Risk Factors Associated with Left-Sided Cardiac Valve Calcification: A Case Control Study

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Key Words

Valvular heart disease · Calcification · Degenerative valve lesions

Abstract

Objectives: To identify risk factors associated with cardiac valve calcification that is easily detectable through routine blood tests in patients who received valve replacement therapy. **Methods:** Four hundred patients with valvular heart disease who underwent valve replacement surgery between December 2009 and January 2013 were enrolled in this study. Of these, 77 had valve calcification; the other 323 did not. Multivariate logistic regression analysis was used to assess for risk factors associated with valve calcification. **Results:** In our study population, rheumatic valve lesions were the most common reason for valve replacement. Degenerative nonstenotic valve lesion was a protective factor and degenerative stenotic valve lesion was a strong risk factor for valve calcification. Serum levels of gamma-glutamyl transferase (GGT) of between 30 and 46 IU/l and >90 IU/l and total bilirubin (TBIL) of between 15 and 20 μ mol/l were positively correlated with valve calcification. Meanwhile, serum calcium (Ca^{2+}) levels of between 2.3 and 2.4 mmol/l were negatively correlated with rheumatic valve cal-

cification. **Conclusions:** Degenerative stenotic lesion is a risk factor and degenerative nonstenotic lesion a protective factor for cardiac valve calcification. Serum GGT and TBIL levels are positively correlated and serum Ca^{2+} levels negatively correlated with rheumatic cardiac valve calcification.

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Introduction

Valvular heart disease (VHD) contributes to cardiac dysfunction and cardiovascular morbidity and mortality, and affects a substantial subset of the global population (2–4% of adults aged >65 years) [1]. Cardiac valve conditions characterized by significant calcification of the leaflets are still among the most common cardiac valve lesions encountered in clinical practice. One such condition, aortic valve (AV) sclerosis, is the third-most common cardiovascular disease in the USA, and is predicted to have an incidence of 2.8% among patients aged ≥ 70 years [2]. Another condition, in which severe calcification and destruction of cardiac valves occurs, is rheumatic fever.

M.J. and L.W. contributed equally to this study.

Table 1. Baseline characteristics of enrolled patients

	Case patients (calcific valve) (n = 77)	Control patients (noncalcific valve) (n = 323)	p value
Age, years	52.97 ± 10.21	48.92 ± 10.69	0.002
Males	37 (48.1)	120 (37.2)	0.079
Type of surgery			
MV replacement	26 (33.8)	170 (52.6)	0.003
AV replacement	20 (25.9)	41 (12.7)	0.004
AV + MV replacement	31 (40.3)	112 (34.7)	0.359
VHD etiology			
Rheumatic	58 (75.3)	247 (76.5)	0.832
Congenital	6 (7.8)	9 (2.8)	0.038
Degenerative	8 (10.4)	6 (1.9)	0.000
Undefined	5 (6.5)	59 (18.2)	0.011
Infective	0	2 (0.6)	0.489

Values denote n (%), except for age, which is expressed as mean ± SD.

Rheumatic fever has an estimated worldwide incidence of between 5 and 30 million people and is responsible for >90,000 deaths annually [3]. It is estimated that >90% of all cases of mitral stenosis, both in developed and developing countries, are due to rheumatic fever [4, 5]. AV sclerosis and rheumatic fever account for the vast majority of calcific valve lesions seen in clinical practice.

Calcification can impair valve function and lead to cardiac dysfunction, necessitating valve replacement therapy. However, the exact reasons for AV and mitral valve (MV) calcification remain largely unknown. Although some risk factors (including older age, male sex, hypercholesterolemia, etc.) appear similar for AV calcification and atherosclerosis, as many as 50% of patients with calcific aortic stenosis do not have clinically significant atherosclerosis [6]. Meanwhile, less is known about the risk factors for MV calcification of various etiologies. In addition, no factors predicting valve calcification have been identified in clinical practice. In this study, we explore the potential risk factors for left-sided cardiac valve calcification via a retrospective analysis of data pertaining to 400 patients who received valve replacement surgery.

Materials and Methods

Patient Population

We screened all patients with VHD receiving single AV or MV surgery or combined AV and MV surgery between December 2009 and January 2013 at the First Affiliated Hospital of Nanjing Medical University, and found 400 qualifying patients. Patients who had received valve replacement with confirmed histopathological examinations were enrolled for this study. Patients who underwent

valvuloplasty and traumatic valve repair without preexisting valve disease were excluded. The presence of valve leaflet calcification was confirmed by histopathological examinations. Data on baseline characteristics, potential etiology and surgical strategies were collected from electronic medical records. The VHD etiology and classification of valve lesions were identified based on the medical history, primary heart diseases and histopathological results recorded in the medical records according to the definition by Rajamannan et al. [7, 8] and Caira et al. [9].

Measurement and Data Collection

Data collected from each patient's medical record included personal information, results of echocardiography, blood chemistry and pathological examination. Rheumatic, degenerative stenotic, degenerative nonstenotic and congenital causes, age and gender as well as serum levels of calcium (Ca^{2+}), alkaline phosphatase (ALP), retinol-binding protein (RBP), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), lipoprotein a [Lp(a)], creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA), gamma-glutamyl transferase (GGT), total bilirubin (TBIL) and direct bilirubin (DBIL) were stratified for purposes of regression analysis.

Statistical Analysis

Distributions of categorical variables were summarized as frequencies and percentages. Continuous data were summarized as mean ± standard deviation (SD). All categorical data were recorded as number (n), rate (%), odds ratio (OR) and 95% confidence interval (CI). Categorical data were analyzed using the Pearson χ^2 test. Continuous variables were analyzed with the Mann-Whitney U test, chosen as a conservative measure due to the challenges of determining true normality with a small dataset. Multivariate logistic regression analysis was used to assess for valve calcification-associated factors. Variables entered into the model included etiology, age, gender and serum levels of Ca^{2+} , ALP, RBP, TC, TG, LDL-C, Lp(a), Cr, BUN, UA, GGT, TBIL and DBIL. SAS for Windows v9.2 was used for all statistical analyses and $p < 0.05$ (2-sided) was considered statistically significant.

Results

Clinical Characteristics

We identified 418 patients who received cardiac valve surgery from December 2009 to January 2013. Eighteen were excluded for the following reasons: 15 received valvuloplasty therapy during the surgery and no pathological examination results were recorded, and 3 had valvular lesions of traumatic origin without pre-existing valve disease. The remaining 400 patients were enrolled in the study.

Baseline characteristics of the calcification group (case patients) and noncalcification group (control patients) are presented in table 1. The mean age of the case patients was older than that of the control patients (52.97 ± 10.21 vs. 48.92 ± 10.69 years, $p = 0.002$). The percentage of males was comparable in both groups. Compared to the noncalcification group, the percentage of patients with a diseased AV was greater in the calcification group, i.e. 25.9% (20/77) versus 12.7% (41/323), $p = 0.004$. The percentage of patients with a diseased MV was less in the calcification group, i.e. 33.8% (26/77) versus 52.6% (170/323), $p = 0.003$. The percentage of patients with diseased AV and MV was similar in the 2 groups. The etiology analysis, which demonstrated the percentage of patients with rheumatic VHD, was comparable between the 2 groups, but the percentage of congenital, degenerative and undefined VHD in the calcification group was greater than in the noncalcification group (table 1). The distribution of the valve lesion types involved according to different etiologies was also analyzed (fig. 1; table 2). In the rheumatic subgroup, the percentages of patients with simple mitral stenosis were higher in the control group than in the calcification group (46.6 vs. 27.6%; $p = 0.024$). The percentages of patients with combined mitral stenosis and aortic stenosis and regurgitation were higher in the calcification group (13.8 vs. 4.9%; $p = 0.033$). In the degenerative VHD group, aortic stenosis (76.9 vs. 7.7%; $p = 0.000$) and MV collapse (41.5 vs. 7.7%; $p = 0.026$) were the predominant lesion types in the calcification and noncalcification groups, respectively. Bicuspid AV stenosis was the predominant type in the congenital calcification group, but the percentage difference was not statistically significant when compared to the noncalcification group. Comparison of echocardiographic data before surgery showed that the aortic root diameter (AOD), left atrium diameter (LAD) and left ventricular (LV) end-diastolic diameter (LVDd), end-systolic diameter (LVDs), ejection fraction (LVEF) and fractional shortening (LVFS) were comparable between the 2 groups according to the different etiolo-

Fig. 1. Distribution of valve lesion types according to the VHD etiologies and the number of cases. **a** Rheumatic lesion group. **b** Congenital group. **c** Degenerative lesion group. AR = Aortic regurgitation; AS = aortic stenosis; AVC = aortic valve collapse; BAV = bicuspid aortic valve; Ca = case group; Ctrl = controls; MR = mitral regurgitation; MS = mitral stenosis; MVC = mitral valve collapse; MVM = mitral valve malformation.

gies. However, in the rheumatic subgroup, the interventricular septal width (IVS) and LV posterior wall thickness (LVPW) in the calcification group were greater than in the noncalcification group (table 3). Hepatic and renal function blood indexes before surgery were also compared.

Table 2. Distribution of valve and lesion types

Valve lesion types (moderate-to-severe)	Rheumatic Ca (n = 58)	Rheumatic Ctrl (n = 247)	p value
MS	16 (27.6)	115 (46.6)	0.024
MS + MR	9 (15.5)	17 (6.9)	0.062
AS	1 (1.7)	0	n.a.
AS + AR	3 (5.2)	8 (3.2)	0.443
MS + AS	4 (6.9)	15 (6.1)	0.767
MS + AR	10 (17.2)	44 (17.8)	1.000
MS + AS + AR	8 (13.8)	12 (4.9)	0.033
MS + MR + AR	6 (10.4)	31 (12.5)	0.824
MS + MR + AS + AR	1 (1.7)	5 (2.0)	1.000
	Congenital Ca (n = 6)	Congenital Ctrl (n = 9)	
MVM	0	2 (22.2)	n.a.
BAV	6 (100)	5 (55.7)	0.103
AVC	0	1 (11.1)	n.a.
MVM + AVC	0	1 (11.1)	n.a.
	Degenerative Ca (n = 13)	Degenerative Ctrl (n = 65)	
MR	0	7 (10.8)	n.a.
MVC	1 (7.7)	27 (41.5)	0.026
AS	10 (76.9)	5 (7.7)	0.000
AR	0	18 (27.7)	n.a.
AR + AVC	0	4 (6.2)	n.a.
AS + MR	1 (7.7)	1 (1.5)	0.307
AR + MR	1 (7.7)	3 (4.6)	0.536
	Infective Ca (n = 0)	Infective Ctrl (n = 2)	
MVP	0	2 (100)	n.a.

Figures in parentheses are percentages. AR = Aortic regurgitation; AS = aortic stenosis; AVC = aortic valve collapse; BAV = bicuspid aortic valve; Ca = case group; Ctrl = control group; MR = mitral regurgitation; MS = mitral stenosis; MVC = mitral valve collapse; MVM = mitral valve malformation; MVP = mitral valve perforation; n.a. = not applicable.

The serum levels of alanine aminotransferase, aspartate aminotransferase, TBIL, DBIL, indirect bilirubin, TC, TG, albumin and Cr were comparable between the 2 groups. The BUN level was higher in the case group than in the control group in rheumatic patients (online suppl. table S1; see www.karger.com/doi/10.1159/000443203 for all online suppl. material). Coronary artery disease (CAD) is often accompanied by calcific aortic stenosis and mitral annulus calcification. The percentage of concomitant CAD was low in the rheumatic group. A higher percentage of CAD was observed in the congenital and degenerative groups, but the differences were not statistically significant between the 2 groups (online suppl. table S2).

Comparison of Groups for Risk Factors

Etiological factors including the presence of rheumatic, degenerative stenotic, degenerative nonstenotic and congenital VHD, age, gender and the levels of Ca^{2+} , ALP, RBP, TC, TG, LDL-C, Lp(a), Cr, BUN, UA, GGT, TBIL and DBIL were identified as potential risk factors for valve calcification, and were stratified for logistic regression analysis. Multivariate analysis identified several statistically significant differences between the 2 groups. In an all-patient analysis group (table 4), degenerative stenotic lesion was a strong risk factor for valve calcification compared to rheumatic lesion (OR 33.997; 95% CI 2.922–395.490; $p = 0.005$). Degenerative nonstenotic lesion ap-

Table 3. Comparison of cardiac lesion structural diameters by echocardiogram

	Case patients (calcific valve)	Control patients (noncalcific valve)	p value
Rheumatic, n	58	247	
AOD, mm	30.22±3.28	29.44±3.55	0.126
LAD, mm	54.12±14.77	52.30±11.06	0.287
LVDd, mm	51.59±7.52	50.51±8.76	0.391
LVDs, mm	34.62±6.46	33.60±7.29	0.328
IVS, mm	10.43±1.63	9.73±1.28	0.000
LVPW, mm	10.07±1.34	9.57±1.10	0.003
LVEF, %	61.71±6.11	62.08±6.36	0.687
LVFS, %	33.64±4.15	33.79±4.54	0.816
Degenerative stenotic, n	10	4	
AOD, mm	30.20±5.20	31.75±3.20	0.594
LAD, mm	37.20±8.19	35.50±3.70	0.702
LVDd, mm	51.00±6.00	50.50±4.65	0.885
LVDs, mm	34.30±5.66	32.75±3.77	0.627
IVS, mm	13.30±2.21	13.00±2.45	0.827
LVPW, mm	13.00±2.16	12.50±1.91	0.695
LVEF, %	62.89±6.74	63.33±6.22	0.913
LVFS, %	34.35±4.68	34.63±5.66	0.931
Degenerative nonstenotic, n	3	61	
AOD, mm	25.67±5.51	32.62±4.74	0.056
LAD, mm	37.33±3.06	43.74±9.88	0.270
LVDd, mm	56.00±4.00	63.95±9.89	0.173
LVDs, mm	35.67±5.86	42.52±9.16	0.206
IVS, mm	10.33±1.15	10.33±1.48	0.995
LVPW, mm	10.00±1.00	10.15±1.36	0.854
LVEF, %	59.03±5.84	62.14±6.78	0.440
LVFS, %	31.83±4.29	33.89±4.70	0.461
Congenital, n	6	9	
AOD, mm	36.00±5.90	32.11±4.08	0.153
LAD, mm	36.17±4.54	37.00±7.71	0.816
LVDd, mm	50.17±13.26	54.11±10.80	0.537
LVDs, mm	33.83±13.56	35.22±8.32	0.808
IVS, mm	12.00±1.79	10.67±1.80	0.183
LVPW, mm	11.50±2.07	10.33±1.50	0.226
LVEF, %	58.48±10.32	61.51±3.63	0.427
LVFS, %	31.47±6.32	33.51±2.54	0.394

Values are expressed as mean ± SD, unless indicated otherwise.

peared to be a protective factor for valve calcification (OR 0.103; 95% CI 0.022–0.481; $p = 0.004$). Both serum GGT and TBIL levels were associated with valve calcification. When compared to serum GGT levels <30 IU/L, levels between 30 and 46 IU/l and >90 IU/l showed significant statistics for valve calcification (OR 3.174; 95% CI 1.094–9.212; $p = 0.034$ and OR 6.627; 95% CI 1.846–23.794; $p =$

Table 4. Associated risk factors for valve calcification via logistic regression analysis in all patients

	OR	95% CI	p value
GGT			
<30 IU/l*			
30 ≤ GGT < 46 IU/l	3.174	1.094–9.212	0.034
46 ≤ GGT < 90 IU/l	2.097	0.671–6.550	0.202
≥90 IU/l	6.627	1.846–23.794	0.004
TBIL			
<15 μmol/l*			
≥15 μmol/l	2.961	1.163–7.536	0.023
≥20 μmol/l	1.016	0.245–4.218	0.982
Etiology of lesions			
Rheumatic*			
Degenerative stenotic	33.997	2.922–395.490	0.005
Degenerative nonstenotic	0.103	0.022–0.481	0.004

* The first level of candidate factors is used as the control for logistic regression.

Table 5. Associated risk factors for valve calcification via logistic regression analysis in the rheumatic group

	OR	95% CI	p value
GGT			
<30 IU/l*			
30 ≤ GGT < 46 IU/l	4.239	1.250–14.377	0.021
46 ≤ GGT < 90 IU/l	2.235	0.643–7.769	0.206
≥90 IU/l	6.807	1.566–29.589	0.011
TBIL			
<15 μmol/l*			
15 ≤ TBIL < 20 μmol/l	3.088	1.121–8.511	0.029
≥20 μmol/l	1.168	0.249–5.475	0.844
Ca ²⁺			
<2.2 mmol/l*			
2.2 ≤ Ca ²⁺ < 2.3 mmol/l	0.565	0.144–2.219	0.414
2.3 ≤ Ca ²⁺ < 2.4 mmol/l	0.214	0.052–0.879	0.032
≥2.4 mmol/l	0.421	0.108–1.638	0.212

* The first level of candidate factors is used as the control for logistic regression.

0.004, respectively). The significant relative risk ratio of TBIL for valve calcification was serum TBIL levels of between 15 and 20 μmol/l (OR 2.961; 95% CI 1.163–7.536; $p = 0.023$). In the rheumatic group (table 5), GGT and TBIL showed a similar relative risk ratio for valve calcification. Interestingly, serum Ca²⁺ levels appeared to have a protective association with rheumatic valve calcifica-

tion. Ca^{2+} levels of between 2.3 and 2.4 mmol/l showed significantly protective effects on valve calcification (OR 0.214; 95% CI 0.052–0.879; $p = 0.032$). Similarly, gender and serum levels of ALP and Cr were negatively associated, and age, RBP, LDL-C and DBIL positively associated with valve calcification, but the differences were not statistically significant in the all-patient group and in rheumatic patients. TC, TG, Lp(a), BUN and UA levels showed no association with valve calcification based on regression analysis.

Discussion

The major findings of our study include: (1) rheumatic VHD was the most common reason for cardiac valve replacement, (2) valve calcification occurred most often in the degenerative stenotic AV but not in nonstenotic valve lesions, (3) serum GGT and TBIL levels were positively correlated with cardiac valve calcification and serum Ca^{2+} level was negatively correlated with rheumatic valve calcification. From this, we concluded that degenerative stenotic aortic lesion is an associated risk factor, that GGT and TBIL are positive predictors for the presence of cardiac valve calcification, that degenerative nonstenotic valve lesion is a negative predictor for valve calcification and that a normal serum Ca^{2+} level is a negative predictor for rheumatic valve calcification.

Degenerative AV stenosis has become the most common type of VHD in developed countries [10], and it often involves valvular calcification. Whether the AV is especially susceptible to calcification is not clear. Our results showed that the percentage of patients who underwent AV replacement in the calcification group was higher than that in the control group, which indicates AV susceptibility to calcification. A distribution assay of valve lesion types according to VHD etiology and logistic regression analysis indicated that calcific valve lesions occurred predominantly in the congenital and degenerative AV stenotic lesions, but that noncalcific valve lesions occurred in rheumatic and degenerative nonstenotic lesions, both in the MV and the AV. Degenerative stenotic valve lesion showed a strong relative risk correlated with valve calcification, but nonstenotic valve lesion was a negative predictor for valve calcification. Our results suggest that any tendency toward calcification of the AV is dependent on the VHD etiology and the type of valve lesion. It has been suggested that calcific AV stenosis and mitral annulus calcification have similar pathogenesis and share common risk factors with atherosclerotic vascular disease

[11]. In this study, the percentage of concomitant CAD in the congenital and degenerative VHD groups was higher in the calcification group, indicating a trend of degenerative AV calcification accompanied by vascular sclerosis. However, the lower percentage of CAD in the rheumatic group indicates that the mechanism of rheumatic valve calcification is different from that of atherosclerotic calcification. In this study, we observed a significantly larger size of IVS and LVPW in the rheumatic calcification group, suggesting cardiomegaly associated with rheumatic valve calcification. Aortic stenotic lesion leads to an increased afterload and results in cardiomegaly, and mitral stenosis decreases the preload of the LV and results in normal or decreased LV size. Since there was a significant composition difference of valve lesion type between the rheumatic calcification and noncalcification groups, the higher aortic stenosis ratio and lower mitral stenosis ratio in the calcification group might have contributed to the increased LVPW.

Serum GGT level is related to many atherosclerotic risk factors, including hypertension, metabolic syndrome and diabetes [12]. GGT is also considered to be a marker for oxidative stress, which contributes to atherosclerosis. Atherosclerosis and calcific AV disease share some common risk factors and histopathological findings [13]. The controversial relationship between GGT and AV calcification was evaluated in 2 previous studies. In a cross-sectional study, Bozbas et al. [14] showed that serum GGT levels are increased in patients with calcific aortic stenosis and that these increases seem to occur in advanced, rather than milder, forms of calcific AV disease. In another study, Cappelli et al. [15] demonstrated the accumulation of GGT in lipid deposits and cellular elements of stenotic AVs and suggested a common mechanism of lesion shaping underlying both atherosclerosis and degenerative AV disease; however, they found a negative correlation between serum and tissue GGT levels and the severity of valve stenosis. In our study, we analyzed the correlation between GGT and both AV and MV calcification. We found that GGT was positively correlated with cardiac valve calcification regardless of valve type, suggesting that it is a risk factor for calcification and acts via a similar mechanism on both valve types. Further perspective cohort studies are needed to determine the exact role of GGT in heart valve calcification.

Bilirubin is a degradation product of heme and partially reflects the activity of heme oxygenase [16]. Bilirubin inhibits reactive oxygen species formation in vascular endothelium and serves as an antioxidant [17, 18]. In selected cohorts and general patient studies, it has been as-

sociated with a decreased risk for cardiovascular events [19, 20]. Two recent cohort studies reported that the serum bilirubin level is inversely associated with coronary artery calcification by CT scanning, suggesting a protective effect of bilirubin on arterial sclerosis and calcification [21, 22]. There is no report of a relationship between serum TBIL and cardiac valve calcification. Contrary to the results of previous studies on coronary artery calcification, our data showed that an elevated serum TBIL level was positively associated with cardiac valve calcification. We speculate that a potential reason for the discrepancy between results is the differences in the study populations. Ruiz-Gaspa et al. [23] reported that bilirubin inhibits bone formation and that a high bilirubin level leads to osteoporosis in end-stage liver disease. As valve calcification is an ectopic mineralization and has similar mechanisms to bone formation [7, 9], we speculate that bilirubin could not prevent the formation of valve calcification; however, a positive relationship between a higher bilirubin level and valve calcification might indicate a compensatory anticalcification effect of bilirubin. Contrary to bilirubin's role as a protective factor in the general population with cardiovascular risk factors, it appears to be a risk reporter for valve calcification in patients with cardiac valve disease.

Serum Ca^{2+} and phosphorus abnormalities are associated with vascular and heart valve calcification [24, 25]. However, most of the data are from populations with advanced chronic renal disease concomitant with significant Ca^{2+} -phosphorus metabolism dysfunction [26]. Two recent cross-sectional studies on patients with normal kidney function reported that elevated serum Ca^{2+} , phosphorus and Ca^{2+} -phosphorus product levels were the risk factors associated with coronary artery calcification [27, 28]. However, these 2 studies did not report the relation of serum Ca^{2+} level to cardiac valve calcification.

Contrary to results on coronary artery calcification in previous studies, our data showed that serum Ca^{2+} level is negatively correlated with rheumatic valve calcification, and that a serum Ca^{2+} level of between 2.3 and 2.4 mmol/l had the lowest OR. The reason for the discrepancy between results is not clear. In our study population, kidney function was nearly normal, and the relationship was only found in rheumatic etiology; we thus speculate that the difference in VHD etiology between our subjects and those in the previous studies may have contributed to the discrepant results.

Limitations

This study has several limitations. First, the data were collected from a single center and the sample size was relatively small. Further, to maintain study power, the logistic regression analyses used were unconditional, meaning that the matching parameters were not specifically accounted for during the analysis of each risk factor. Finally, the small sample size precluded a multivariable analysis, meaning that independent risk factors were not demonstrated and confounding may therefore have been present.

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Conflict of Interest

The authors have declared that no conflicts of interest exist.

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