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Heart Valve Calcification in Patients with Type 2 Diabetes and Nonalcoholic Fatty Liver Disease

Running Title: Valvular calcification and NAFLD in diabetes

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ABSTRACT

Purpose: Aortic valve sclerosis (AVS) and mitral annulus calcification (MAC) are two powerful predictors of adverse cardiovascular outcomes in patients with type 2 diabetes, but the aetiology of valvular calcification is uncertain. Nonalcoholic fatty liver disease (NAFLD) is an emerging cardiovascular risk factor and is very common in type 2 diabetes, but whether NAFLD is associated with valvular calcification in this group of patients is presently unknown.

Methods: We undertook a cross-sectional study of 247 consecutive type 2 diabetic outpatients with no previous history of heart failure, valvular heart diseases (aortic stenosis, mitral stenosis, moderate or severe aortic and mitral regurgitation) or hepatic diseases. Presence of MAC and AVS was detected by echocardiography. NAFLD was diagnosed by ultrasonography.

Results: Overall, 139 (56.3%) patients had no heart valve calcification (HVC-0), 65 (26.3%) patients had one valve affected (HVC-1) and 43 (17.4%) patients had both valves affected (HVC-2). 175 (70.8%) patients had NAFLD and the prevalence of this disease markedly increased in patients with HVC-2 compared with either HVC-1 or HVC-0 (86.1% *vs*. 83.1% *vs*. 60.4%, respectively; $p<0.001$). NAFLD was significantly associated with AVS and/or MAC (unadjusted-odds ratio 3.51, 95%CI 1.89–6.51, $p<0.001$). Adjustments for age, sex, waist circumference, smoking, blood pressure, hemoglobin A1c, LDL-cholesterol, kidney function parameters, medication use and echocardiographic variables did not appreciably weaken this association (adjusted-odds ratio 2.70, 95%CI 1.23-7.38, $p<0.01$).

Conclusions: Our results show that NAFLD is an independent predictor of cardiac calcification in both the aortic and mitral valves in patients with type 2 diabetes.

Key-words: aortic valve sclerosis; heart valve calcification; mitral annulus calcification; nonalcoholic fatty liver disease; type 2 diabetes

List of abbreviations:

AVS, aortic valve sclerosis
BMI, body mass index
eGFR, estimated glomerular filtration rate
HbA1c, hemoglobin A1c
HVC, heart valve calcification
IHD, ischemic heart disease
IVRT, isovolumetric relaxation time
LV, left ventricular
MAC, mitral annulus calcification
NAFLD, nonalcoholic fatty liver disease
SAC, systemic arterial compliance
SVR, systemic vascular resistance

INTRODUCTION

Aortic valve sclerosis (AVS) is present in approximately 30% of adults >65 years of age [1,2]. For decades, this disease was thought to be a passive process of little or no clinical significance in which the valve degenerated with age in association with calcium accumulation. However, AVS shares histopathological and epidemiological features with coronary atherosclerosis [2], and large prospective studies have now documented that AVS is independently associated with increased cardiovascular mortality and morbidity both in the general population and in non-diabetic high-risk patient individuals [3-6]. Mitral annulus calcification (MAC) is common in the elderly (~15%) and is also now known to be associated with adverse cardiovascular outcomes [6-8]. Recently, we have also shown that the presence of AVS and MAC, singly or in combination, is very common in patients with type 2 diabetes (occurring in up to ~45% of patients), and AVS and MAC are associated with an increased risk of all-cause and cardiovascular mortality, independently of established risk factors [9]. Notably, we showed that the combined presence of AVS and MAC is more strongly associated with increased risk of all-cause and cardiovascular mortality than the presence of either AVS or MAC alone [9].

In parallel, nonalcoholic fatty liver disease (NAFLD) has emerged as a public health problem of epidemic proportions worldwide [10]. NAFLD is highly prevalent in people with type 2 diabetes (occurring in up to 70% of patients) [11-13], and is not only associated with liver-related mortality and morbidity, but is also associated with an increased risk of developing ischemic heart disease (IHD), abnormalities of myocardial function and structure, and cardiac arrhythmias (*e.g.*, atrial fibrillation) [14-16]. Preliminary evidence also suggests that NAFLD is associated with the presence of AVS, independently of established cardiovascular risk factors, in both non-diabetic and type 2 diabetic individuals [17,18]. However, to our knowledge, no studies have tested associations between NAFLD and MAC, or associations between NAFLD and AVS, to determine whether similar (or different) associations occur between NAFLD and AVS compared with NAFLD and MAC, in patients with or without type 2 diabetes. This issue is of clinical significance, because the aortic and mitral valves are

different anatomically and are exposed to different intra-myocardial pressure gradients during the cardiac cycle.

Thus, the aim of this study was to examine whether NAFLD is associated with AVS and MAC (singly or in combination) in a large sample of patients with type 2 diabetes.

MATERIALS AND METHODS

Patients

We studied 247 white consecutive outpatients with type 2 diabetes, who regularly attended the diabetes clinics of the University of Verona and the "Sacro Cuore" Hospital of Negrar. Some data from a part of these patients (n=180) reporting associations between NAFLD and AVS was published previously [18]. For the present analyses, we excluded patients with: (1) a prior history of chronic heart failure, heart valve diseases or prosthetic heart valves, atrial fibrillation, cancer and overt nephropathy; and (2) a prior history of cirrhosis of any etiology or other known causes of chronic liver diseases, including viral hepatitis, hemochromatosis and excessive alcohol intake (defined as >20 g/day for women and >30 g/day of alcohol intake for men, respectively). All women were postmenopausal and did not take hormonal replacement therapy.

The local Ethics Committee approved the study protocol. All participants gave their written informed consent for participation in this research.

Clinical and Laboratory Data

Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Waist circumference was measured at the level of the umbilicus. Blood pressure was measured with a standard mercury sphygmomanometer (at the right upper arm using an appropriate cuff size). Information on alcohol consumption, smoking status and current use of medications was obtained from all patients via interviews during medical examinations.

Venous blood samples were drawn in the morning after an overnight fast. Serum liver enzymes, creatinine (measured using a Jaffé rate-blanked and compensated assay) and other biochemical blood measurements were determined using standard laboratory procedures (DAX 96; Bayer Diagnostics, Milan, Italy). Most participants had serum liver enzymes within the reference ranges in our laboratory, which for serum aminotransferases were 10 to 40 U/L for women and 10 to 50 U/L for men, respectively. Low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald equation. Hemoglobin A1c (HbA1c) was measured by an automated high-performance liquid chromatography analyzer (HA-8140; Menarini Diagnostics, Florence, Italy); the upper limit of normal for the laboratory was 5.6%. The glomerular filtration rate (eGFR) was estimated by the four-variable Modification of Diet in Renal Disease study equation [19]. Albuminuria was measured by an immuno-nephelometric method on a morning spot urine sample and expressed as the albumin-to-creatinine ratio; abnormal albuminuria was defined as an albumin-to-creatinine ratio ≥30 mg/g creatinine.

Presence of IHD was defined as a documented history of myocardial infarction, angina or coronary revascularization procedures. Presence of internal or common carotid artery stenoses was ascertained by echo-Doppler scanning. In all participants, the presence of microvascular complications, such as retinopathy (by fundoscopy after pupillary dilation), peripheral sensory neuropathy (by biothesiometer) and nephropathy (by eGFR and albuminuria measurements) were also recorded.

Echocardiography and Liver Ultrasonography

A 12-lead standard resting electrocardiogram and a transthoracic echocardiographic Doppler evaluation with spectral tissue Doppler analysis (Vivid 7, GE Vingmed, Horten, Norway) were performed within ~1 month of liver ultrasonography in all patients by two experienced cardiologists, who were blinded to the participants' details, including liver ultrasound data. Conventional echocardiography was used to measure left ventricular (LV) diameters, wall thickness, and mass according to standard criteria. LV end-diastolic and end-systolic volumes and ejection fraction at rest were measured at the apical 4-chamber and 2-chamber views (by modified Simpson rule) [20]. Left

atrial maximal volume was measured at the end of LV systole from the apical 4-chamber and 2-chamber views (by modified Simpson rule) [20,21]. Pulsed-wave Doppler was used to measure trans-mitral peak early diastolic velocity (E), peak late diastolic velocity (A) and E-wave deceleration time (Dte). Pulsed-wave tissue Doppler echocardiography of the septal and lateral mitral annulus was also used to measure the early peak (e') and late (a') annular diastolic and systolic (s') tissue velocities [22,24].

Presence of AVS was defined according to international guidelines as either focal or diffuse calcification of the aortic leaflets without significant obstruction to left ventricular outflow, with a trans-aortic peak instantaneous velocity <2.6 m/s [2-9,20]. Presence of MAC was defined as an intense echocardiographic-producing structure with highly reflective characteristics that was located at the junction of the atrio-ventricular groove and the posterior or anterior mitral leaflet on the parasternal long-axis, short-axis, or apical 4-chamber view [6-9,20].

Liver ultrasonography was performed in all participants by two experienced radiologists, who were blinded to the participants' details, including echocardiographic data. Hepatic steatosis was diagnosed on the basis of characteristic ultrasonographic features, *i.e.*, evidence of diffuse hyper-echogenicity of the liver relative to the kidneys, ultrasound beam attenuation, and poor visualization of intra-hepatic vessel borders and diaphragm [25]. It is known that liver ultrasonography has a good sensitivity and specificity for detecting moderate and severe hepatic steatosis (~90-95%), but its sensitivity is reduced when the hepatic fat infiltration identified by liver biopsy is <30% [10,25]. Semi-quantitative ultrasonographic scoring for the degree of hepatic steatosis (mild, moderate or severe) was not available in this study. Grading of hepatic fat content using ultrasonography has been used in previous studies but remains somewhat subjective [25].

Statistical Analysis

Data are expressed as means±SD or proportions. We calculated a HVC score combining the presence of calcium at different valve sites as follows: HVC-0: absence of any calcification at the level of the aortic and mitral valve; HVC-1 presence of either isolated AVS or isolated MAC; HVC-2: coexistence of AVS and MAC [6,9]. Differences in clinical/biochemical characteristics and echocardiographic parameters among patients stratified by the HVC score were tested with the one-way ANOVA for normally distributed variables and the Kruskal-Wallis test for non-normally distributed variables (*i.e.*, diabetes duration, liver enzymes and triglycerides). The X^2 test was used for categorical variables to study differences in proportions or percentages among the groups. Binary logistic regression analysis was also used to examine the association between NAFLD and the HVC score, and to identify the factors that were independently associated with the presence of AVS and/or MAC, which was included as the dependent variable (HVC-0 *vs*. combined HVC-1 and HVC-2). Four forced-entry logistic regression models were performed: an unadjusted model; a model adjusted for age and sex (model 1); a regression model further adjusted for waist circumference, smoking history, HbA1c, LDL-cholesterol, eGFR, diastolic blood pressure and current use of any hypoglycemic, lipid-lowering and anti-hypertensive drugs (model 2); and, finally, a regression model adjusted for the same variables included in model 2 *plus* the E/e' ratio (model 3). The covariates for multivariate regression models were chosen as potential confounding factors based on their significance in univariable regression analyses or based on their biological plausibility (*i.e.*, age, sex, waist circumference, smoking and LDL-cholesterol). Interaction terms were also generated between NAFLD and sex, age and waist circumference in terms of valvular calcification. None of these interaction terms was statistically significant in the fully adjusted regression models. P-values <0.05 were considered statistically significant.

RESULTS

Of the 247 patients included in the study, 175 (70.8%) patients met the clinical criteria for a diagnosis of NAFLD (*i.e.*, hepatic steatosis on ultrasonography among patients who did not have excessive alcohol consumption, viral hepatitis or other known causes of hepatic steatosis) and 72 patients did not. No patients had clinical, biochemical characteristics (including platelet count, liver enzymes, albumin and prothrombin time) or ultrasonographic findings suggestive of cirrhosis or portal hypertension (coarse liver texture or splenomegaly).

Overall, 139 (56.3%) patients had no heart valve calcification (HVC-0), 65 (26.3%) patients had either isolated AVS or isolated MAC (HVC-1), of whom 42 patients had isolated AVS and 23 had isolated MAC. The remaining 43 (17.4%) patients had combined AVS and MAC (HVC-2). No patients had aortic stenosis (*i.e.*, defined as a trans-aortic peak instantaneous velocity ≥2.6 m/s), bicuspid aortic valve disease and mitral stenosis. In addition, a few patients had mild aortic/mitral regurgitation, but none of them had moderate or severe aortic and mitral regurgitation.

Table 1 shows the clinical and biochemical characteristics of patients with type 2 diabetes stratified by HVC scores. Diastolic blood pressure, HbA1c and serum gamma-glutamyltransferase levels increased, whereas eGFR levels decreased across HVC scores. Similarly, compared with those with HVC-0, patients with HVC-2 had a greater prevalence of chronic kidney disease (*i.e.*, eGFR <60 ml/min/1.73 m^2 and/or abnormal albuminuria), diabetic retinopathy and insulin treatment, and were more likely to be treated for dyslipidemia and hypertension (especially ACE-inhibitors, angiotensin receptor blockers or calcium-channel blockers). Notably, the prevalence of NAFLD markedly increased in patients with HVC-2 compared with either HVC-1 or HVC-0 (86.1% *vs*. 83.1% *vs*. 60.4%, respectively; p<0.001). Sex, age, BMI, waist circumference, duration of diabetes, systolic blood pressure, pulse pressure, smoking, fasting glucose levels, lipids, serum aminotransferases, presence of carotid artery stenoses, IHD and peripheral sensory neuropathy, and the proportion using beta-blockers and diuretics did not differ significantly among the three groups of patients.

Table 2 shows the main echocardiographic parameters of patients grouped according to their HVC score. Left atrial volume index and E/e' ratio (*i.e.*, an index of LV diastolic filling pressure) increased progressively across the HVC score. No significant differences were observed in LV volumes, LV-ejection fraction, E/A ratio, Dte, tau, IVRT, SAC and SVR index among the three groups of patients.

Figure 1 shows the prevalence of different levels of HVC score among patients with and without NAFLD. Of note, approximately 50% of patients with NAFLD had any valvular calcification at the level of aortic and mitral valves compared with ~20% of patients who did not have NAFLD.

Table 3 shows the effect of the adjustment for potential confounders on the association between NAFLD and the presence of AVS, MAC or both. In univariable regression analysis, NAFLD was associated with a 3.5-fold increased rate of valvular calcification (unadjusted-odds ratio 3.51, 95% CI 1.89-6.51). Adjustments for age, sex, waist circumference, smoking, HbA1c, LDL-cholesterol, eGFR, diastolic blood pressure and use of medications (model 2) did not appreciably weaken this association. Further adjustment for the E/e' ratio (model 3) did not attenuate the significant association between NAFLD and valvular calcification. Almost identical results were found when patients with a prior history of IHD (*n*=25) were excluded from analysis. Interestingly, as also shown in **Table 3**, other independent predictors of valvular calcification were higher HbA1c and lower eGFR levels. Results remained unchanged even when we additionally adjusted for LV mass index and left atrial volume index (data not shown).

Table 4 shows the effect of the adjustment for the same set of covariates we used in Table 3 on the relationship between NAFLD and AVS or MAC, separately. Consistent with the other results, NAFLD was associated with an approximately 2.5-fold to 3-fold higher risk of having AVS or MAC after adjustment for potential confounders.

DISCUSSION

The major finding of our study is that the prevalence of NAFLD was highest in patients with coexisting AVS and MAC (*i.e.*, HVC-2), intermediate in those with either isolated AVS or isolated MAC (HVC-1), and lowest in those without valvular calcification (HVC-0). Notably and most importantly, the association between NAFLD and HVC score was independent of multiple cardiovascular risk factors and potential confounders, including diabetes-related variables, eGFR, use of medications, and echocardiographic parameters. Of note, other independent predictors of valvular calcification in these patients were lower eGFR values and poor glycemic control.

To date, very little information is currently available regarding the association between NAFLD and presence of calcification of the aortic and mitral valves, especially in people with type 2 diabetes, and it is uncertain whether similar associations exist between both NAFLD and AVS and NAFLD and MAC. It is known that the process of calcification of the aortic and mitral valves is associated with established risk factors for atherosclerosis, and that valvular calcification shares many of its histological and molecular characteristics with atherosclerosis [1,2]. It is also known that the presence of diabetes is predictive of poor prognosis in native valve disease and of faster degeneration of implanted bio-prosthetic aortic valves [26,27]. Similarly, increasing evidence indicates that valvular calcification independently predicts adverse cardiovascular outcomes among patients with end-stage kidney disease [28,29]. However, it is important to note that the Framingham Heart Study investigators have recently shown that decreased kidney function is significantly associated with the presence of valvular calcification before the onset of end-stage kidney disease in middle-aged individuals [30]. Similar findings were also noted by the investigators of the Cardiovascular Health Study in a community-based cohort of older adults [31] and by the investigators of the Multi-Ethnic Study of Atherosclerosis in a racially and ethnically diverse middle-aged population [32].

Our findings expand to diabetic patients the recent observations by Markus *et al.* who showed that fatty liver on ultrasonography is associated with an increased prevalence of AVS, independently of multiple cardiovascular and metabolic risk factors, in a cohort of 2,212 German men and women (aged

45–81 years) [17]. However, these authors did not analyze the association between fatty liver and MAC, or between fatty liver and combined AVS and MAC. In addition, they did not perform separate statistical analyses stratifying by diabetes status. More recently, in a sample of 180 patients with type 2 diabetes, we found that NAFLD and AVS were strictly inter-related after adjustment for major confounders [18]. In that study, however, we did not observe any significant association between NAFLD and the presence of MAC (unadjusted-OR 1.32, 95% CI 0.6-2.9, p=0.52) [18]. Although it is possible that our failure to observe an association between NAFLD and MAC may have been due to the relatively small sample size and inadequate power to detect an association, it was also plausible that different associations exist between NAFLD and MAC as exist for NAFLD and AVS.

To our knowledge, this is the largest cross-sectional study reporting a significant association between NAFLD and MAC, and between NAFLD and combined AVS and MAC in a sample of type 2 diabetic individuals with preserved LV systolic function.

Large prospective studies reported that AVS and MAC are independently associated with an increased risk of future cardiovascular events [3-8]. In addition, the presence of MAC, but not of AVS, is independently associated with an increased risk of incident atrial fibrillation (AF) and improves AF risk prediction [33]. This suggests that the presence of MAC may not only mark increased cardiovascular risk, but in some cases may increase the risk of incident AF. Overall, therefore, our findings suggest that calcification of the aortic and mitral valves may represent a further link underpinning the increased risk of developing both cardiovascular disease and AF observed in patients with NAFLD. These findings further highlight the clinical importance of evaluating risk of cardiovascular disease among patients with NAFLD [10,14-16]. Our findings also suggest that the echocardiographic detection of MAC and/or AVS in type 2 diabetic patients with NAFLD may identify a subgroup of patients, who are at higher risk of developing cardiovascular disease and AF than had previously been appreciated. Another clinical implication of our findings is that once diagnosis of echocardiographic AVS or MAC has been made, an aggressive management of modifiable cardiovascular risk factors is essential also to reduce the risk of progression of these

valvular diseases. Future studies are needed to examine whether a change in NAFLD status (either development of new fatty liver, progression to cirrhosis, or resolution of existing fatty liver) over time can modify the risk of progression or regression of AVS or MAC.

To date, the underlying mechanisms responsible for the observed associations between NAFLD and AVS or MAC are incompletely understood. The most obvious explanation for our findings is that the association between NAFLD and calcification of the aortic and mitral valves is simply a marker of coexisting cardiovascular risk factors, including poor glycemic control, decreased eGFR, hypertension, dyslipidemia, smoking and prior IHD. However, in our analyses, the association between NAFLD and valvular calcification persisted after adjustment for these potential confounders. Furthermore, the association between NAFLD and valvular calcification was essentially unaltered after excluding patients with IHD. This suggests that additional mechanisms, beyond classic cardiovascular and metabolic risk factors, could, in part, be responsible for the observed association. Accumulating clinical and experimental evidence indicates that NAFLD may exacerbate hepatic/peripheral insulin resistance, induces atherogenic dyslipidemia and releases a variety of pro-atherogenic mediators (*e.g*., C-reactive protein, interleukin-6, tumor growth factor-beta, osteopontin, plasminogen activator inhibitor-1 and other pro-coagulant and pro-fibrogenic mediators) that play important roles in the pathophysiology of vascular and valvular calcification [10,11,14,16,34-37]. Preliminary evidence also suggests that the coexistence of obesity-related increases in fat accumulation in the myocardium may additionally exert local adverse effects that result in myocardial and valvular alterations [16]. Mahmod *et al.* showed that myocardial steatosis, as detected by proton magnetic resonance spectroscopy, was common in patients with severe aortic stenosis, and that myocardial steatosis was significantly associated with LV systolic dysfunction among these patients [38]. However, in a recent elegant study assessing the effect of different ectopic fat depots on LV function in non-diabetic men with NAFLD, Granér *et al*. reported that only intra-hepatic triglyceride content and visceral adipose tissue were independent predictors of LV diastolic function, whereas myocardial steatosis, epicardial and pericardial fat were not significantly associated with diastolic function measures [39]. This further supports the possibility that the association between NAFLD and

valvular calcification may be because of toxic systemic effects. However, further studies are needed in order to improve understanding of these issues.

Collectively, although the potential role of NAFLD in the pathophysiology of vascular and valvular calcification requires further confirmation in larger well-designed studies, we believe that this is a promising field of research to explore, and that the pathways that involve the contribution of NAFLD *per se* to chronic inflammation, hypercoagulation and activation of Wnt signaling pathway might provide a potential therapeutic target for the treatment and prevention of vascular and valvular calcification in diabetic people with NAFLD [1,36,37,40-42]. In line with our data, it is also possible to hypothesize that another underlying mechanism by which NAFLD may promote vascular and valvular calcification could be partly mediated by the development of kidney damage [43,44].

Our study has several important strengths, including the large sample size, the completeness of the dataset, and the ability to adjust for multiple risk factors and potential confounders, including diabetes-related variables, kidney function parameters and use of medications. In addition, our patients were free of cirrhosis; we believe that the inclusion of patients with such complication would have confounded the interpretation of data.

Despite the comprehensive nature of the data set, there are some important limitations to our study. Firstly, the cross-sectional design of the study limits our ability to establish the temporality of the observed associations. Secondly, the diagnosis of NAFLD was based on ultrasound imaging and the exclusion of other known aetiological factors of chronic liver diseases, but was not confirmed by liver biopsy. Although some non-differential misclassification of NAFLD on the basis of ultrasonography is likely (*i.e.*, some of our diabetic control patients could have underlying NAFLD despite fairly normal serum liver enzymes and negative ultrasonography examination), this limitation would serve to attenuate the magnitude of our effect measures toward null; thus, our data can probably be considered conservative estimates of the relationship between NAFLD and valvular calcification. Finally, because

our sample comprised white type 2 diabetic individuals who were followed at outpatient clinics, our results may not necessarily be generalizable to other non-white diabetic populations.

In conclusion, these results provide further information about the relationship between NAFLD and heart valve calcification, demonstrating that NAFLD is strongly and independently associated with AVS and MAC (singly or in combination) in patients with type 2 diabetes. Future studies are needed to elucidate whether NAFLD may predict the development and progression of valvular calcification.

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Author contributions: A.M. researched data, contributed to discussion and reviewed/edited the manuscript. M.P., C.B., S.B., P.L., F.V., L.B., I.P., M.D. and L.Z. researched data, reviewed/edited the manuscript. G.Z., E.B., C.D.B. and E.B. contributed to discussion and reviewed/edited the manuscript. G.T. analysed the data, wrote the manuscript, and is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data.

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Table 1. Clinical and biochemical characteristics of patients with type 2 diabetes stratified by status of heart valve calcification (HVC).

	HVC-0 (n=139)	***HVC-1 (n=65)***	***HVC-2 (n=43)***	***P value***
Sex (male/female)	105/34	45/20	29/14	0.46
Age (years)	67.6±7	69.1±8	69.7±5	0.12
Body weight (kg)	82.1±14	82.6±14	81.6±15	0.93
Body mass index (kg/m^2)	28.8±5	28.6±4	29.1±5	0.88
Waist circumference (cm)	97.8±13	97.1±16	98.6±16	0.43
Diabetes duration (years)	13±10	14±10	14±9	0.49
Systolic blood pressure (mmHg)	142±17	145±15	141±13	0.34
Diastolic blood pressure (mmHg)	77±8	81±9	79±8	<0.05
Pulse pressure (mmHg)	63±14	64±14	64±12	0.98
Mean arterial pressure (mmHg)	98±10	101±9	99±10	0.21
Heart rate (bpm)	74±11	75±12	73±8	0.79
Smoking history (%)	30.2	29.2	32.6	0.80
Fasting glucose (mmol/l)	8.1±2.3	7.8±2.1	8.1±2.5	0.55
Hemoglobin A1c (%)	7.1±0.9	7.4±1.6	7.6±1.4	<0.01
Total cholesterol (mmol/l)	4.44±1.0	4.50±1.1	4.33±1.0	0.50
HDL-cholesterol (mmol/l)	1.26±0.3	1.27±0.3	1.22±0.2	0.59
LDL-cholesterol (mmol/l)	2.57±0.8	2.54±0.8	2.41±0.8	0.47
Triglycerides (mmol/l)	1.54±0.7	1.51±0.8	1.76±0.9	0.18
eGFR (ml/min/1.73 m^2)	84.1±19	83.9±18	66.5±24	<0.001
AST (U/l)	20±5	23±6	26±7	0.19
ALT (U/l)	23±5	26±7	30±9	0.08
GGT (U/l)	24±7	33±12	36±14	<0.05
Hypertension (%)	77.4	81.5	91.0	0.08
eGFR <60 ml/min/1.73 m^2 (%)	7.9	7.7	30.2	<0.001
Abnormal albuminuria (%)	12.2	32.3	25.7	<0.05

Diabetic retinopathy, any degree (%)	14.4	15.3	34.9	<0.005
Diabetic sensory neuropathy (%)	13.0	13.8	13.8	0.64
Carotid artery stenosis ≥50 % (%)	17.3	17.0	25.6	0.43
History of IHD (%)	10.0	9.2	11.6	0.92
Oral hypoglycemic drug users (%)	79.1	86.2	62.8	<0.05
Insulin users (%)	36.7	35.3	46.5	<0.05
ACE-inhibitor/ARB users (%)	73.4	72.3	90.7	<0.05
Calcium-channel blocker users (%)	32.4	23.1	44.2	0.07
Diuretic users (%)	40.3	35.4	41.9	0.74
Beta-blocker users (%)	21.6	21.5	18.6	0.91
Statin users (%)	76.2	72.3	90.7	<0.05
NAFLD (%)	60.4	83.1	86.1	<0.001
AVS (%)	0	64.6	100	ND
MAC (%)	0	35.4	100	ND

Sample size, *n*=247. Data are expressed as means±SD or percentages. ND, not determined.

Hypertension was defined as blood pressure ≥140/90 mmHg and/or use of any anti-hypertensive drugs.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ARB, angiotensin receptor blockers; AVS, aortic valve sclerosis; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HVC, heart valve calcification; IHD, ischemic heart disease; MAC, mitral annular calcification.

Table 2. Main echocardiographic characteristics of patients with type 2 diabetes stratified by status of heart valve calcification (HVC).

	HVC-0 (n=139)	***HVC-1 (n=65)***	***HVC-2 (n=43)***	***P value***
LV end-diastolic volume (ml)	105±22	111±26	109±36	0.23
LV end-systolic volume (ml)	38±14	42± 5	43±24	0.16
LV ejection fraction (%)	64±7	62±7	62±8	0.21
LV mass index (g/m^2)	107±27	106±18	111±30	0.62
LA volume index (ml/m^2)	29±9	33±9	35±15	<0.01
E/A wave ratio	0.75±0.2	0.77±0.2	0.77±0.2	0.54
Dte (ms)	259±66	241±61	249±56	0.17
E/e' ratio	8.30±2.3	9.52±2.7	9.97±3.0	<0.001
IVRT (ms)	85±15	85±17	88±15	0.54
Tau (ms)	45±14	47±12	51±14	0.16
SAC (mmHg/ml)	1.10±0.3	1.15±0.4	1.11±0.4	0.64
SVR index ($dyne/s/cm^5$)	2497±792	2504±819	2691±847	0.25

Sample size, *n*=247. Data are expressed as means±SD.

HVC, heart valve calcification; IVRT, isovolumetric relaxation time; LA, left atrial; LV, left ventricular; SAC, systemic arterial compliance; SVR, systemic vascular resistance; Tau, time constant of isovolumic relaxation.

Table 3. Independent predictors of the presence of valvular calcification (defined as presence of AVS, MAC or both) in patients with type 2 diabetes.

Logistic Regression Models	**Odds ratio(s)**	**95% CI**	***P* value**
NAFLD (yes *vs*. no)			
Unadjusted model	3.51	1.89-6.51	<0.001
Adjusted model 1	3.29	1.76-6.16	<0.001
Adjusted model 2	3.88	1.69-8.94	<0.001
Adjusted model 3	2.70	1.23-7.38	<0.01
After excluding patients (*n*=25) with a prior history of ischemic heart disease			
Unadjusted model	3.77	1.95-7.26	<0.001
Adjusted model 1	3.55	1.83-6.90	<0.001
Adjusted model 2	4.12	2.01-9.02	<0.001
Adjusted model 3	3.48	1.44-8.54	<0.005
Other independent predictors of valvular calcifications in adjusted model 2			
HbA1c (%)	1.32	1.05-1.69	<0.05
eGFR (ml/min/1.73 m^2)	0.98	0.97-0.99	<0.005

Sample size, *n*=247 (except for the regression model 3 in which E/e' ratio measurements were available in 238 patients).

Data are expressed as odds ratios±95% confidence intervals (CI) as assessed by either univariable (unadjusted) or multivariable logistic regression analyses.

Other covariates included in multivariable regression models, along with NAFLD, were as follows: **model 1**: age and sex; **model 2**: age, sex, waist circumference, smoking history, HbA1c, LDL-cholesterol, eGFR, diastolic blood pressure, use of any hypoglycemic, lipid-lowering and anti-hypertensive drugs; **model 3**: adjustment for the same variables included in model 2 *plus* E/e' ratio.

Table 4. Independent predictors of the presence of either aortic valve stenosis (AVS) or mitral annulus calcification (MAC) in patients with type 2 diabetes.

Logistic Regression Models for AVS	**Odds ratio(s)**	**95% CI**	***P* value**
NAFLD (yes *vs*. no)			
Unadjusted model	3.58	1.80-7.12	<0.001
Adjusted model 1	3.35	1.67-6.75	<0.001
Adjusted model 2	3.82	1.78-8.14	<0.001
Adjusted model 3	2.65	1.07-6.31	=0.035
Logistic Regression Models for MAC	**Odds ratio(s)**	**95% CI**	***P* value**
NAFLD (yes *vs*. no)			
Unadjusted model	2.54	1.24-5.21	<0.01
Adjusted model 1	2.42	1.18-4.99	<0.01
Adjusted model 2	3.06	1.35-6.98	<0.005
Adjusted model 3	3.01	1.12-7.99	=0.026

Sample size, *n*=247 (except for the regression model 3 in which E/e' ratio measurements were available in 238 patients).

Data are expressed as odds ratios±95% confidence intervals (CI) as assessed by either univariable (unadjusted) or multivariable logistic regression analyses.

Other covariates included in multivariable regression models, along with NAFLD, were as follows: **model 1**: age and sex; **model 2**: age, sex, waist circumference, smoking history, HbA1c, LDL-cholesterol, eGFR, diastolic blood pressure, use of hypoglycemic, lipid-lowering and anti-hypertensive drugs; **model 3**: adjustment for the same variables included in model 2 *plus* the E/e' ratio.

FIGURE LEGENDS

Figure 1. Prevalence of HVC score in patients with type 2 diabetes stratified by NAFLD status. *P*-value <0.001 for the difference by the X^2 test.

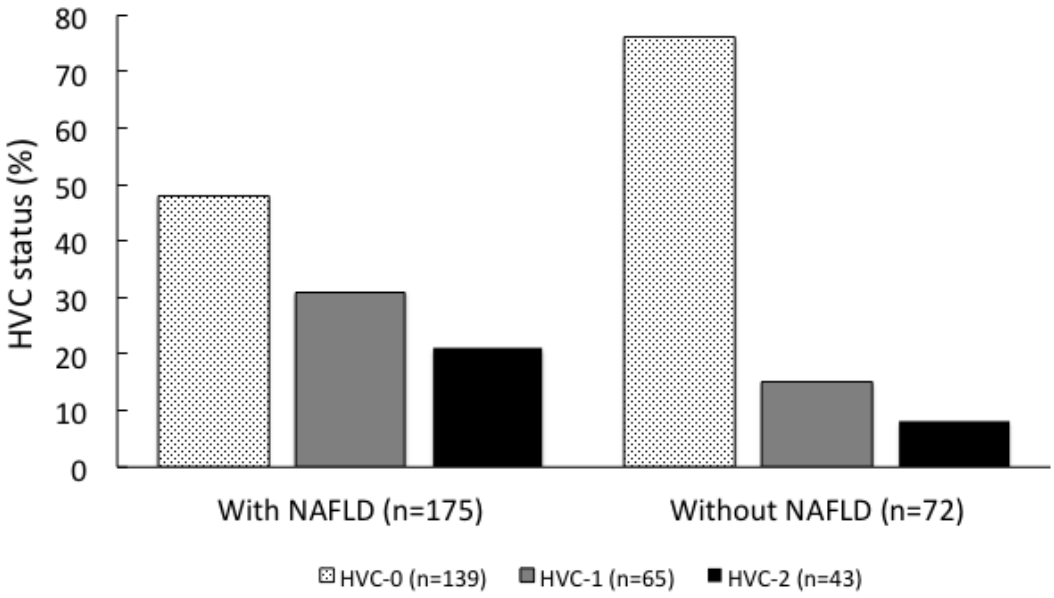

Figure 1