initiates thrombus formation. Under high shear stress, the platelet collagen receptors (glycoprotein VI [GPVI], $\alpha_2\beta_1$) are unable to support platelet adhesion; the initial platelet adhesion to endothelia requires the interaction between immobilized von Willebrand factor (vWF) on the surface of the endothelium or in the subendothelial matrix with its platelet receptor, the GPIb-IX-V complex. When shear force increases, vWF multimers unfold, resulting in the binding of the vWF A1 domain to platelet GPIbα. Two mechanosensitive domains in GPIbα unfold by vWF-mediated pulling force, and the anchoring of GPIbα to actin filaments via filamin-A allows resisting shear force during platelet adhesion.[30] This interaction mediates platelet tethering and translocation on the endothelium. Subsequent $\alpha_{IIb}\beta_3$ integrin inside-out activation and release of platelet granule content leads to platelet arrest and irreversible aggregation.[31] On granule release, the ATP P2X1 receptor and the 2 ADP receptors, P2Y1 and P2Y12, play central roles in the amplification of shear-dependent platelet aggregation.[32] Concomitant activation of the coagulation cascade leads to thrombin generation, which further activates and recruits platelets to the formation of thrombi and produces fibrin that consolidates the clot.

Under normal conditions, endothelial cells produce antithrombotic substances such as nitric oxide (NO) and prostaglandin I_2 (PGI_2) that inhibit platelet adhesion, activation, and aggregation, and thrombomodulin, which inactivates thrombin. In addition, the ectonucleotidase

CD39 degrades ATP and ADP, 2 main platelet agonists, thereby preventing platelet activation. It is conceivable that such mechanisms also contribute to inhibiting platelet adhesion and activation on aortic valve endothelia. However, the specificities of platelet interactions with aortic valve endothelial cells remain totally unknown. In AS, few available data concur with the new concept that platelets would contribute to the disease through mechanisms that differ from those involved in hemostasis.[33] According to a recent study, platelets may be involved in AS progression by promoting valvular calcification. Activated platelets would participate in valve interstitial cell mineralization through lysophosphatidic acid production and autotaxin activity.[34] In addition, the contribution of platelets to inflammation, including their ability to interact with immune cells, could represent another mechanism underlying the AS-associated osteogenic process.[35] On activation, platelets release soluble mediators from their granules, which further promotes platelet recruitment and activation but also mediates immune and inflammatory responses. Platelet α-granules contain hemostatic factors (ie, coagulation factor V, vWF, fibrinogen), growth factors (ie, platelet-derived growth factor, transforming growth factor β), cytokines and chemokines (ie, interleukin-1β, platelet-activating factor, platelet factor 4, CCL5), and metalloproteinases (ie, MMP9, TIMP1).[36] Dense granules comprise small molecules such as serotonin, calcium, ADP, and ATP. It is therefore possible that on platelet activation, released platelet granule content contributes to valvular extracellular matrix remodeling and fibrosis. Furthermore, inflammation itself might also dictate platelet contribution to AS pathophysiology. Indeed, in addition to platelet contribution to disease pathophysiology, diseases can modify platelets.[37] On the one hand, the disease environment can induce changes in megakaryocytes that produce platelets with modified RNA or protein content. On the other hand, platelet content can also become modified in circulation. Platelets are able to take up proteins and small molecules from blood and release them locally at sites of activation. For instance, platelets accumulate circulating acute-phase proteins, such as C-reactive protein and serum amyloid A, which are produced by the liver during inflammation.[38] Dyslipidemia or hyperglycemia also modifies platelet phospholipid content, which may result in platelet hyperreactivity and subsequent enhanced thrombosis.[39] However, to date, how and when platelets intervene in AS pathophysiology has remained unclear.

HEMOSTASIS IMBALANCE IN AORTIC STENOSIS

In terms of hemostasis, AS patients display both a mild bleeding and a high thrombotic risk.[40] This dual clinical picture is inherent to the disease condition. Indeed, high shear stress through stenosed aortic valve induces vWF unfolding and subsequent GPIbα-mediated platelet activation and release of platelet granule content.[41] Cleavage of unfolded vWF by ADAMTS-13 leads to secondary loss of high molecular weight vWF multimers (HMWM), which results in acquired vWF disease (VWD) and Heyde syndrome (ie, lower gastrointestinal bleeding from angiodysplasia). Indeed, cleaved vWF shows less affinity for platelets and collagen than HMWM, which makes their hemostatic activity much less efficient. Because platelets are very sensitive to and are activated by shear stress, it is likely that, depending on stenosis severity and disease stage, AS differentially alters platelet phenotype, which, in addition to VWD, may contribute to the risk of bleeding or thrombosis. Shear causes shedding of GPIbα and GPVI, resulting in secondary platelet hyporeactivity and potential bleeding.[42] Interestingly it has been shown that in vitro, under high shear stress conditions, GPVI shedding occurs independently of vWF/GPIbα engagement, and does not require $\alpha_{IIb}\beta_3$ integrin activation or platelet aggregation.[43] GPVI shedding, triggered by brief and transient shear exposure, results in progressive accumulation of circulating soluble GPVI. Thus, these platelet responses to shear might all represent novel markers of AS severity and/or prognosis. Concomitant with the VWD-associated bleeding risk, AS is characterized by increased activation of coagulation with concurrent hypofibrinolysis, which may be responsible for fibrin deposition on the aortic valve. Markers of coagulation, thrombin-antithrombin complexes (TAT) and prothrombin factor 1+2 (F1+2), as well as soluble markers of platelet activation, soluble CD40 ligand and β-thromboglobulin, were found to be elevated in patients with lower percentages of HMWM and more severe stenosis (see **Table 1**).[44] Platelets may thus be activated by thrombin generated as a result of coagulation activation. Markers of impaired systemic fibrinolysis, such as level of plasminogen activator inhibitor-1, the most important regulator of plasminogen activation and plasmin generation, are also elevated.[45] It has also been shown that platelet activation, assessed by measuring surface expression of P-selectin and activated $\alpha_{IIb}\beta_3$ integrin, increased in parallel with plasma serotonin elevation in patients with severe AS.[41] Overall, these data indicate hemostasis

imbalance in AS, leading to both mild bleeding tendency and a high thrombotic risk.[40] However, a clear understanding of platelet contribution to AS and associated bleeding or thrombosis will necessitate detailed investigation of platelet phenotype during disease initiation and progression. Importantly, such studies might reveal new therapeutic avenues of AS and help in defining more tailored antithrombotic management of these patients. The advanced age and comorbidities of most AS patients makes their antithrombotic management highly challenging. Hence, thorough characterization of circulating platelets would seem essential for accurate assessment of patients' hemostatic status.

CIRCULATING BIOMARKERS OF PROSTHESIS VALVE DYSFUNCTION

Bioprosthesis are prone to structural valve deterioration (SVD). Despite major improvements in valve design and surgical procedures, SVD is still a major limiting factor to the durability of bioprostheses. Biomarkers may help to the identification of causal factors for SVD and clinical decision making. Higher calcium-phosphorus product is a strong predictor of bioprosthesis calcification and patients with less than 30 mL/min of preoperative creatinine clearance are at higher risk of SVD compared to patients with a clearance greater than 60 ml/min.[22,46]

Lipid-related biomarkers are other plasma biomarkers implicated in SVD. Patients with total cholesterol of at least 200mg/dL or triglycerides levels higher than 150mg/dL are at greater risk for re-operation for structural valve failure.[47] A study reported that in patients younger than 57 years, total cholesterol level higher than 240mg/dL or triglycerides level higher than 123 mg/dL are predictors of re-intervention for valve failure.[48] Higher levels of Apo-B, ApoB/ApoA-I ratio, Homeostatic model assessment (HOMA) are also associated with increased risk of SVD. High plasma level of the proprotein convertase subtilisin/kenin 9 (PCSK9), a positive regulator of LDL cholesterols, and/or associated with high level of oxidized-LDLs are associated with higher risk of SVD.[49,50] Lipoprotein-associated phospholipase A2 (Lp-PLA2) which enzymatically produce free fatty acids from oxidized-LDLs and promoting inflammation is expressed in explanted bioprostheses for SVD50 in colocation with macrophages (CD68), and oxidized-LDLDs. Plasma Lp-PLA2 activity have also been associated with the occurrence of SVD (see **Table 1**).[49] Lipid insudation found in explanted bioprosthesis for SVD exposed lipid-laden macrophages featuring foam cells that can precipitate SVD in the long-term, even in the absence of mineralization.[51] CD14, a membrane glycoprotein present at the surface of monocytes and macrophages, can be secreted by these cells or the liver, and the circulating soluble form of CD14 has been related to SVD (see **Table 1**).[52]

CIRCULATING BIOMARKER TO IDENTIFY PARAVALVULAR LEAK

Previous studies have shown that loss of HMWM of vWF is observed in patients with AS or regurgitation and is corrected after AVR.40 HMWM defect is predictive of the presence of postprocedural paravalvular regurgitation after TAVI and is associated with increased 1-year mortality.[53] Point-of-care measurement of vWF-dependent platelet function using PFA-200 (Siemens) not only predicted PVAR but also MLTB at 30 days post TAVI.[54] These data are still to be confirmed in larger patient cohorts from independent centers. It is also worth noting that PFA-200 data do not accurately reflect overall platelet reactivity and, because data are influenced by medication, low platelet count, hematocrit, and levels of vWF antigen, the specificity of this technology is a major limitation.

SUMMARY

Lipids, including Lp(a) and the apoB/apoA-I ratio, angiotensin II, and calcium-phosphorus product, may represent valuable markers of AS disease progression and/or bioprosthesis deterioration. However, the implementation of these biomarkers in clinical practice will require further validation in large multicenter patient cohorts. In addition, more basic and translational research is definitely required to clarify AS disease mechanisms to uncover multibiomarker-based diagnostic and prognostic tools that might be useful during the natural progression of AS and after aortic valve replacement. Markers of hemostasis and further demonstration of a role for platelets in aortic valve calcification represent other promising avenues that might not only help the assessment of AS progression but also the management of antithrombotic therapy while preserving hemostasis.

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