Aortic and Mitral Annular Calcification Are Predictive of All-Cause and Cardiovascular Mortality in Patients With Type 2 Diabetes

Andrea Rossi, MD
Giovanni Targher, MD
Giacomo Zoppini, MD
Mariantonietta Cicoira, MD
Stefano Bonapace, MD
Carlo Negri, MD
Vincenzo Stoico, MD
Pomppilo Faggiano, MD
Corrado Vassanelli, MD
Enzo Bonora, MD

OBJECTIVE—To examine the association of aortic valve sclerosis (AVS) and mitral annulus calcification (MAC) with all-cause and cardiovascular mortality in type 2 diabetic individuals.

RESEARCH DESIGN AND METHODS—We retrospectively analyzed the data from 902 type 2 diabetic outpatients, who had undergone a transthoracic echocardiography for clinical reasons during the years 1992–2007. AVS and MAC were diagnosed by echocardiography, and a heart valve calcium (HVC) score was calculated by summing up the AVS and MAC variables. The study outcomes were all-cause and cardiovascular mortality.

RESULTS—At baseline, 477 (52.9%) patients had no heart valves affected (HVC-0), 304 (33.7%) had one valve affected (HVC-1), and 121 (13.4%) had both valves affected (HVC-2). During a mean follow-up of 9 years, 137 (15.2%) patients died, 78 of them from cardiovascular causes. Compared with patients with HVC-0, those with HVC-2 had the highest risk of all-cause and cardiovascular mortality, whereas those with HVC-1 had an intermediate risk (P < 0.0001 by the log-rank test). After adjustment for sex, age, BMI, systolic blood pressure, diabetes duration, A1C, LDL cholesterol, estimated glomerular filtration rate, smoking, history of myocardial infarction, and use of antihypertensive and lipid-lowering drugs, the hazard ratio of all-cause mortality was 2.3 (95% CI 1.1–4.9, P < 0.01) for patients with HVC-1 and 9.3 (3.9–17.4, P < 0.001) for those with HVC-2. Similar results were found for cardiovascular mortality.

CONCLUSIONS—Our findings indicate that AVS and MAC, singly or in combination, are independently associated with all-cause and cardiovascular mortality in type 2 diabetic patients.
on chronic complications in type 2 diabetic outpatients attending the diabetes clinic at the University Hospital of Verona (13). Briefly, for this analysis we retrospectively analyzed the electronic records of all Caucasian type 2 diabetic outpatients, who regularly attended our diabetes clinic during the years 1992–2007. Of these, we selected all patients with type 2 diabetes who had undergone a first trans-thoracic echocardiography for clinical reasons (e.g., dyspnea, palpitations, chest pain, heart murmur, history of myocardial infarction, electrocardiographic abnormalities, assessment of left ventricular wall thickness, presence of multiple CVD risk factors) at our institution during the same period of time. On the basis of these criteria, 937 patients with type 2 diabetes were identified and included in the final analysis.

The local ethics committee approved the study protocol. The informed consent requirement for this study was exempted by the ethics committee because researchers only accessed retrospectively a deidentified database for analysis purposes.

Clinical and laboratory data
BMI was measured as kilograms divided by the square of height in meters. A physician measured blood pressure in duplicate with a mercury sphygmomanometer (at the right upper arm using an appropriate cuff size) after the patient had been seated quietly for at least 5 min. Subjects were considered to have arterial hypertension if their blood pressure was $\geq 140/90$ mmHg or if they were taking any antihypertensive drugs. Information on medical history and smoking status was obtained from all patients via interviews during medical examinations.

Venous blood samples were drawn in the morning after an overnight fast. Serum lipids, creatinine, and other biochemical blood measurements were determined in the same laboratory using standard laboratory procedures (DAX 96; Bayer Diagnostics, Milan, Italy). LDL cholesterol was calculated by the Friedewald equation. A1C was measured by an automated high-performance liquid chromatography analyzer (Bio-Rad Diamat; Bio-Rad, Milan, Italy); the upper limit of normal for our laboratory was 5.6%.

Glomerular filtration rate was estimated from the four-variable Modification of Diet in Renal Disease study equation (14). Urinary albumin excretion rate was measured from a 24-h urine sample using an immunonephelometric method. The presence of abnormal albuminuria (defined as albumin excretion rate $\geq 30$ mg/day) was confirmed in at least two of three consecutive urine samples (14). The diagnosis of diabetic retinopathy was based on fundoscopy by a single ophthalmologist.

Conventional echocardiography
All echocardiographic examinations were performed at our institution by experienced cardiologists (A.R. and S.B.). Left ventricular (LV) chamber dimensions and wall thickness were measured from M-mode recordings as suggested by the American Society of Echocardiography, and LV mass was calculated using a necropsy validated equation (15). LV ejection fraction (LV-EF) was measured from LV diameters or two-dimensional area changes in systole and diastole (16). Left atrial (LA) diameter was measured from M-mode tracing. Regional LV function was evaluated by means of wall motion score index (WMSI). The left ventricle was divided into 16 segments. Each segment was analyzed individually and scored on the basis of its motion and systolic thickening. LV segments were scored as follows: normal, 1; hypokinetic, 2; akinetic, 3; and dyskinetic or aneurysmatic, 4. WMSI was derived as the sum of all scores divided by the number of LV segments visualized (15). MAC was defined by increased echodensity located at the junction of the atrioventricular groove and posterior mitral leaflet on the parasternal long-axis, short-axis, or apical four-chamber view. AVS was defined as focal or diffuse calcification and thickening of the aortic leaflets with or without restriction of leaflet motion on echocardiography (17). A trans-aortic peak instantaneous velocity $\geq 2.5$ m/s was considered as aortic stenosis.

Mortality follow-up
Vital status on 30 September 2007 was ascertained for all participants through examination of the electronic databases of the Social Health Unit of the Veneto Region, which include all records of deaths that occurred within the Veneto Region as well as the specific causes of death. Causes of death were identified in 100% of subjects. Death certificates were coded by trained nosologists using the ICD-9. Deaths were attributed to CVD causes when ICD-9 codes were 390–459. A selected sample of death certificates was reviewed manually to validate the process.

Statistical analysis
Data are expressed as means $\pm$ SD or percentages. Skewed variables were logarithmically transformed to improve normality prior to analysis (duration of diabetes, triglycerides, and albuminuria). In accord with a previous study (3), we calculated a heart valve calcium (HVC) score combining the presence of calcium at the level of aortic and mitral valve as follows: HVC-0, absence of any thickening or calcification; HVC-1, presence of either isolated AVS or isolated MAC; and HVC-2, coexistence of AVS and MAC. Patients were stratified according to the HVC score. Comparisons between groups were made using one-way ANOVA (for continuous variables) and the $\chi^2$ test (for categorical variables). Univariate survival analysis was performed by the Kaplan-Meier analysis, and the overall significance was calculated by the log-rank test. Cox regression analysis was used to study the association between the HVC score and the risk of all-cause and CVD mortality after adjustment for potential confounders. The model assumptions for the Cox proportional hazards regression models were checked by visual inspection of proportional hazard assumption, Schoenfeld residuals, and covariance matrix. Four forced-entry Cox regression models were performed: an unadjusted model; a model adjusted for age and sex (model 1); a model adjusted for age, sex, BMI, duration of diabetes, smoking status, systolic blood pressure, A1C, LDL cholesterol, estimated glomerular filtration rate (eGFR), history of myocardial infarction, and use of any antihypertensive and lipid-lowering medications (model 2, where the number of subjects with available data for analysis was 792); and a model adjusted for age, sex, LV mass, LV-EF $<50\%$, WMSI, and LA diameter (model 3, where all subjects had available data for analysis). The covariates included in these multivariate regression models were chosen as potential confounding factors on the basis of their significance in univariate analysis or on the basis of their biological plausibility. Results of Cox proportional hazards models are presented as HR (95% CI). All analyses were performed using statistical package SPSS 19.0, and statistical significance was assessed at the two-tailed 0.05 threshold.

RESULTS
Baseline characteristics
Of the initial sample of 937 type 2 diabetic patients, 35 who had either aortic valve prosthesis or rheumatic or congenital aortic valve disease were excluded from analysis.
Thus, 902 (362 women) patients with type 2 diabetes represent the final analytical sample.

Overall, 477 (52.9%) patients were free of any thickening or calcification at the valvular level (i.e., HVC-0), and 304 (33.7%) patients had either isolated MAC or isolated AVS (HVC-1); i.e., 43 (4.8%) patients had isolated MAC and 261 (28.9%) patients had AVS, and 17 of them had aortic stenosis. The remaining 121 (13.4%) patients had combined AVS and MAC (HVC-2), and 39 of them had aortic stenosis.

As shown in Table 1, age, systolic blood pressure, LDL cholesterol, albuminuria, and the prevalence of hypertension significantly increased, whereas BMI, triglycerides, and eGFR decreased across the HVC score. Sex, smoking history, duration of diabetes, frequency of diabetic retinopathy, fasting plasma glucose, A1C, and HDL cholesterol did not significantly change across the HVC score. Among the echocardiographic parameters, only LA diameter increased across the HVC score, whereas LV mass, LV-EF, and WMSI were not significantly different among the groups.

**Survival analysis**

During a mean follow-up period of 9 years (median 7.6 years), 137 (15.2% of total) patients died (78 of these from CVD causes). As shown in Fig. 1A, the HVC score was strongly associated with an increased risk of death from all causes and from CVD causes. During the follow-up period, patients with HVC-2 had an ~20% overall survival rate compared with ~80% of those with HVC-0 and with an intermediate survival rate (~40%) in those with HVC-1. The differences among these curves were statistically significant (P < 0.0001 by the log-rank test).

Similar results were found for CVD mortality (Fig. 1B).

In Cox univariate survival analysis (see unadjusted models in Tables 2 and 3), both HVC-1 and HVC-2 were associated with an increased risk of all-cause and CVD mortality. As also shown in Tables 2 and 3, the results remained essentially unchanged after adjustment for age, sex, BMI, smoking status, systolic blood pressure, duration of diabetes, A1C, LDL cholesterol, eGFR, history of myocardial infarction, and current use of any antihypertensive and lipid-lowering medications (regression models 1 and 2) or after adjustment for age, sex, and other baseline echocardiographic parameters, such as LV mass, LV-EF <50%, WMSI, and LA diameter (model 3).

Notably, other independent predictors of CVD and all-cause mortality (P < 0.01–0.001) were older age, longer duration of diabetes, smoking status, lower eGFR, and greater LV mass (data not shown). The significant, graded association between the HVC score and the risk of both all-cause and CVD mortality was consistent in men versus women (as shown in Supplementary Tables 1–4) and in younger versus older individuals (data not shown).

Almost identical results were also found when separate analyses were performed in those with (n = 210) and those without (n = 692) a previous history of myocardial infarction. In both of these subgroups, increasing HVC score predicted all-cause and CVD mortality, independently of potential confounders. In particular, among those with a history of myocardial infarction, the HVC score was associated with both all-cause mortality (HR 4.9 [95% CI 2.4–10.4], P < 0.001 for HVC-1, and 7.5 [2.7–20.6], P < 0.001 for HVC-2) and CVD mortality (4.2 [1.7–10.9], P < 0.005, and 9.0 [2.6–31.1], P < 0.001) even after adjustment for age, sex, LV mass, LV-EF <50%, WMSI, and LA diameter.

The strong association between the HVC score and mortality was further reinforced by the finding of a positive, graded relationship between the extent of mitral and aortic annular calcification and the clinical outcomes. Indeed, the HR for all-cause mortality was more than doubled in those with both valves affected (unadjusted HR 7.2 [95% CI 4.5–11.4] for those with HVC-2) compared with those with either isolated AVS (3.2 [2.2–4.8]) or isolated MAC (2.4 [1.3–4.1]), respectively. In addition, a stronger association with the risk of mortality was also observed with the amount of calcium at the level of aortic valve; the HR for all-cause mortality increased from 3.2 (95% CI 2.2–4.8) in those with isolated AVS to 4.4 (2.2–7.7) in those with aortic stenosis at baseline. Notably, after exclusion of patients who had aortic stenosis at baseline (n = 56) or developed aortic stenosis during the follow-up (n = 13), the value of HVC score in predicting all-cause mortality remained essentially unchanged (adjusted HR 2.3 [95% CI 1.5–3.5] for HVC-1 and HR 5.1 [3.1–8.5] for HVC-2, respectively). Consistent results were observed for CVD mortality (data not shown).

**Table 1—Baseline clinical, biochemical, and echocardiographic characteristics of type 2 diabetic patients, stratified by HVC score**

<table>
<thead>
<tr>
<th></th>
<th>HVC-0</th>
<th>HVC-1</th>
<th>HVC-2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>477</td>
<td>304</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Female sex</td>
<td>38</td>
<td>41</td>
<td>47</td>
<td>0.18</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66 ± 9</td>
<td>71 ± 8</td>
<td>73 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 5</td>
<td>28 ± 5</td>
<td>28 ± 5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Current smokers</td>
<td>26</td>
<td>26</td>
<td>28</td>
<td>0.81</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>15 ± 9</td>
<td>16 ± 9</td>
<td>17 ± 8</td>
<td>0.09</td>
</tr>
<tr>
<td>Hypertension</td>
<td>91</td>
<td>93</td>
<td>94</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>137 ± 19</td>
<td>139 ± 18</td>
<td>143 ± 20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 ± 9</td>
<td>80 ± 9</td>
<td>80 ± 10</td>
<td>0.84</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>9.0 ± 2.6</td>
<td>8.7 ± 2.7</td>
<td>9.1 ± 2.8</td>
<td>0.54</td>
</tr>
<tr>
<td>A1C</td>
<td>7.6 ± 1.6</td>
<td>7.6 ± 1.5</td>
<td>7.9 ± 1.5</td>
<td>0.28</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>4.9 ± 0.9</td>
<td>5.1 ± 1.0</td>
<td>5.2 ± 0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.75 ± 1.1</td>
<td>1.71 ± 1.4</td>
<td>1.50 ± 0.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>66 ± 19</td>
<td>63 ± 19</td>
<td>63 ± 18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Albuminuria (mg/day)</td>
<td>53 ± 124</td>
<td>75 ± 157</td>
<td>94 ± 180</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diabetic retinopathy</td>
<td>30</td>
<td>33</td>
<td>41</td>
<td>0.18</td>
</tr>
<tr>
<td>LA diameter (mm)</td>
<td>39 ± 5</td>
<td>40 ± 5</td>
<td>42 ± 5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV ejection fraction</td>
<td>59 ± 13</td>
<td>58 ± 12</td>
<td>60 ± 12</td>
<td>0.61</td>
</tr>
<tr>
<td>WMSI</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>0.86</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>246 ± 77</td>
<td>243 ± 92</td>
<td>259 ± 99</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Data are means ± SD or percent. Cohort size, n = 902. P values refer to one-way ANOVA or the χ² test (for categorical variables). Hypertension was defined as blood pressure ≥140/90 mmHg or use of any antihypertensive drug treatment.
**CONCLUSIONS**—To our knowledge, this study is the first to specifically address the value of AVS and MAC in predicting all-cause and CVD mortality in a large sample of type 2 diabetic patients referred for clinically indicated echocardiograms.

The major finding of this “real-world” study is that AVS and MAC, singly or in combination, predicted an increased risk of CVD and all-cause mortality, independently of traditional risk factors, diabetes-related variables, kidney function parameters (eGFR), and other baseline echocardiographic variables (LV mass, LV-EF, WMSI, and LA diameter). Notably, the combined presence of AVS and MAC was more strongly associated with an increased risk of all-cause and CVD mortality than the presence of either item alone. Collectively, the results of this study complement and expand previous observations on the value of AVS and MAC for predicting mortality both in the general population and in other nondiabetic high-risk patient populations (1,4–9,18).

The biological mechanisms accounting for the association of AVS and MAC with an increased risk of all-cause and CVD mortality have still not been fully elucidated. Patients with MAC or AVS, especially those with AVS, may progress to aortic stenosis, which is a pathologic condition that is associated with an increased risk of mortality (19). However, it should also be noted that in our study the strength of the association between the HVC score and the clinical outcomes remained essentially unaltered even after exclusion of patients who either had aortic stenosis at baseline or developed aortic stenosis during the follow-up. Thus, in accord with the results of previous studies (1–3,20,21), we believe that a plausible explanation for our findings is that AVS and MAC are reliable markers of the severity of systemic atherosclerosis. It is known that there are similarities in the histopathologic lesions of AVS, MAC, and coronary atherosclerosis and that these three diseases share multiple CVD risk factors (e.g., hypertension, smoking, dyslipidemia, and diabetes) and common pathophysiologic mechanisms (1–3,18,21). More recent studies suggested that AVS and MAC are not only associated with coronary atherosclerosis but also with atherosclerosis in other vascular beds (21–25). However, since in our study the HVC score strongly predicted CVD mortality independently of traditional CVD risk.

Data are n (%) or HR (95% CI) as determined by means of univariate and multivariate Cox proportional hazard models. Cohort size, n = 902 (except for regression model 2, in which 792 subjects with available data were included). Regression model 1: adjusted for age and sex. Regression model 2: model 1 plus BMI, diabetes duration, smoking history, systolic blood pressure, A1C, LDL cholesterol, eGFR, previous history of myocardial infarction, and use of any antihypertensive and lipid-lowering drugs. Regression model 3: model 1 plus LV mass, LV-EF <50%, WMSI, and LA diameter as detected by means of echocardiography.
factors and other potential confounders, it is also possible to speculate that AVS and MAC, singly or in combination, reflect not only the burden of systemic atherosclerosis (1–3,21,26,27) but also the presence of other underlying conditions, which may predispose patients to active and accelerated atherosclerosis, such as systemic chronic inflammation (21,28), platelet aggregation abnormalities (21,29), and genetic predisposition (30).

Our findings may have important clinical implications. CVD risk stratification is of paramount importance in routine clinical practice. The use of risk estimation systems such as the Framingham Risk Score has been recommended as the cornerstone for the estimation of the CVD risk in persons without previous CVD events (31,32), but their ability to identify patients who will develop future CVD events and death remains limited, particularly in those with type 2 diabetes (33,34).

Imaging techniques have been proposed to improve specificity of CVD risk stratification. Great attention has recently been paid to coronary artery calcium (CAC) score, as measured by computed tomography (35,36). However, the CAC evaluation has some important disadvantages such as its high costs and radiation exposure. Moreover, the incremental value of CAC score to the prediction of major CVD events and death is marginal (37). Conversely, the echocardiographic evaluation of AVS and MAC is easy, reproducible, safe, and cheap (15). Overall, our findings provide strong evidence that AVS and MAC, singly or in combination, are powerful independent predictors of all-cause and CVD mortality in patients with type 2 diabetes. The echocardiographic detection of “asymptomatic” MAC and AVS can identify a subgroup of patients with higher likelihood of diffuse vascular atherosclerotic processes and should prompt a careful evaluation of conventional CVD risk factors and the institution of proper prevention or treatment strategies.

The major limitations of this study are its retrospective, longitudinal design (which does not allow us to draw any firm conclusion about causality) and a possible selection bias of excluding the patients who had missing echocardiographic data at baseline. There was also a relatively small number of clinical events during the follow-up (i.e., the cumulative incidence of all-cause mortality in the whole sample was ~15% [137 deaths]), and, therefore, the results should be interpreted with some caution. In addition, although our statistical models were extensive, unmeasured confounders (e.g., plasma inflammatory biomarkers) could potentially explain the observed associations. Finally, because our sample comprised white type 2 diabetic individuals who were followed at an outpatient diabetes clinic and who were referred for echocardiography for clinical reasons, our results may not necessarily be generalizable to other diabetic populations. The strengths of our study include the relatively large number of participants of both sexes, the long duration of the follow-up, the complete nature of the dataset, and the ability to adjust for several established risk factors and potential confounders.

In conclusion, this study is the first to demonstrate a graded and independent association of AVS and MAC with the risk of all-cause and CVD mortality in a sample of type 2 diabetic patients referred for clinically indicated echocardiograms. Further follow-up studies on larger cohorts of patients are needed to confirm the reproducibility of our results and to determine whether intervening in the progression of calcific annular or valvular disease can reduce the incidence of CVD events and death in patients with type 2 diabetes.

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Heart valve sclerosis and mortality in diabetes


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