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# Insights into the Use of Biomarkers in Calcific Aortic Valve Disease

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## Abstract

Calcific aortic valve disease (CAVD) is the most common acquired valvular disorder in developed countries. CAVD ranges from mild thickening of the valve, known as aortic valve sclerosis (AVSc), to severe impairment of the valve motion, which is termed aortic valve stenosis (AVS). The prevalence of CAVD is nearing epidemic status: its preceding stage, in which there is aortic sclerosis without obstruction of the left ventricular outflow, is present in nearly 30% of adults over 65 years of age. Since there is no existing medical therapy to treat or slow the progression of CAVD, surgery for advanced disease represents the only available treatment. Aortic valve replacement is the second most frequently performed cardiac surgical procedure after coronary artery bypass grafting. Therefore, CAVD represents a major societal and economic burden.

The pathophysiological development of CAVD is incompletely defined. At the present time, the major methods to diagnose CAVD are clinical examination, echocardiography and cardiac catheterization. Due to the multiple biological pathways leading to CAVD, there are many potential biomarkers that might be suitable for deriving clinically useful information about the presence, severity, progression and prognosis of CAVD. Although the data available does not permit recommendations for clinicians at this time, they do support a paradigm of screening patients based on multiple biomarkers to provide the information necessary to optimize future therapeutic interventions.

This review summarizes the results of several studies investigating the value of potential biomarkers that have been used to predict the severity, progression and prognosis of CAVD.

### Keywords

Degenerative Aortic Valve Disease; Aortic Valve Stenosis; Aortic Valve Sclerosis; Biomarkers

## INTRODUCTION

Calcific aortic valve disease (CAVD) is a slow but progressive pathological condition of the aortic valve characterized by dystrophic calcification of the valve leaflets [1,2]. Initial phases of the disease include mild thickening of the valve, known as aortic valve sclerosis (AVSc); whereas more advanced stages comprise serious impairment of leaflet motion with subsequent

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limitation of blood flow through the valve [1] [Figure 1]. These two medical conditions are known as aortic valve sclerosis (AVSc) and aortic valve stenosis (AVS), respectively.

With an estimated prevalence of 5.2 million affected people in the United States, CAVD represents the most common type of valvular disease [3]. The prevalence increases with age, so that approximately 30% of all individuals aged 65+ years have AVSc and 4% have AVS [4]. CAVD is the number one cause in the USA of 95,000 surgical valve replacements performed annually, with studies showing a steady increase in the number of operations over the past few decades [1,5,6]. As a result of a steady population growth and rising life expectancy, one can expect that the demand for aortic valve operations will significantly increase in the future [Figure 2].

The current treatment of choice for AVS is surgical valve replacement [4], either with mechanical or biological prostheses. Other treatment options are aortic valvuloplasty or percutaneous valve replacement. Balloon aortic valvuloplasty is a well-established, and well-studied procedure with nontrivial complication rates, very high rates of recurrent stenosis and moderately high rates of aortic insufficiency [7]. Percutaneous aortic valve replacement is an evolving therapy that is being actively examined in the PARTNER trial [8]. This technique has the potential to have a favorable risk-benefit ratio compared with surgical AVR, but may not be suitable for all patients [9,10]. Long term performance of these prostheses remain unknown at the present time.

Since there is no medical therapy available for aortic valve stenosis, surgery represents the only definitive therapy. Several studies have tried to medically alter the progression of AS using statins or RAAS antagonists [11] with uniformly unimpressive results. Also, it is important to note that despite surgical treatment, the underlying mechanism of the valvular degeneration is left untreated.

In addition, the current understanding of the pathophysiological mechanisms underlying CAVD is still not fully elucidated [1,2,12]. For a long time, the essential pathophysiological mechanism of CAVD was believed to be a simple degenerative process with passive accumulation of calcium in the cusps. However, recent data suggests that CAVD is an active cellular process that develops within the valve leaflet. Mechanical stress on the aortic valve in addition to atherosclerotic risk factors, leads to valvular endothelial dysfunction/leakage, followed by deposition of lipids and other compounds. This triggers inflammation, which in turn activates valvular myofibroblasts resulting in their osteoblastic transdifferentiation. These events provide the basis for further changes involving extracellular matrix remodeling and neo-vascularization, ultimately leading to active calcification [1,2] [Figure 3]. These calcific changes primarily occur at the aortic side of the valve leaflets, which is the region with the highest turbulence suggesting that mechanical stress might be one of the causes triggering calcification [1,2].

Although this process shares many characteristics with the development of atherosclerosis, these two diseases are not synonymous [13]. Despite the fact that in both conditions there is evidence of plaque formation and vascular calcification, the behavior is very different being atherosclerotic plaque instability the trademark of atherosclerosis and plaque size and build up the one of CAVD. For more detailed information on the pathogenesis of CAVD, the interested reader is referred to some recent and comprehensive reviews on this topic [1,2,12]. This article reviews the results of all published series on the different biomarkers that have been used to predict the severity, progression and prognosis of CAVD.

## REVIEW OF THE LITERATURE ON CURRENTLY USED BIOMARKERS

In the following section, we focus on all the published biomarkers currently researched for the screening of CAVD and their putative clinical value. When a large number of studies have been published on a particular biomarker, we have divided this data into three parts as whether it pertains to the severity, progression or prognosis of CAVD. Table 1 gives an overview of the studies discussed in this section. To enhance the significance of each of these different biomarkers and to make this presentation more comprehensible, we have decided to group the different biomarkers into the different stages of evolution of CAVD understanding that such arbitrary classification does not necessarily represent the physiologic continuum of this disease.

### MARKERS OF ENDOTHELIAL DYSFUNCTION/LEAKAGE

**Asymmetric dimethylarginine—**In 1992, Asymmetric dimethylarginine (ADMA) was identified as a physiological inhibitor of tissue nitric oxide (NO) synthase, and since then it has emerged as a marker, mediator and regulator of endothelial cell dysfunction [14]. ADMA has been linked to the development of several pathological conditions, including homocysteinemia, diabetes mellitus, chronic kidney disease and cardiovascular diseases.

Ngo and co-workers investigated the role of ADMA in CAVD [15]. This study compared the plasma ADMA levels of 42 patients with moderate to severe AVS to an equivalent number of age-matched control subjects. Univariate statistical analysis revealed neither a significant difference in ADMA levels between both groups nor a correlation of ADMA levels and the degree of AVS. Backward stepwise multiple linear regression showed that the diagnosis of AVS was independently linked to increased plasma ADMA levels.

Although this study showed an association between AVS and ADMA, the use of this protein as a putative biomarker and predictor of CAVD has several limitations. As Ngo and colleagues only investigated the role of ADMA in patients with end-stage AVS, it would be of interest to elucidate the plasma ADMA levels in a cohort with AVSc or mild AVS. In addition, future studies could also address if ADMA levels correlate with the progression of CAVD.

In addition, Ngo et al, recently reported that Aortic sclerosis inversely correlate with platelet NO. On multiple regression, AVSc was associated with impaired platelet NO responsiveness, a finding that provide a potential mechanism for the propensity for acute coronary syndromes in this condition [16].

**Homocysteine—**Similar to ADMA, homocysteine is also associated with endothelial dysfunction [17]. Although there is no strong correlation between plasma ADMA and homocysteine levels, both proteins are considered to be cardiovascular risk factors, especially for coronary artery disease (CAD). Novaro et al. [18] retrospectively analyzed the data of 17 patients with AVS, 32 subjects with AVSc and 27 control individuals. Bivariate statistical analysis showed a significant correlation between median plasma homocysteine levels and the severity of CAVD. The median homocysteine concentration was also significantly elevated in CAVD patients compared to healthy individuals. However, after multiple logistic regressions modeling analysis of the data, homocysteine was found not to be a predictor of AVS.

Another study analyzed the data of 58 patients with AVS +/− CAD and 47 control individuals [19]. Although subjects with AVS showed higher mean homocysteine levels than the control group, this difference was statistically not significant. Further analysis of the test group revealed that mean homocysteine levels are increased in patients with CAD compared to subjects who suffer from AVS only, but again the data lacked statistical power. Both studies show a correlation of blood homocysteine levels and CAVD, however, this relationship was

lost following multivariate analysis. Given the poor statistical power, further work is needed to figure out whether homocysteine can serve as a biomarker for CAVD.

**Tissue plasminogen activator—**The enzyme tissue plasminogen activator (tPA) is secreted by endothelial cells and catalyzes the conversion of plasminogen to plasmin, resulting in fibrinolysis [20,21]. Besides this physiological function, recombinant tPA is also commonly used as a therapeutic agent to treat myocardial infarction, ischemic stroke, deep venous thrombosis and pulmonary embolism. Glader and colleagues measured the plasma tPA levels of 101 patients suffering from severe AVS and 101 matched controls [22]. The authors reported that increased levels of tPA are present in patients with AVS. The results were statistically significant.

High levels of blood tPA are associated with the presence of AVS, thus representing a potential biomarker for CAVD. Further studies are needed to investigate whether tPA also correlates with early stages of CAVD and disease progression.

## MARKERS OF LIPID DEPOSITION AND OTHER COMPOUNDS

**Lipids—**For a long time, hypercholesterolemia has been a well-known risk factor for atherosclerosis and the development of CAD [23]. More recently, hypercholesterolemia has also been associated with the pathogenesis of CAVD. To this date, no studies have demonstrated a significant benefit on the use of lipid-lowering agents in patients with CAVD in terms of disease progression.

**Severity:** Wilmshurst et al. conducted a case-control study, in which total cholesterol levels of 20 patients with severe AVS and 20 controls were measured [24]. Although this trial comprised only a small number of patients, the results showed a statistically significant correlation between the presence of AVS and elevated concentrations of cholesterol. Supporting data has been published by other groups [25–27].

In contrast to these results, other studies have not shown a correlation between hyperlipidemia and CAVD [28–31]. Mohler et al. [29] retrospectively analyzed the data of 120 patients undergoing aortic valve replacement for AVS. The results showed that only gender and smoking, but not cholesterol levels, were statistically significant risk factors for degenerative AVS. Further, Glader et al. also showed an association of lipoprotein (a) and the presence of severe AVS [22]. Côte and colleagues reported that oxidized LDL was associated with worse fibrocalcific remodeling of valve tissue of patients suffering from AVS [32].

**Progression:** Messika-Zeitoun and co-workers prospectively studied the acquisition and progression of early aortic valve calcification in 262 volunteers via electron-beam-computed tomography [33]. In multivariate analysis, LDL-cholesterol independently determined the acquisition of aortic valve calcification, whereas higher baseline scores for aortic valve calcification determined faster progression of already existing aortic valve calcification. Corroborating results have been obtained by other groups [34–36].

Contrary data has been reported by other researchers [37]. Bellamy et al. conducted a trial in which 156 patients with AVS underwent Doppler echocardiography and measurement of cholesterol levels at baseline as well as after a mean period of 3.7 years. Data analysis did not reveal a correlation between blood cholesterol concentrations and the progression of AVS. Supporting results have been obtained by three other major studies, SEAS, SALTIRE and ASTRONOMER. Besides the observation that statins do not slow down the progression of AVS, these prospective trials did not detect a relationship between LDL levels and the progression of AVS either [38–40].

**Prognosis:** Rosenhek and coworkers investigated predictors in the outcomes of asymptomatic but severe AVS [41]. Using multivariate analysis LDL was not found to be an independent predictor for the outcome AVS.

In summary, although there is a large number of studies available that focus on the role of lipids in CAVD, their actual value as markers for CAVD is unclear at the current time. Even if a correlation between lipoproteins and CAVD becomes manifest, the actual clinical use of these molecules would be limited by the fact that hypercholesterolemia is present in up to 85% of the United States population [42]. This high prevalence makes cholesterol level an uncertain screening tool for CAVD.

**Leptin—**Leptin is produced by adipocytes and represents a key hormone in energy homeostasis [43]. It has also been linked to cardiovascular diseases, such as myocardial infarction [44]. Glader et al. examined whether this hormone is also associated with CAVD [22]. This study comprised 101 patients undergoing surgery for AVS and an equivalent number of age- and sex-matched healthy individuals. Leptin was analyzed with a double antibody radio-immunoassay (RIA). Significantly higher plasma levels of leptin have been found in patients compared to control subjects. Leptin levels were significantly associated with valvular AS in both univariate and multivariate analysis.

Although this study revealed an association of increased leptin levels and the presence of AVS, the role of this protein in the pathogenetic process of CAVD remains unclear. Further, this study demonstrated only a correlation between leptin and the presence of severe AVS; whether leptin is also associated with mild/moderate AVS or the progression of CAVD remains elusive.

## MARKERS OF INFLAMMATION

**C-reactive protein—**C-reactive protein (CRP) is found in the human blood and belongs to the class of acute-phase proteins. [45] It is released by hepatic adipocytes in response to a great variety of inflammatory processes, such as infections, atherosclerosis or auto-immune diseases. Although it is a rather unspecific marker, it represents a very useful tool to monitor the activity of such inflammatory conditions.

**Severity:** Being the most commonly studied marker for atherosclerosis activity, researchers also investigated the role of CRP in CAVD. Galante et al. [46] showed that CRP levels were elevated in 68 patients with severe AVS in comparison to 92 control individuals. Although CRP was independently associated with AVS, no association has been observed for aortic valve area, degree of calcification and aortic jet velocity.

Whereas the relationship between CRP and AVS has been confirmed by other groups [47, 48], the situation seems to be more elusive for patients suffering from AVSc only. In a community-based study, Agmon et al. showed that there is only a weak association between CRP levels and asymptomatic AVSc [49]. In contrast, Jevanantham et al. [50] report that CRP levels are increased in patients with AVS and AVSc in comparison to healthy individuals.

**Progression:** Imai et al. [51] set out to investigate whether plasma CRP levels correlate with disease progression of AVS. In this study, 135 asymptomatic patients were diagnosed with mild, moderate or severe AVS on echocardiogram. A subgroup of 47 patients was observed over a period of 1 year and was divided into slow or fast progressors. The results showed that subjects with severe AVS had significantly higher baseline CRP levels than individuals with only mild or moderate AVS. CRP was identified as an independent marker of severe AVS. Also, fast progressors showed higher CRP levels than slow progressors. Other investigators have corroborated this data [51].

Opposing results have been published by Novaro et al. [52]. This large population-based trial investigated the progression of CAVD over five years. Interestingly, CRP was not associated with disease progression to AVSc or AVS.

**Prognosis:** Imai et al. [48] also investigated whether CRP can predict the long-term outcome of patients suffering from AVS. The mean follow-up time was 23 months. Indeed, patients with high CRP levels showed lower long-term survival rates than individuals with low CRP levels. The data available for CRP seems to be somewhat inconsistent. Whereas most authors agree that there is a correlation between CRP and the severity of AVS, the situation is less clear for AVSc. Furthermore, it is also ambiguous whether CRP can reflect the progression of CAVD.

Besides these points, the actual clinical use of CRP as a marker for CAVD is limited by the protein's unspecific mode of release. Since CRP is secreted during almost all inflammatory conditions making its use as a screening tool for CAVD difficult.

## MARKERS OF OSTEOBLASTIC TRANSDIFFERENTIATION

**Fetuin-A—**Fetuin-A is a glycoprotein that is produced by the liver and found in high concentrations in the blood serum [53]. *In vitro* studies have shown that fetuin-A is capable of inhibiting calcification. Wang et al. [54] have shown that serum fetuin-A levels are associated with valvular calcification in patients suffering from end-stage renal disease. Ix et al. [55] extended this information to patients without renal disease. A total of 970 individuals underwent echocardiographic assessment of mitral and aortic valve calcification as well as measurement of serum fetuin-A concentrations. Among these people, 79 subjects exhibited AVS. Data analysis showed an inverse association between serum fetuin-A levels and the prevalence of AVS in non-diabetic patients, but not in diabetic patients. Additionally, Koos et al. concluded that non-dialyzed patients with lower fetuine-A levels showed an increase in aortic valve calcification, and perhaps that this effect is not associated with renal function [56].

It remains unclear why fetuin-A is not associated with CAVD in diabetic patients. Also, future work is needed to investigate whether blood fetuin-A levels also correlate with the severity and progression of AVS.

**Calcium-phosphorus product—**The product of serum calcium and serum phosphorus is also known as the calcium-phosphorus product or CaxP. In nephrology, it is commonly used as a predictor of calcification [57]. It has been shown previously that CaxP is associated with AVS in patients with end-stage renal disease [58]. Mills et al. [59] set out to investigate whether CaxP is also related to the severity of AVS in patients with normal renal function. In a cross-sectional, retrospective study, 107 patients were evaluated for their serum CaxP levels and their degree of AVS. Indeed, CaxP was inversely related to the aortic valve area and positively related to both peak and mean transvalvular gradients. Although this study showed a correlation between CaxP and the severity of AVS, the reason for this association remains elusive. Since serum calcium and phosphorus levels can be influenced by a variety of other medical conditions, it is not clear whether CaxP can function as a specific marker or for CAVD. Furthermore, it is not clear if CaxP can reflect the progression of AVS.

**Osteopontin—**Osteopontin is a multifunctional glycophosphoprotein that is probably best known for its regulatory function in bone remodeling [60]. Osteopontin is known to play a crucial role in the differentiation and stimulation of osteoclasts. Besides its function in native bone tissue, osteopontin is also implicated in a variety of acute as well as chronic inflammatory

processes, including wound healing, fibrosis and atherosclerosis. Furthermore, osteopontin is involved in the biomineralization of dystrophic and ectopic sites, including aortic valve tissue.

Against this background, we carried out a study to explore whether high plasma osteopontin levels are associated with the presence of CAVD [61]. In this study, 23 patients with AVS and 7 control subjects underwent echocardiographic assessment of the aortic valve area and degree of calcification, followed by measurement of plasma osteopontin levels. Indeed, individuals with no or mild aortic valve calcification showed lower osteopontin levels in comparison to patients suffering from moderate to severe aortic valve calcification. Furthermore, patients with aortic stenosis had higher osteopontin levels than healthy subjects. Thus, this study demonstrated, for the first time, a correlation between plasma osteopontin levels and the severity of CAVD.

Although the data was statistically significant, one limitation of this study is the small number of patients and the possibility of type one error. Also, we did not investigate whether osteopontin levels are capable of reflecting the progression of CAVD nor did we correlate osteopontin levels with other biomarkers. Finally, this study was limited to the quantitative assessment of plasma osteopontin levels. Since it is known that post-translational modifications of osteopontin can have a great impact on the protein's biological function [62], it would be of major interest to include this criterion in a future trial.

## MARKERS OF CLINICAL PROGRESSION

**Gamma-glutamyl transferase—**Commonly known as a liver enzyme, gamma-glutamyl transferase (GGT) is physiologically found at the cellular plasma membrane and counteracts oxidative stress by cleaving glutathione [63]. Being an enzyme commonly used in clinical practice as a marker for hepato-biliary dysfunction and alcohol abuse, GGT has also been linked to cardiovascular diseases [64].

Against this background, Bozabs et al. investigated whether GGT is also linked to CAVD [65]. In a retrospective study, the authors analyzed the serum GGT levels of 383 patients in regard to their echocardiographically determined degree of CAVD. 133 patients with mild valve thickening, 126 patients with AVS and 124 control individuals were matched for gender and age. Subjects positive for alcohol abuse or hepatic disease were excluded. Results showed a significant correlation between the blood GGT levels and the presence of AVS. Although the median GGT concentration was elevated in patients with AVSc, this increase failed to show statistical significance in comparison to the AVS group and the control group, respectively.

The usage of GGT as a predictor for CAVD is limited since there is only a significant correlation between serum GGT levels and severe AVS, but this correlation is lost in early stages of CAVD. In addition, it is not clear whether GGT can reflect the disease progression in CAVD. The putative clinical use of GGT as a biomarker for CAVD is furthermore complicated by the fact that blood GGT levels can also be compromised by hepatic disease or alcohol intake, thus restricting the utilization of GGT as a potential biomarker to an even smaller patient population. The molecular biological or pathophysiological cause for elevated concentrations of GGT in CAVD remains very unclear.

**Natriuretic peptides—**B-type natriuretic peptide (BNP) and its prohormone NT-proBNP are released from the myocardial wall in response to pressure and volume overload [66]. Whereas NT-proBNP represents the inactive cleavage product, BNP is the biologically active hormone mediating vasodilatation. Being well-established markers for congestive heart failure [67], both peptides have recently been linked to CAVD as well.

**Severity:** Several studies have shown a correlation between the plasma level of natriuretic peptides and the severity of AVS [68–72]. This is the case for both BNP and NT-proBNP. Although there is a correlation between transvalvular gradients and natriuretic NT-proBNP levels [65], more recent data suggests that the best correlation between natriuretic peptides levels and severity of AVS is observed when the aortic valve area is used as a categorical variate [73–75].

**Progression:** Natriuretic peptides are also capable of marking the progression of AVS [74, 76]. Gerber and colleagues observed that asymptomatic AVS patients with elevated baseline NT-proBNP levels develop more often symptoms during an 18-month follow-up period than subjects with normal NT-proBNP levels [76]. In addition, the annual increase of NT-proBNP in patients who became symptomatic was greater than the increase in asymptomatic subjects.

**Prognosis:** Weber et al. [77] observed a total of 159 patients over a median period of 902 days; whereas 102 patients underwent Aortic valve replacement (AVR) and 57 were treated conservatively. Baseline NT-proBNP levels were higher in individuals who died of cardiac causes or experienced cardiac complications compared to survivors. Further, multivariate analysis revealed that NT-proBNP was an independent predictor for negative outcome in conservatively-treated patients. Other studies reported similar results for both natriuretic peptides [70,78–80].

## CONCLUSIONS AND FUTURE DIRECTIONS

Early stages of CAVD usually remain asymptomatic for a long time. When patients finally complain about symptoms, the disease has already progressed to an advanced stage. After the onset of symptoms, 90% of patients with untreated severe AVS have a life expectancy of less than 10 years [81] and if the presenting symptom is heart failure the mortality is over 50% in the first year [82] Aortic valve replacement is an invasive and costly procedure and remains the treatment of choice for these patients.

The use of biomarkers and screening programs to assess the risk of future disease has helped improve outcomes across a wide spectrum of disease states. Patients at risk of developing CAD are currently evaluated with a combination of clinical and laboratory data to determine the need to initiate therapy; the same could be done for patients found to be at risk of developing degenerative aortic valve disease.

The general purposes of biomarkers include disease identification, grading disease severity, providing pathophysiological clues, prognostic information, and assessing the effects of different therapeutic interventions. Keeping these goals in mind we have selected a group of biomarkers that may help provide some of these answers as it pertains to CAVD [table 2]

The processes implicated in CAVD development and progression are often present outside the valve tissue and blood sampling will usually not be able to define the source of a particular biomarker (atheroma, valve, other inflamed site, wound healing and/or endothelium anywhere). Given this reality, we know that any putative biomarkers with sufficient sensitivity to detect degenerative aortic valvular lesions have the risk of low specificity due to abnormalities elsewhere in the body. This sensitivity-specificity tradeoff could be overcome by the study of multiple biomarker candidates in future series of patients with CAVD at different stages of the disease process. This should be the first step before medical therapeutic intervention is entertained for the treatment of CAVD. Clearly, CAVD is a complex multi-factorial process; the analysis of the different stages of aortic valve degeneration cannot be solely based on the quantification of a single biomarker per series.

Furthermore and as evidenced by the data presented, not all biomarkers seem to be good candidates. We think that asymmetric dimethylarginine, fetuin-A, CaxP, natriuretic peptides and osteopontin are the most promising candidates at the present time. They have demonstrated the best potential in the published literature up to date. We have selected asymmetric dimethylarginine as its level correlate with the degree of AVS and is involved in endothelial cell dysfunction [14], which represents an important part of process in the pathogenesis of CAVD. Fetuin-A is interesting due to its *in vitro* ability to inhibit calcification [53]. Natriuretic peptides seem to be very promising candidates, as there is great consistency in the literature for their ability to reflect the activity and progression, as well as to predict the prognosis of CAVD. Although CaxP has only been correlated with the severity of CAVD, it seems to be a good candidate as high levels of calcium and phosphorus have been linked to increased calcification [58]. Osteopontin is of special interest as a biomarker for CAVD, since it is the only molecule directly involved in the ectopic calcification phenomenon that occurs in the latter stages of CAVD [61].

Another fundamental part of any further studies will have to include grouping patients by their clinical stages (mild, moderate and severe AVS) and echocardiographic features (normal aortic valve, aortic valve sclerosis, aortic valve stenosis) and correlate the levels of the suggested biomarkers as they evolve in the disease process. It is expected that many of these patients have been or will receive different medical treatments for other conditions that are commonly associated with CAVD. A careful analysis will evaluate all these variables to set the stage for future therapeutic interventions. This is currently our line of research in collaboration as it pertains to the study of degenerative aortic valve disease.

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## Abbreviation and Acronyms List

| | |
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| ADMA | Asymmetric dimethylarginine |
| AVS | Aortic stenosis |
| AVSc | Aortic valve sclerosis |
| BNP | B-type natriuretic peptide |
| CAD | Coronary artery disease |
| CAVD | Calcific aortic valve disease |
| CRP | C-reactive protein |
| GGT | Gamma-glutamyl transferase |
| LDL | Low density lipoprotein |
| tPA | Tissue plasminogen activator |
| RAAS | Renin Angiotensin Aldosterone System |

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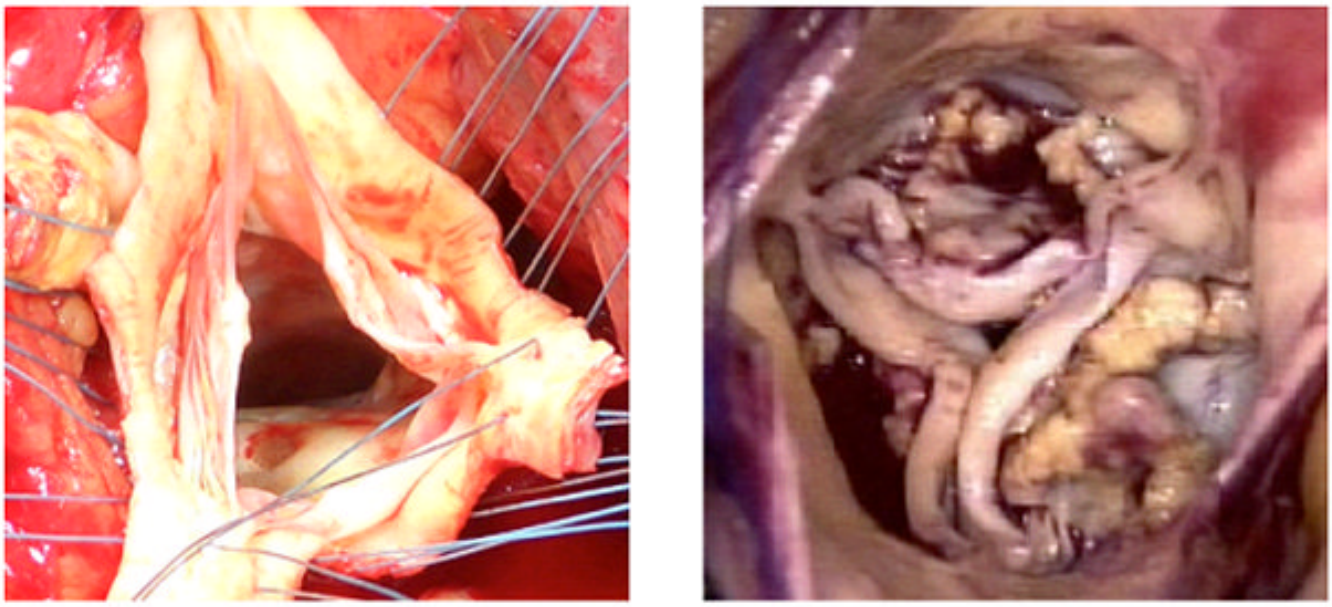

**Figure 1. Macroscopic Appearance of a Healthy and a Diseased Aortic Valve**

The left panel shows normal aortic valve leaflets with no calcification or sclerosis, the right panel displays a stenosed valve with leaflet and annular fibrosis and heavy leaflet calcification.

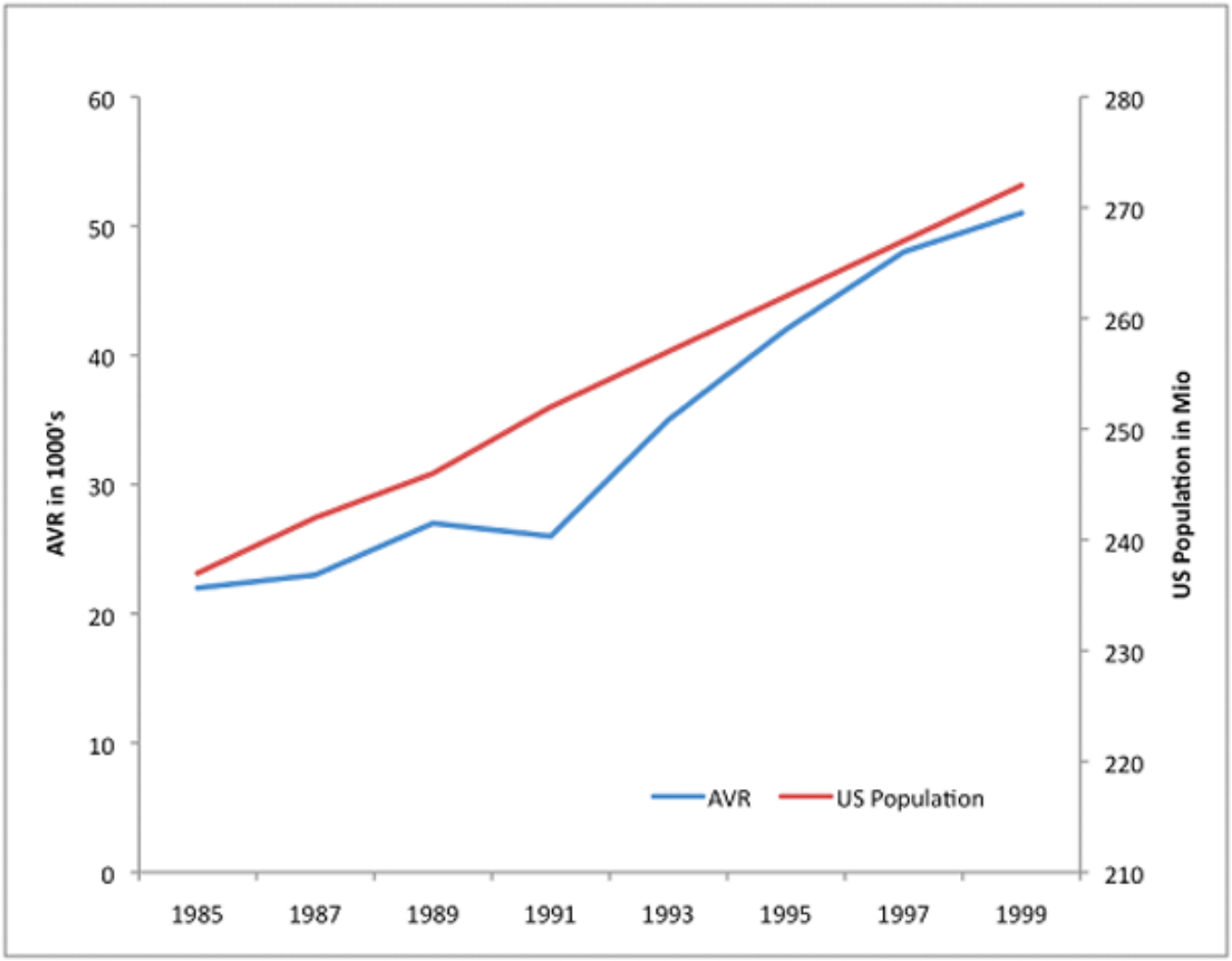


**Figure 2. Trends of Aortic Valve Replacements and US Population Growth**

Number of annually performed AVRs and population growth in the USA between 1985 and 1999. Data obtained from the National Center for Health Statistics (ICD-9-CM) and the US Census Bureau. AVR = Aortic valve replacement

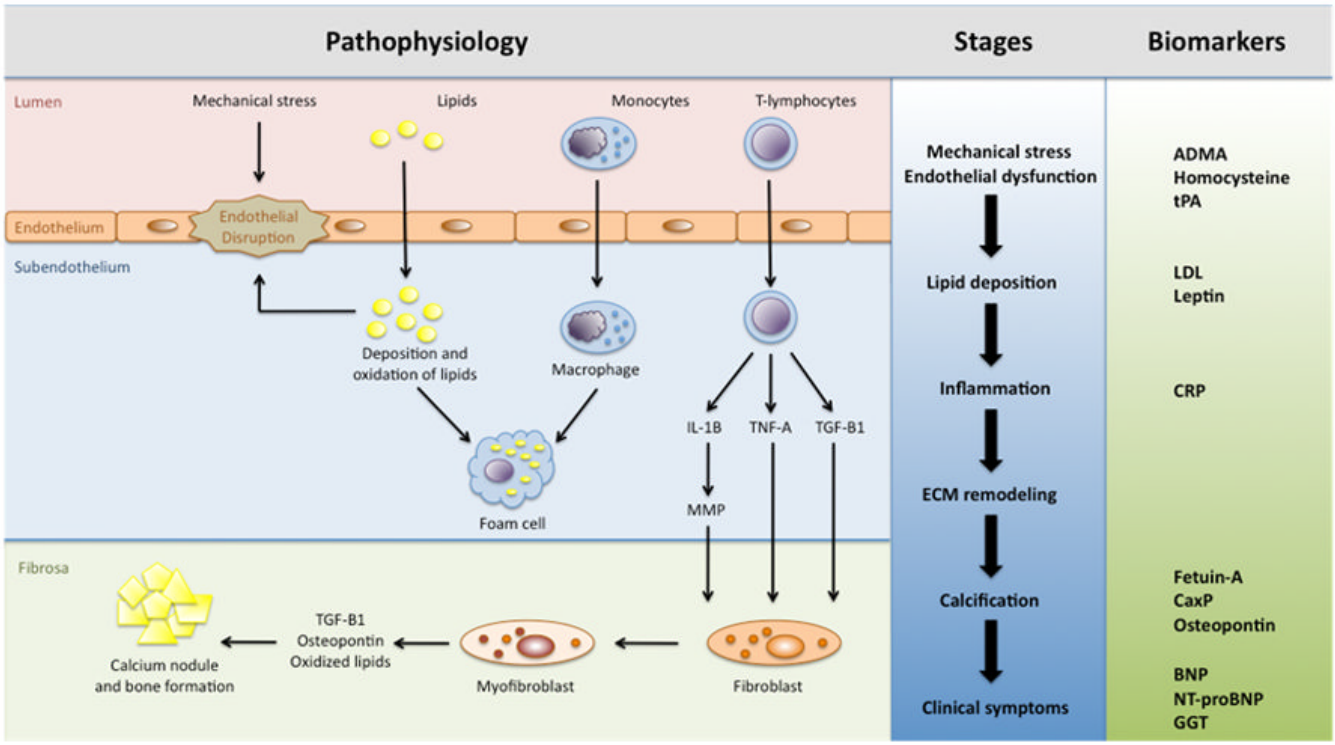


**Figure 3. Pathogenesis of Calcific Aortic Valve Disease**

Mechanical stress on the aortic valve in addition to atherosclerotic risk factors, leads to valvular endothelial dysfunction/leakage, followed by deposition of lipids and other compounds in the subendothelium where they are oxidized. Blood monocytes infiltrate the valve tissue and phagocytize the modified lipids, thus becoming foam cells. T-lymphocytes secrete cytokines, which promote inflammation and remodeling of the extracellular matrix. Fibroblasts transdifferentiate into valvular myofibroblast with an osteoblast-like phenotype, thus leading to active calcification and bone formation. TNF-α=Tumor necrosis factor α, TGF-β1=Transforming growth factor β1, IL-1β=Interleukin-1β

**Table 1**

**Overview of Studies on Biomarkers for Calcific Aortic Valve Disease**

This table gives an overview of trials investigating potential biomarkers for CAVD. Of note, there is a large number of studies available that focus on natriuretic peptides, lipids and CRP. Therefore, only selected trials are included in the table.

| Author | Year | n | Biomarker | Study groups | Results |
|---|---|---|---|---|---|
| Ngo et al.[15] | 2007 | 82 | ADMA | AVS vs. control | Elevated ADMA levels independently associated with AVS |
| Novaro et al. [18] | 2004 | 86 | Homocysteine | AVS vs. AVSc vs. control | Homocysteine level do not associated with presence and degree of CAVD |
| Gunduz et al. [19] | 2005 | 105 | Homocysteine | AVS vs. control | No statistically significant difference detected |
| Bozbas et al. [65] | 2008 | 383 | GGT | AVS vs. AVSc vs. control | GGT elevated in patients with AVS, but no significant correlation between GGT and AVSc |
| Ix et al. [55] | 2007 | 970 | Fetuin-A | AVS vs. MC vs. control | Inverse association between fetuin-A and the presence of AVS in non-diabetics |
| Weber et al. [72,75,77] | 2006 | 159 | NT-proBNP | AVS | NT-proBNP correlates with the severity of AVS and is of prognostic value in conservatively treated patients |
| Bergler-Klein et al. [374] | 2007 | 69 | BNP | AVS | BNP can predict outcome in low-flow AVS |
| Cowell et al. [38] | 2005 | 155 | LDL | AVS | No relationship between serum LDL levels and the progression of AVS |
| Messika-Zeitoun et al. [33] | 2007 | 262 | LDL | AVC | High LDL ilevels are associated with fast progression of AVC |
| Glader et al. [22] | 2003 | 202 | Leptin | AVS vs. control | Increased leptin levels are associated with the presence of AVS |
| Glader et al. [22] | 2003 | 202 | tPA | AVS vs. control | High levels of tPA are associated with the presence of AVS |
| Imai et al. [48] | 2008 | 135 | CRP | AVS | CRP predicts the severity, progression and prognosis of subjects with asymptomatic AVS |
| Novaro et al. [18] | 2007 | 5621 | CRP | AVS vs. AVSc vs. control | CRP is not associated with the progression of CAVD |
| Mills et al.[59] | 2004 | 107 | CaxP | AVS | CaxP levels correlate with the severity of AVS |
| Yu et al. [61] | 2009 | 30 | Osteopontin | AVS vs. control | Elevated Osteopontin levels found in patients with AVS |

MC = mitral calcification, AVC = aortic valve calcification

## Table 2
### Summary of the Value of current Biomarkers for Calcific Aortic Valve Disease

This table is based on the data reported by the studies reviewed in this article.

| Biomarker | Presence/Severity | Progression | Prognosis |
|---|---|---|---|
| ADMA | + | n/a | n/a |
| Homocysteine | ? | n/a | n/a |
| GGT | +/–° | n/a | n/a |
| Fetuin-A | + | n/a | n/a |
| Natriuretic peptides | + | + | + |
| LDL | ? | ? | – |
| Leptin | + | n/a | n/a |
| tPA | + | n/a | n/a |
| CRP | +/?* | ? | + |
| CaxP | + | n/a | n/a |
| Osteopontin | + | n/a | n/a |

A "+" indicates positive correlation, "–" indicates no correlation, "?" indicates contrary published results and "n/a" indicates that no studies were published on this issue.

° GGT correlates with AVS but not AVSc,

* CRP correlates with AVS but unclear results for AVSc