

Disease of the Aortic Valve or Aorta

Aortic Valve Sclerosis Is Associated With Systemic Endothelial Dysfunction

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OBJECTIVES	We sought to examine the association between aortic valve sclerosis (AVS) and systemic endothelial manifestations of the atherosclerotic process.
BACKGROUND	Clinical and experimental studies suggest that AVS is a manifestation of the atherosclerotic process. Systemic endothelial dysfunction is an early sign of the atherosclerotic process and can be assessed by ultrasonography of the brachial artery.
METHODS	A total of 102 in-hospital patients (76 men; mean age 63.5 ± 9.7 years) referred to the stress echocardiography laboratory underwent: 1) transthoracic echocardiography, with specific assessment of AVS (thickened valve leaflets with a transaortic flow velocity <2.5 m/s); 2) stress echocardiography; 3) coronary angiography, with evaluation of the Duke score (from 0 [normal] to 100 [most severe disease]); and 4) an endothelial function study, with assessment of endothelium-dependent, post-ischemic, flow-mediated dilation (FMD).
RESULTS	Aortic valve sclerosis was present in 35 patients (group I) and absent in 67 (group II). Groups I and II were similar in terms of the frequency of stress-induced wall motion abnormalities (35.3% vs. 19.4%, $p = \text{NS}$) and the angiographic Duke score (33.8 ± 28.6 vs. 35.2 ± 29.1 , $p = \text{NS}$). Patients with AVS showed a markedly lower FMD than those without AVS ($2.2 \pm 3.5\%$ vs. $5.3 \pm 5.3\%$, $p < 0.01$). On multivariate analysis, only FMD was highly predictive of AVS, with an odds ratio of 1.18 for each percent decrease in FMD (95% confidence interval 1.05 to 1.32; $p = 0.01$).
CONCLUSIONS	Aortic valve stenosis is associated with systemic endothelial dysfunction. This observation may provide a mechanistic insight into the emerging association between AVS and cardiovascular events. (J Am Coll Cardiol 2003;41:136–41) © 2003 by the American College of Cardiology Foundation

Early atherosclerosis involves the endothelium of many arteries. Vascular dysfunction has been implicated as an early event in atherogenesis (1). Moreover, vascular dysfunction associated with vascular injury has been postulated as the precursor of atherosclerosis (2). Endothelial dysfunction is thought to be an important factor in the development of atherosclerosis, hypertension, and heart failure.

Atherosclerosis risk factors (3,4) and clinical atherosclerotic cardiovascular disease (5) are independently associated with aortic valve sclerosis (AVS), suggesting that AVS represents an atherosclerosis-like process involving the aortic valve (6). Aortic valve sclerosis in the absence of obstruction of ventricular outflow affects 21% to 26% of adults >65 years old (4,7). It appears to have a similar etiology to that of mitral annular calcification (MAC), which has a highly significant association with atherosclerosis of the vascular system, including coronary artery disease (CAD) (8,9).

Over the past decade, a noninvasive technique has

evolved to evaluate flow-mediated dilation (FMD), an endothelium-dependent function, in the brachial artery after occlusion (10–13). This stimulus provokes the endothelium to release nitric oxide, with subsequent vasodilation that can be imaged and quantitated as an index of vasomotor function (14).

Ultrasound imaging of the brachial artery during reactive hyperemia is a widely used tool for quantifying endothelium-dependent vasomotion (15). Impaired endothelium-dependent vasomotion is a diffuse disease process resulting in abnormal regulation of blood vessel tone and loss of several atheroprotective effects of the normal endothelium (16), and impaired peripheral endothelial function may also be a marker of increased future cardiovascular risk (17).

Initial preliminary clinical and experimental studies suggest that AVS is a manifestation of the atherosclerotic process (18). Systemic endothelial dysfunction is an early sign of the atherosclerotic process and can be assessed by brachial artery ultrasonography. The present study's hypothesis is that AVS is associated with systemic endothelial dysfunction. Therefore, we examined the association between AVS and systemic endothelial manifestations of the atherosclerotic process in patients with known or suspected CAD.

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Abbreviations and Acronyms

AVS	= aortic valve sclerosis
CAD	= coronary artery disease
FMD	= flow-mediated dilation
LV	= left ventricle/ventricular
MAC	= mitral annular calcification
MI	= myocardial infarction
TTE	= transthoracic echocardiography

METHODS

Study patients. The study population consisted of 102 hospitalized patients (76 men and 26 women; mean age 63.5 ± 9.7 years) with suspected or known CAD, referred to the stress echocardiography laboratory and scheduled for subsequent coronary angiography. Seventy-two patients had stable angina pectoris, 35 had a previous myocardial infarction, and 29 had a previous revascularization procedure (13 with coronary angioplasty and 16 with coronary artery bypass graft surgery). After taking a clinical history, with special attention to risk factor identification, all patients underwent, on different days, in a variable sequence, and within 15 days: 1) complete transthoracic echocardiography (TTE); 2) stress echocardiography; 3) an endothelial function study; and 4) coronary angiography. All patients had stopped nitrate treatment at the time of the endothelial function study. Each of the four tests was interpreted by observers who were unaware of the results of the other tests.

Patients with aortic stenosis (transaortic flow velocity >2.5 m/s), rheumatic valvular disease, prosthetic valves, bicuspid aortic valves, congenital heart disease, bacterial endocarditis, or hypertrophic obstructive cardiomyopathy, as well as those on hemodialysis, were excluded from the study. Patients with a poor acoustic window ($n = 2$) and acute, unstable coronary syndromes were also excluded.

The hospital's Institutional Review Board approved the study, and all patients gave written, informed consent.

Risk factors. The cardiac risk factors considered in this study were age, gender, family history of CAD, diabetes mellitus, hypertension, hypercholesterolemia, and history of smoking. Diabetes was defined as hyperglycemia requiring previous or ongoing pharmacologic therapy. Hypertension was defined as either a systolic or diastolic elevation of blood pressure ($>140/90$ mm Hg) or ongoing antihypertensive pharmacologic therapy. Hypercholesterolemia was defined as a total cholesterol level >200 mg/dl or current treatment with lipid-lowering medication. Current cigarette smoking was defined as active smoking within the past 12 months.

Transthoracic echocardiography. Complete TTE studies were performed in all patients, by use of a commercially available system (Acuson Sequoia C256, Mountain View, California; Hewlett-Packard SONOS 5500, Andover, Massachusetts).

We defined AVS as a focal area of increased echogenicity and thickening of the aortic valve leaflets without restriction

of leaflet motion, with a transaortic flow velocity <2.5 m/s on TTE, using the criteria of Stewart et al. (4) and Otto et al. (5).

The TTE criteria for MAC included an intense echo-producing structure located at the junction of the atrioventricular groove and posterior mitral valve leaflet on the parasternal long-axis, apical four-chamber, or parasternal short-axis view, using the criteria of Adler et al. (19).

All studies were recorded digitally in the DICOM format or on super-VHS videotape and evaluated by experts in echocardiography who were blinded to the results of coronary angiography.

Peak transaortic flow velocities (average of triplicate measurements) were measured during the TTE examination by continuous-wave Doppler imaging (20). Presence of aortic regurgitation (21) was also noted.

Stress echocardiography. Stress echocardiography was performed with dipyridamole (up to 0.84 mg/kg body weight over 10 min, with atropine up to 1 mg, as needed), dobutamine (up to 40 g/kg per min, with atropine up to 1 mg, as needed), or upright bicycle exercise, according to standard protocol (22). Commercially available imaging systems with digital acquisition were used. All standard echocardiographic views were obtained when possible. The left ventricle (LV) was divided into 16 segments, as suggested by the American Society of Echocardiography (23). Segmental wall motion was graded as follows: normal = 1; hypokinetic = 2; akinetic = 3; and dyskinetic = 4. Inadequately visualized segments were not scored. The stress echocardiogram was considered positive when one LV segment was increased by one grade or more at peak stress. The wall motion score index was derived by dividing the sum of individual visualized segment scores by the number of visualized segments. Appearance of ST-segment depression >1 mm at 0.08 s after the J point, as determined by the physician, was taken as evidence of a positive stress electrocardiogram (ECG). Intra- and inter-observer agreement has already been shown to be high ($>90\%$) in our laboratory (24).

Endothelial function study. All patients were studied at least 4 h after their last meal, according to a standard protocol previously described in detail (25). Briefly, the patients were instructed to lie quietly in a supine position for 10 min before the study. All studies were performed in a temperature-controlled room (20 to 25°C). The diameter of the brachial artery was measured from two-dimensional ultrasound images. In each study, scans were taken at rest, during reactive hyperemia, at rest again, and after sublingual nitrate administration. The brachial artery was scanned in the longitudinal section, on the dominant arm, 2 to 15 cm above the elbow. The focus zone was set to optimize images of the lumen-arterial wall interface, and machine-operating parameters were not changed during the rest of the study. The arterial diameter was measured at a fixed distance from an anatomic marker, such as a bifurcation. Measurements were taken from the anterior to posterior "m" line at end

diastole, incident with the R-wave on the ECG. Three cardiac cycles were analyzed for each scan, and measurements were averaged. Following the baseline measurements, a pneumatic tourniquet was inflated below the elbow to a pressure of 250 mm Hg; forearm cuff occlusion was maintained for 4.5 min. Sustained maximal vessel dilation and maximal flow change occurred after 4.5 min of cuff occlusion; a higher cuff occlusion time does not provide a greater response (14). A shorter duration of cuff occlusion provides a less intense stimulus and fails to achieve sustained vasodilation 1 min after cuff release. Therefore, the arterial diameter was measured at 1 min after cuff deflation. After 10 min of vessel recovery, rest scan and flow measurements were repeated. Sublingual nitrate (0.3 mg glyceryl trinitrate [Trinitrina by Pharmacia, Peapark, New Jersey]) was then administered to evaluate endothelium-independent vasodilation. The last set of scans was performed 3 min after nitrate intake. Endothelium-dependent, post-ischemic FMD was determined by the maximal brachial artery diameter after exactly 60 s of reactive hyperemia, compared with the baseline vessel diameter, and was expressed as percent FMD. Endothelium-independent peripheral (nitrate mediated dilation) vasodilation is expressed as the percent change in brachial artery diameter 3 min after sublingual nitrate administration, using the baseline rest diameter as a reference. Intra- and inter-observer variabilities in our laboratory have been evaluated at 2.5% and 2.0%, respectively (25).

Coronary angiography. Coronary angiography in multiple views was performed according to the standard Judkins or Sones technique. At least five views, including two orthogonal views, were acquired for the left coronary artery and at least two orthogonal views for the right coronary artery. All angiographic studies were performed by experienced observers who ignored the results of noninvasive stress testing. The percent diameter stenosis was determined by quantitative coronary angiography, using an automated edge-detection system (Mipron, Kontron, Germany). In our laboratory, this method's intra- and inter-observer variabilities were 7% and 6%, respectively (26). A vessel was considered to have significant obstruction if its diameter was narrowed by $\geq 50\%$, with respect to the pre-stenotic tract. A Duke score was calculated in each patient, according to a previously described method (27). Briefly, this prognostically validated index describes the extent and severity of CAD using a scale from 0 to 100, from nonsignificant CAD to severe left main coronary artery disease. It takes into account the number of major diseased vessels and the location and severity of stenosis.

Statistical analysis. Data were entered into Excel and analyzed with SPSS version 10.0 (SPSS, Inc., Chicago, Illinois). Continuous measures are expressed as the mean value \pm SD. When appropriate, the 95% confidence intervals (CIs) are given. Continuous variables are analyzed by using the Student *t* test. Dichotomous variables are compared by chi-square analysis.

Multivariate stepwise logistic regression analysis (forward selection with likelihood ratio criterion for selection variables: 0.05 to enter; 0.10 to remove) was used to estimate the relationship between FMD and AVS and to evaluate for potential confounders, including age, gender, presence of hypertension, hypercholesterolemia, diabetes mellitus, and smoking. In all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

Sixty-six patients (64.7%) had a history of stable angina; 35 (34.3%) had a previous myocardial infarction; and 29 (28.4%) had a previous revascularization procedure (with bypass surgery in 16 patients, angioplasty in 13 patients, and both in 26 patients). Sixty-six patients (64.7%) were receiving anti-ischemic therapy (beta-blockers and/or calcium antagonists) at the time of stress echocardiography and endothelial testing.

Aortic valve sclerosis was present in 35 patients (group I) and absent in 67 patients (group II). The characteristics of the study groups are listed in Table 1. There were no significant differences in baseline characteristics between the groups.

Aortic sclerosis and valvular regurgitation. Aortic regurgitation was detected in 33 subjects (32.4%). Aortic regurgitation was trivial to mild in 19 patients (18.6%) and moderate in 4 (3.9%). The proportion of patients with aortic regurgitation was significantly higher among patients with AVS (34.3%) than among patients with morphologically normal aortic valves (16.4%; $p = 0.04$). Mitral regurgitation was detected in 27 patients with AVS (77.1%) and in 49 patients with morphologically normal aortic valves (73.1%; $p = \text{NS}$).

Aortic sclerosis and echocardiographic parameters. End-diastolic volume in patients with AVS was significantly greater than that in patients with morphologically normal aortic valves (166.4 ± 95.9 vs. 127.6 ± 44.9 ml, $p = 0.006$). The LV mass index was significantly higher in patients with AVS than in those with no evidence of AVS (130.7 ± 48.5 vs. 113.5 ± 40.0 g/m², $p = 0.05$). There were no significant differences in end-systolic volume, LV ejection fraction, or wall thickness between the groups. There was also no significant relationship between AVS and MAC. The proportion of patients with MAC was 11.4% in patients with AVS and 7.5% in patients without AVS ($p = \text{NS}$).

Aortic sclerosis and CAD. Significant CAD during coronary angiography was found in 66 patients (64.7%). Among patients with AVS, 21 had CAD (60.0%), and among those without AVS, 45 had CAD (67.2%; $p = \text{NS}$). Groups I and II had similar angiographic Duke scores (33.8 ± 28.3 vs. 31.7 ± 25.8 ; $p = \text{NS}$).

Stress echocardiography. A positive result of stress echocardiography was found in 17 patients (16.7%). Among patients with AVS, eight had a positive test, and among patients without AVS, nine had a positive result. Groups I

Table 1. Patient Characteristics (n = 102)

	Group I* (n = 35)	Group II† (n = 67)	p Value
Age (yrs)	64.6 ± 9.1	62.9 ± 10.0	NS
Gender (M/F)	26/9 (74.3%/25.7%)	50/17 (74.6%/25.4%)	NS
Family history of CAD	15 (43%)	31 (46%)	NS
Diabetes mellitus	6 (17.1%)	11 (16.4%)	NS
Systemic hypertension	16 (45.7%)	37 (55.2%)	NS
Hypercholesterolemia	14 (40.0%)	31 (46.3%)	NS
Previous MI	10 (28.6%)	25 (37.3%)	NS
CABG	3 (8.6%)	13 (19.4%)	NS
PTCA	2 (5.7%)	11 (16.4%)	NS
Nitrate treatment	17 (48.6%)	39 (58.2%)	NS
Beta-blocker treatment	12 (34.3%)	24 (35.8%)	NS
Calcium antagonist treatment	13 (37.1%)	29 (43.3%)	NS
ACE inhibitor treatment	11 (31.4%)	16 (23.9%)	NS
Lipid-lowering drug treatment	6 (17.1%)	16 (23.9%)	NS
Smoking	16 (45.7%)	35 (52.2%)	NS
Body mass index (kg/m ²)	27.2 ± 3.3	26.7 ± 3.5	NS
Total cholesterol (mg/dl)	220.8 ± 51.9	200.2 ± 34.8	NS
LDL cholesterol (mg/dl)	124.4 ± 57.8	133.1 ± 38.9	NS
Triglycerides (mg/dl)	170.2 ± 136.1	178.1 ± 101.9	NS

*Patients with aortic valve sclerosis (AVS). †Subjects without AVS. Data are presented as the mean value ± SD or number (%) of patients.

ACE = angiotensin-converting enzyme; CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; LDL = low-density lipoprotein; MI = myocardial infarction; NS = not significant; PTCA = percutaneous transluminal coronary angioplasty.

and II had a similar frequency of a positive stress echocardiogram (22.9% vs. 13.4%; p = NS), rest wall motion score index (1.09 ± 0.14 vs. 1.27 ± 0.46; p = NS), and peak stress wall motion score index (1.19 ± 0.23 vs. 1.30 ± 0.45; p = NS).

Aortic sclerosis and endothelial dysfunction. Flow-mediated dilation was significantly lower (2.2 ± 3.5%) in patients with AVS than in those with morphologically normal aortic valves (5.3 ± 5.3%; p < 0.002) (Fig. 1).

Endothelium-independent vasomotion was not statistically different between patients with AVS and those with normal aortic valves (nitrate medicated dilation: 7.4 ± 5.2% vs. 9.1 ± 6.0%; p = NS).

Multivariate analysis was performed using stepwise logistic regression. Flow-mediated dilation was highly predictive of AVS, with an odds ratio of 1.18 for each percent decrease in FMD (95% CI 1.05 to 1.32; p = 0.005). Age (p = 0.95), hypertension (p = 0.23), hypercholesterolemia (p = 0.76), smoking (p = 0.62), and diabetes (p = 0.9) were not predictors of AVS in this analysis.

DISCUSSION

Previous reports have noted an association between AVS and clinical atherosclerotic cardiovascular disease (5,28). To our knowledge, this study is the first to demonstrate an independent association between aortic sclerosis and systemic endothelial dysfunction, suggesting that these disorders represent related processes in the spectrum of atherosclerotic cardiovascular disease.

Comparison with previous studies. Several pathologic and echocardiographic studies (3,4,7,21) have demonstrated a strong association between AVS and risk factors such as

age (29–32), male gender (4,33), hypertension (34), cholesterol, diabetes, smoking (3,4), and MAC (19). Previous studies have also shown that patients with AVS undergoing coronary angiography have a higher prevalence of CAD (19,35). In our study, the sample size was powered to detect a difference in FMD, but not in the prevalence of CAD or risk factors for CAD. The sample size is too small to expect an association with clinical risk factors or coronary angiographic findings. Previous studies have shown that AVS is associated with an increase of ~50% in the risk of cardio-

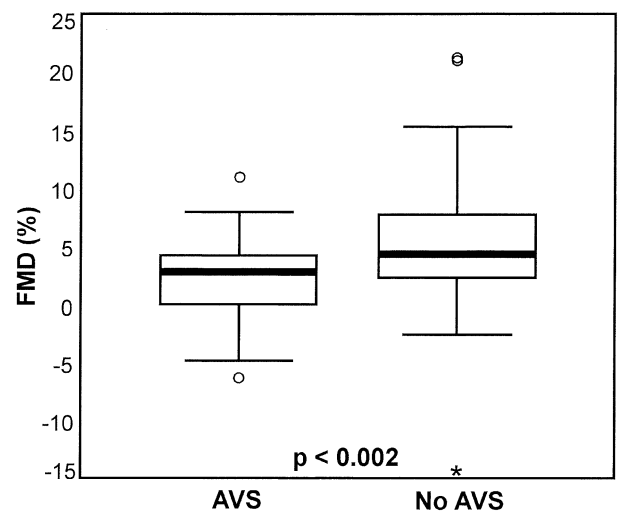


Figure 1. Endothelium-dependent flow-mediated dilation (FMD) of the brachial artery during reactive hyperemia (endothelium-dependent vasomotion) is significantly higher in subjects with no evidence of aortic valve sclerosis (AVS) than in patients with AVS. Data are expressed as the median value (25% and 75% and outliers, in a “box-and-whiskers” plot). *p < 0.002.

vascular death or myocardial infarction in adults >65 years old (5).

Aronow et al. (36) reported that older patients with valvular aortic sclerosis have a higher risk (1.8 times) of new coronary events than older subjects without valvular aortic sclerosis, after controlling for the confounding effects of other prognostic variables. This supports the hypothesis that valvular calcification is a manifestation of systemic atherosclerotic processes. Our data showing an association between AVS and systemic endothelial dysfunction may add to our understanding of the relationship between aortic sclerosis and adverse clinical outcomes in adults with CAD. In fact, endothelial dysfunction can lead to cardiovascular events through several mechanisms. One possible mechanism is myocardial ischemia secondary to endothelial dysfunction, even in the absence of obstructive CAD (37,38). Another possible mechanism by which coronary endothelial dysfunction may contribute to cardiac events is through acceleration of coronary atherosclerosis, as evidenced by the development of obstructive CAD (39). There is also initial evidence linking ultrasonically assessed systemic endothelial dysfunction to a worse prognosis in patients undergoing vascular surgery (40). The association between ultrasonically assessed systemic endothelial dysfunction and AVS may provide a mechanistic insight into the emerging association between AVS and cardiovascular events.

Study limitations. The size of our study population ($n = 102$) may have limited our ability to detect significant, albeit relatively less important, risk factors for AVS. We selected patients who were scheduled to undergo coronary angiography and stress echocardiography for a clinical indication. These inclusion criteria might have skewed the spectrum of the population toward more advanced forms of CAD than studies in which mostly asymptomatic subjects were enrolled (3,4,34). However, these same selection criteria allowed us to gain insight into multiple markers of the atherosclerotic process, both early (such as systemic endothelial dysfunction) and advanced (such as a positive stress echocardiogram and angiographically assessed stenosis) (41).

We did not use a digitized method to identify AVS and MAC. This could have caused a verification bias and may have affected the reproducibility in identifying cardiac calcifications. Nevertheless, the qualitative “eyeball” method is the one currently adopted in everyday clinical echocardiographic practice.

Sixty-five percent of patients were receiving anti-ischemic therapy at the time of testing, and it is known that this may affect stress test sensitivity (42) and, at least for some calcium antagonists, endothelial function. Nevertheless, withholding of therapy in all patients would have been impractical and/or unethical.

Conclusions. Aortic valve sclerosis is associated with systemic endothelial dysfunction. This observation may provide a mechanistic insight into the emerging association between AVS and cardiovascular events.

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