Aortic stenosis (AS) is common, and its prevalence increases with aging. Degenerative AS is due to aortic valve thickening with calcium deposition that causes cusp stiffness and decreased aortic valve area (AVA). Recent data showed that aortic valve calcification (AVC) is not passive but is integral to AS formation, appearing early in valve lesions and accumulating through active and highly regulated mechanisms similar to ossification. This mechanistic AVC-AS association has led to attempts at validating for coronary artery calcification (CAC) quantification and potentially can quantify AVC. Pilot studies using EBCT suggested potential value for diagnosis, severity assessment, progression monitoring, and therapeutic evaluation in AS. However, these observations are limited by lack of validation and outcomes data and by small samples. Furthermore, it is uncertain whether AVC is merely a surrogate for AVA, which is routinely and accurately measured by Doppler echocardiography, or whether AVC load in severe AS negatively affects clinical outcome. These seminal observations are important, but methodological uncertainties regarding AVC assessment hindered their generalizability and use in clinical practice. Recently, electron-beam computed tomography (EBCT) has enabled the noninvasive, objective definition of calcifications and the quantification of calcification load. EBCT is extensively validated for coronary artery calcification quantification and potentially can quantify AVC. Pilot studies using EBCT showed a strong association and diagnostic value for severe AS. The curvilinear relationship between AVC and AVA suggests these measures are complementary, and indeed, AVC provides independent outcome information. Thus, AVC is an important measurement in the evaluation of patients with AS. (Circulation. 2004;110:356-362.)

Key Words: stenosis • calcium • echocardiography • tomography
and AVA are sufficiently independent that AVC may provide incremental information. It is essential to resolve these uncertainties, because surgery for severe AS may entail serious risks, and diagnosis may be difficult, which underscores the importance of improved diagnostic and prognostic assessment.

The aims of the present study were to validate EBCT AVC measurement, to examine the hemodynamic correlates and diagnostic value of AVC for AS, and to assess outcome implications of quantitative AVC measurement by EBCT.

Methods

Pathology Data

Aortic valve complete specimens (preserved in formalin-filled bags) of 30 consecutive aortic valve replacements (for AS or regurgitation) at the Mayo Clinic were used without prerequisite with regard to calcification load or type of lesion. EBCT of specimens was obtained in random order and interpreted independently before pathological examination. A validated soft tissue removal method (timed bleaching technique) from formalin-fixed valves allowed tissue digestion. Then, residual calcium was rinsed with water, dried overnight, and directly weighed the following day by a pathology technician unaware of EBCT results.

Clinical Patients

Eligible patients were the first 100 consecutive patients who underwent prospective EBCT for cardiac calcification assessment and quantitative Doppler echocardiography. Fifty-six patients were enrolled after undergoing prospective assessment of cardiac calcification in the population (the Epidemiology of Coronary Artery Calcification study) and Doppler echocardiography clinically required for cardiac murmur within 4 months of the EBCT. Forty-four other patients clinically evaluated for AS underwent prospective EBCT and Doppler echocardiography during the same episode of care. Patients with associated valvular, pericardial, or congenital heart diseases (and children with congenital AS), cardiomyopathy, or acute myocardial infarction were excluded. EBCT and echocardiographic measurements were performed blinded to other test results and to clinical data.

Electron-Beam Computed Tomography

EBCT was performed with an Imatron C-150. A scan run consisted of acquisition of 40 3-mm-thick contiguous transverse slices. Acquistion time was 100 ms/slice, triggered by electrocardiography at 80% of the RR interval. Measurements were obtained offline with Analyze Software. Calcification was defined as 4 adjacent pixels with density >130 Hounsfield units. AVC score was calculated with density of pixels. Area and volume of calcifications were calculated per slice and summated. Valve specimens in formalin-filled bags were scanned by the same process. Aortic valve calcium was directly weighed the following day by a pathology technician unaware of EBCT results.

Statistical Analysis

Data are presented as mean ± SD or percentages. Comparison of AVC by EBCT with pathological calcium weight used linear regression. Comparisons between groups or methods used ANOVA, t test, or χ² test as appropriate. AVC (EBCT absolute score and log transformation) association with AS hemodynamic severity was tested with linear and nonlinear regressions, and the best fit was retained. AVC diagnostic value for severe AS (AVA <1 cm²) was analyzed; sensitivity, specificity, and positive and negative predictive values were calculated for various thresholds; and area under the receiver operator characteristic (ROC) curve (AUC) was calculated. Agreement between echocardiographic calcification class and AVC quartiles used the κ-test (systematic differences tested by McNemar test). Event-free survival was defined as survival without dyspnea, angina, syncope, heart failure, or need for surgery and was analyzed for global events (at any time during follow-up) and for late events (occurring after the first month of follow-up), overall (any event) and for AS-related events, estimated by the Kaplan-Meier method. Cox proportional-hazard analyses evaluated the predictive value of AVC for event-free survival univariately, with adjustment for AVA alone and for age, gender, AVA, symptoms, and EF.

Results

Methodological Assessment of AVC Measurement by EBCT

Anatomic Validation

The 30 aortic valve specimens were obtained from consecutive patients, 19 of whom had undergone surgery for AS and 11 of whom had surgery for mixed aortic valve disease or aortic regurgitation. The aortic valve disease was degenerative in 23 patients, bicuspid valve in 5, and endocarditis in 2. AVC score by EBCT (1125 ± 1294, range 0 to 4751) and calcium weight by pathology (653 ± 748 mg, range 1 to 3034 mg) showed a strong linear correlation (r = 0.96, P < 0.0001; score = 41 + 1.66 × weight of calcium; Figure 1). Estimated calcium weight calculated as score/1.7 was not different from the measured calcium weight (662 ± 761 mg, P = 0.8 versus measured) and showed a strong linear correlation with measured weight (r = 0.96, P < 0.0001) with a small error of estimate (53 mg). Compared with AVC score, AVC area and volume by EBCT showed correlations > 0.99 and no im-

Figure 1. Correlation between AVC score measured by EBCT and weight of calcium measured by tissue digestion in 30 aortic valve specimens.
provement in correlation to calcium weight, so that AVC score was the measure reported.

Reproducibility
AVC score intraobserver and interobserver variability each were assessed in 10 randomly selected clinical patients. For the same observer, variability between EBCT runs increased with higher score \((P<0.0001)\) and was 4.4±6.5%. Using 2-run averages, intraobserver variability was 3.7±4.3%, and interobserver variability was 4±10%.

Clinical AVC Measurement
Baseline characteristics are summarized in Table 1. Atrial fibrillation and history of coronary disease (angina and/or myocardial infarction) were present in 12% and 13% of patients, respectively. Aortic valves were bicuspid in 11 and tricuspid in 89 patients. Median interval between EBCT and echocardiography was 37 days. Wide ranges were observed for EF (35% to 79%), AVC score (0 to 7226), and AVA (0.5 to 4.1 cm²). The valve diagnosis was innocent murmur or valve sclerosis without stenosis in 42 patients, mild AS in 11, moderate AS in 18, and severe AS in 29.

AVC Score and Hemodynamic Severity
Characteristics according to AVC quartiles are presented in Table 1. AS severity and LV mass increased with AVC quartiles. The association between AVC and AVA was strong \((r=-0.79, P<0.0001)\) but had a curvilinear regression (Figure 2A). The AVC log transform fit best to AVA linearly \((r=-0.79, P<0.0001)\), which confirmed a nonlinear AVC-AVA association with a precipitous AVA decrease for small AVC increments at low calcification loads; larger AVC increments were linked to transition from moderate to severe AS. A similar curvilinear relation was observed between AVC and the AVA index \((r=0.83, P<0.0001)\). The dimensionless index \((r=0.87, P<0.0001)\) and peak aortic velocity \((r=0.86, P<0.0001; \text{Figure 2B})\) also showed strong associations, with curvilinear regression to AVA. For diagnosis of severe AS, the ROC curve of the AVC score showed a high AUC of 0.89 (Figure 3). Diagnostic values of various AVC scores for severe AS are indicated in Table 2. A threshold of 1100 Agatston units (AU) was associated with the highest combination of sensitivity and specificity. Thresholds of 500 and 2000 AU provided high sensitivity (100%) and high specificity (92%), respectively.

Echocardiography calcifications were absent in 17% of patients, mild in 27%, moderate in 24% and severe in 32%. The AVC score (mean±SD) differed \((P<0.0001)\) between

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall</th>
<th>First Quartile</th>
<th>Second Quartile</th>
<th>Third Quartile</th>
<th>Fourth Quartile</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>70±10</td>
<td>66±9</td>
<td>68±11</td>
<td>72±7</td>
<td>73±12</td>
<td>0.06</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>58</td>
<td>48</td>
<td>68</td>
<td>36</td>
<td>80</td>
<td>0.008</td>
</tr>
<tr>
<td>NYHA class III</td>
<td>93</td>
<td>100</td>
<td>92</td>
<td>96</td>
<td>84</td>
<td>0.21</td>
</tr>
<tr>
<td>LV EF, %</td>
<td>64±8</td>
<td>66±8</td>
<td>64±6</td>
<td>66±6</td>
<td>61±10</td>
<td>0.19</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>202±64</td>
<td>172±13</td>
<td>200±14</td>
<td>217±13</td>
<td>222±14</td>
<td>0.04</td>
</tr>
<tr>
<td>AVA, cm²</td>
<td>1.8±0.9</td>
<td>2.7±0.6</td>
<td>2.5±0.6</td>
<td>1.1±0.3</td>
<td>0.9±0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dimensionless index</td>
<td>0.46±0.24</td>
<td>0.74±0.15</td>
<td>0.65±0.14</td>
<td>0.31±0.08</td>
<td>0.23±0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peak aortic valve velocity, m/s</td>
<td>2.8±1.4</td>
<td>1.5±0.5</td>
<td>1.8±0.7</td>
<td>3.6±0.7</td>
<td>4.4±0.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Severe AS, %</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>48</td>
<td>68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AVC score, AU</td>
<td>1316±1749</td>
<td>1±2</td>
<td>129±166</td>
<td>1217±376</td>
<td>3916±1460</td>
<td>...</td>
</tr>
</tbody>
</table>

NYHA indicates New York Heart Association.
*Score ranges appear in parentheses.

Figure 2. Association between AVC and hemodynamic severity of AS in 100 clinical patients: AVC score (x-axis) measured by EBCT and Doppler echocardiographic measurement (y-axis) of AVA (A) and peak aortic valve velocity (B). Note strong, curvilinear association between AVC load and hemodynamic severity of AS.
these echocardiographic classes (no calcification: 4.1±12.7, range 0 to 49.5; mild calcification: 71.5±133, range 0 to 541; moderate calcification: 812±555, range 10 to 1945; and severe calcification: 3094±1731, range 1080 to 7226) but had a wide overlap. When AVC score was classified by quartiles, agreement between methods was modest (κ=0.60).

Echocardiographic assessment of calcification was more extreme (P<0.0001), higher by ≥1 class in 20% of patients and lower by ≥1 class in 12%. Patients with no or mild calcification by EBCT had higher echocardiographic estimation of calcification by ≥1 class in 44% of cases. Use of empiric thresholds (20, 500, and 1100 AU) for AVC grades by EBCT did not improve the κ-statistic (0.58) or differences in severity assessment (P<0.0001).

**AVC and Clinical Outcome**

**Overall Event-Free Survival**

During follow-up of 2.0±2.3 years, 22 events occurred, which included 4 deaths, 13 patients who developed new symptoms (dyspnea or congestive heart failure in 11, angina in 1, and syncope in 1) and 5 patients who underwent aortic valve replacement without preceding symptoms for critical AS in 3, rapid progression of AS in 1, and progression of aortic regurgitation with ventricular dysfunction in 1. A total of 18 patients required aortic valve replacement during follow-up. Event-free survival was 74±5% at 2 years and 69±6% at 5 years. Comparison of patients with and without events during follow-up and association of baseline characteristics with time to event are presented in Table 3. Univariate, AVC was the strongest predictor of event-free survival, with 5-year rates of 90±4% (AVC <500 AU) versus 29±14% (AVC ≥500 AU; P<0.0001). After adjustment for age, sex, AVA, baseline symptoms, and EF, AVC independently predicted event-free survival, with an adjusted relative risk (RR) of 1.06 (95% CI 1.02 to 1.10) per 100-AU increment (P<0.0001). AVC independently predicted event-free survival in patients with no or minimal symptoms (RR 1.09, 95% CI 1.05 to 1.15 per 100 AU; P<0.0001) and in patients with AVA <1.5 cm² (RR 1.05, 95% CI 1.01 to 1.09 per 100 AU; P<0.01). When we focused on late events to minimize potential referral bias, AVC was predictive of event-free survival univariately (at 5 years, 92±4% and 40±18% in patients with AVC below and ≥500 AU, respectively; P=0.0002; Figure 4), independent of age, sex, AVA, symptoms, and EF (RR 1.11, 95% CI 1.03 to 1.23 per 100 AU; P=0.006). The addition of echocardiographic scoring to the model did not affect AVC significance but was of borderline statistical significance (P=0.08).

**AS-Related Events**

Of 22 follow-up events, 17 were AS related, with AS-related event-free survival of 80±5% at 2 years. Patients with events had higher AVC scores (3839±1942 versus 800±1170 AU, P<0.0001). In this series, 4 patients presented with AVA ≤1.5 cm² and LV dysfunction (EF <50%). AVA was 1.0±0.3 cm², EF 42±7%, and mean gradient 32±14 mm Hg. In contrast to low gradient, AVC score was high at 4010±2200 AU. Aortic valve replacement was performed on 3 patients, and severe AVC and AS were confirmed by surgical inspection. AVC score was independently predictive of events (RR 1.05 per 100-AU increment, 95% CI 1.01 to 1.09; P<0.01). The addition of echocardiographic calcification class to the model did not reach statistical significance (P=0.16).

**Discussion**

The present study provides evidence that AVC scoring is an important measure of AS severity, offering essential diagnostic, physiological, and outcome insights, and that it may be an important clinical tool in the routine management of patients with AS. Indeed, AVC score by EBCT accurately and reproducibly estimates calcium weight on aortic valves. AVC load is robustly associated with AS hemodynamic severity, with strong, sensitive diagnostic value in predicting the presence of severe AS. However, the AVC association with AS hemodynamic severity is curvilinear, which suggests that these 2 measures, which evaluate respectively lesion severity and hemodynamic load, are complementary in assessing AS severity and in predicting outcome after diagnosis.

**Quantitative Assessment of AVC**

The ability of EBCT to measure cardiac calcium has been established for coronary artery calcium compared with
pathology for the entire coronary system or specific vessels or segments. Histomorphometry (calcium areas in sequential coronary artery sections) and determination of the weight of mineral ashes are indirect methods that may overestimate true calcium weight. The present study is the first to use tissue digestion to measure calcium weight within cardiac tissue, demonstrating EBCT score accuracy in estimating calcium weight. The AVC score accounts for voxel density and logically reflects calcium weight, which can be simply estimated as score/1.7. Thus, EBCT directly and quantifiably measures AVC load. Qualitative echocardiographic density correlates modestly with AVC by EBCT and tends to produce a more severe grade. Echocardiography is simple but subjective and possibly reflects both fibrosis and calcification, which suggests that both methods should be evaluated. EBCT reintroduces clinically a classic method of AS assessment but improves on it by providing absolute and quantitative AVC measurement with the potential to affect AS diagnosis and prognosis.

### AVC and Hemodynamic AS Severity

Pilot studies reported correlations between AVC measured by EBCT and AS hemodynamic severity, but small sample sizes, narrow ranges of hemodynamic severity, and contradictory interpretations limited the clinical implications. In the present study, a strong negative association between AVC score and AS hemodynamic severity was observed. This provides powerful diagnostic value for severe AS, as revealed by the high area under the ROC curve. EBCT is sensitive in detecting severe AS beginning with a score of 500 AU (≈300 mg of calcium), with the highest sum of sensitivity and specificity for a score of 1100 AU (≈650 mg of calcium) and with a highly specific score threshold of 2000 AU (≈1200 mg of calcium). Such independent valve lesion measurement is particularly important in patients with reduced LV function, in whom a low gradient raises the concern of functionally low AVA without authentically severe AS. These patients are at high risk for surgery, and even stress echocardiography may not provide diagnostic certainty. Patients with AS, reduced EF, and low gradient in the present series show that AVC measurement may particularly enhance the diagnostic armamentarium, but larger series are required to confirm this. Importantly, the curvilinear relationship between AVA and AVC suggests that they provide complementary measures of AS. The physiological implication for progression is that for mild AS, small AVC accumulation results in a large AVA decline, whereas large AVC increments are required to reach severe to critical AS. This concept is in agreement with recent observations that the rate of AVA decline is higher for milder AS. Another implication of the nonlinear AVC-AVA association is that AVC may provide prognostic information independently of AVA.

### AVC and Clinical Outcome

Recent data suggest that calcification load is an important outcome determinant in AS. The present study is the first to

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**TABLE 3. Baseline Characteristics According to Clinical Outcome and Univariate Association With Time to Events**

<table>
<thead>
<tr>
<th>Variable</th>
<th>With Events (n=22)</th>
<th>Event Free (n=78)</th>
<th>P</th>
<th>RR (95% CI) Increment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>70±9</td>
<td>70±10</td>
<td>0.93</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>50</td>
<td>60</td>
<td>0.39</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>NYHA class ≥III, %</td>
<td>27</td>
<td>1</td>
<td>0.01</td>
<td>10.2 (3.5–26.3)</td>
<td>Present 0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>133±15</td>
<td>138±20</td>
<td>0.27</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>EF, %</td>
<td>60±10</td>
<td>65±7</td>
<td>0.01</td>
<td>0.94 (0.90–0.98)</td>
<td>1 point 0.007</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>211±72</td>
<td>200±61</td>
<td>0.52</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>AVA, cm²</td>
<td>1.20±0.65</td>
<td>1.98±0.97</td>
<td>0.001</td>
<td>0.83 (0.76–0.89)</td>
<td>0.1 cm²/2 &lt;0.0001</td>
</tr>
<tr>
<td>AVC index, cm²/m²</td>
<td>0.68±0.46</td>
<td>1.02±0.47</td>
<td>0.003</td>
<td>0.74 (0.64–0.84)</td>
<td>0.1 cm²/2 &lt;0.0001</td>
</tr>
<tr>
<td>Dimensionless index</td>
<td>0.28±0.17</td>
<td>0.51±0.24</td>
<td>0.001</td>
<td>0.42 (0.25–0.60)</td>
<td>0.1 &lt;0.0001</td>
</tr>
<tr>
<td>Peak aortic valve velocity, m/s</td>
<td>3.8±1.4</td>
<td>2.6±1.3</td>
<td>0.001</td>
<td>3.1 (2.1–4.8)</td>
<td>1 m/s &lt;0.0001</td>
</tr>
<tr>
<td>AVC score, AU</td>
<td>3059±2277</td>
<td>824±1186</td>
<td>0.0001</td>
<td>1.07 (1.05–1.09)</td>
<td>100 AU &lt;0.0001</td>
</tr>
<tr>
<td>Coronary calcification score, AU</td>
<td>1025±1436</td>
<td>666±1063</td>
<td>0.20</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

NYHA indicates New York Heart Association.
analyze the AVC score quantitatively by EBCT over a wide range of AVA and to evaluate its outcome impact. A higher AVC load results in lower event-free survival, both overall and for late events, in asymptomatic patients and for AS-related events, independent of all other predictors of outcome, especially AVA and EF. This observation will require confirmation in larger series but is essential in suggesting that measurement of AVC is of clinical significance in patients with aortic valve disease.

**Clinical Implications**

EBCT, by enabling the quantitative, accurate, and reproducible measurement of AVC, provides additional noninvasive AS evaluation. Various thresholds provide sensitive or specific diagnostic criteria for severe AS. It may be particularly helpful whenever diagnosis is uncertain, with poor echocardiographic images, or in difficult clinical situations, such as in patients with LV dysfunction or low cardiac output. In addition, for any given AVA, AVC displays notable range and carries independent prognostic implication. Thus, patients with the highest AVC scores are at higher risk and should be offered closer follow-up. Finally, the accuracy, reproducibility, and simplicity of the AVC score make it an important tool in monitoring AS progression. Recent data suggest that statutes may slow AS progression, and AVC scoring appears to be an excellent tool for determining the efficacy of statins in future clinical trials.

**Study Limitations**

EBCT and Doppler echocardiography were not performed simultaneously, but the median delay between the 2 tests (37 days) was small with regard to the slow progression of AS. In addition, the results may be subject to verification bias because both tests were required for eligibility, and large prospective studies using both tests are necessary in view of the present results. The present study was not designed to compare echocardiographic and EBCT assessment of AVC. The physics of ultrasound and x-rays suggest that these 2 tests detect different components of tissue alteration. Future prospective studies will be needed to evaluate the outcome implications of EBCT and Doppler echocardiography in AS. The present data strongly suggest that these 2 tests provide important and complementary information.

**Conclusions**

EBCT is an accurate and reproducible method to quantify AVC. AVC is strongly and negatively associated with AS hemodynamic severity, and EBCT can provide additional noninvasive information for the diagnosis of severe AS. EBCT may be particularly useful in difficult clinical situations, such as in patients with poor echocardiographic windows or severe LV dysfunction. However, the relationship between AVC and AVA is curvilinear, which demonstrates that these 2 measures provide complementary information on the severity of AS, a concept further supported by the powerful prognostic information independently provided by AVC. Therefore, EBCT measurement of AVC is important in the clinical evaluation of patients with AS.

**Acknowledgments**

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Evaluation and Clinical Implications of Aortic Valve Calcification Measured by Electron-Beam Computed Tomography

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