Influence of cusp morphology on quantitative valve composition in severe aortic stenosis

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Running title: Cusp morphology and stenotic aortic valve composition

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Abstract

Aims

Aortic stenosis is characterized by fibrosis and calcification of the valve, with a higher proportion of fibrosis observed in women. Stenotic bicuspid aortic valves progress more rapidly than tricuspid valves which may also influence the relative composition of the valve. We aimed to investigate the influence of cusp morphology on quantitative aortic valve composition quantified from contrast-enhanced computed tomography angiography in severe aortic stenosis.

Methods and results

Patients undergoing transcatheter aortic valve implantation with bicuspid and tricuspid valves were propensity matched 1:1 by age, sex, and comorbidities. Computed tomography angiograms were analyzed using semi-automated software to quantify fibrotic and calcific scores (volume/valve annular area) and the fibro-calcific ratio (fibrotic score/calcific score). The study population (n=140) was elderly (76±10 years, 62% male) and had a peak aortic jet velocity of 4.1±0.7 m/s. Compared to those with tricuspid valves (n=70), patients with bicuspid valves (n=70) had higher fibrotic scores (204 [interquartile range 118-267] versus 144[99-208] mm^3/cm^2, p=0.006) with similar calcific scores (p=0.614). Women had greater fibrotic scores than men in bicuspid (224[181-307] versus 169[109- 247] mm^3/cm^2; p=0.042) but not tricuspid valves (p=0.232). Men had greater calcific scores than women in both bicuspid (203[124-355] versus 130[70-182] mm^3/cm^2; p=0.008) and tricuspid (177[136-249] versus 100[62-150] mm^3/cm^2; p=0.004) valves. Among both valve types, women had greater fibro-calcific ratio compared to men (tricuspid 1.86[0.94-2.56] versus 0.86[0.54-1.24], p=0.001 and bicuspid 1.78[1.21-2.90] versus 0.74[0.44-1.53], p=0.001).

Conclusions

In severe aortic stenosis, bicuspid valves have proportionately more fibrosis than tricuspid valves, especially in women.

Key words: aortic stenosis, bicuspid aortic valve, biology, fibrosis, calcification, sex

Abbreviations

CT- Computed tomography

TAVI- Transcatheter aortic valve implantation

Introduction

Aortic stenosis is caused by a complex cycle of pathological insults involving endothelial damage, lipid deposition and inflammation, which eventually leads to fibrosis and calcification (1). The latter are responsible for the characteristic features of leaflet thickening and restricted excursion. Among patients with tricuspid aortic valves, valve calcification has been extensively studied using computed tomography (CT) calcium scoring. It is an established prognostic marker in aortic stenosis (2–5) and provides valuable diagnostic utility in patients with discordant echocardiographic findings (6) where sex-specific thresholds for severe aortic stenosis have been established (7). However, in certain patient populations, valve calcification can show discrepancies with aortic stenosis severity (8) as it ignores the hemodynamic influence of fibrosis. Recent advances in CT angiography and image analysis software now allow the burden of both fibrosis and calcification to be simultaneously measured in the aortic valve. Interestingly, valve fibrosis appears to be of particular importance to the pathophysiology of aortic stenosis in female patients (4,9–11).

Bicuspid valves are common, affecting ~1% of the population (12,13) and patients are at increased risk of developing aortic stenosis (14). Whilst many patients are young, there is an increasing number of elderly patients with multiple comorbidities and bicuspid valves. These patients are often treated with transcatheter aortic valve implantation (TAVI). Whilst some studies have suggested that fibrosis may play a more prominent role in the pathogenesis of aortic stenosis in subjects with bicuspid valves, this has not been systematically investigated, especially in elderly patients. A better understanding of valve composition may improve diagnostic thresholds of grading aortic stenosis severity in bicuspid valves, optimize the timing of valve intervention, and enable the development and targeted deployment of medical therapies. In the present study, our aim was to compare valve composition quantified by CT

angiography, in particular the contribution of fibrosis and calcification, in patients with stenotic bicuspid and tricuspid valves being assessed for TAVI.

Methods

Study population

Patients for this study were selected from a single-center registry of consecutive patients who underwent TAVI between April 2015 and October 2020 at St Bartholomew's Hospital, London, United Kingdom. Patients with bicuspid valves were identified and propensity matched 1:1 to patients with tricuspid valves. Bicuspid valve morphology was defined by the Siever's classification using CT angiography (16). The bicuspid subtype was assessed independently by two readers (KP and GE). Co-variates used for matching included age, sex, previous myocardial infarction or stroke, diabetes mellitus, chronic kidney disease, pulmonary disease, frailty score, and urgency of TAVI (based on whether the patient was admitted for acute decompensated aortic stenosis and had an urgent or elective TAVI). Chronic kidney disease was defined as an estimated glomerular filtration rate <60 mL/min/1.73 m^2. Pulmonary disease was defined as any chronic lung disease that results in abnormal lung function tests. Multivessel coronary artery disease was defined as two or more epicardial coronary stenosis greater than 70% stenosis or left main stem stenosis greater than 50%. Frailty was defined as a Rockwood clinical frailty score greater than 5 (16).

The study was approved by a UK research ethics committee (North-West: Greater Manchester South Research Ethics Committee, United Kingdom; reference number: 21/NW/0182) that waived the need for informed consent given its retrospective observational nature and was secondary use of routinely collected data.

Computed tomography angiography protocol

Pre-interventional planning CT angiography was performed on a Somatom FORCE scanner (Siemens Healthineers, Erlangen, Germany) using a peak tube voltage of 120 kV and collimation of 128 × 0.625 mm. A 100-mL bolus injection of iohexol (Omnipaque 300, GE Healthcare, Chicago, Illinois) contrast was used with bolus triggering in the ascending aorta. Images were acquired craniocaudally, using a FLASH whole-body acquisition (lung apices down to the lesser trochanters). Patients did not receive rate-limiting medications for the purpose of their scans.

Computed tomography angiography image analysis

Semi-automated software (Autoplaque version 2.5, Cedars-Sinai Medical Center, Los Angeles, CA, USA) was used to quantify the tissue composition of the aortic valve using a mediastinal window from pre-TAVI CT angiograms (11) (Figure 1). The best diastolic phase was selected at 70% of the R-R interval. Multiplanar reconstructions were reorientated to the aortic valve plane and the annulus defined (Supplementary Figure 1). Volumes of interest for the valve were contoured around its perimeter on cross-sectional images within this plane and adjusted to exclude the aortic wall. The z-axis was defined between the annulus (defined as a plane linking the most ventricular points of each cusp) and the origin of the coronary ostia. volume. Adaptive scan-specific Hounsfield unit thresholds for fibrotic and calcific tissue components were automatically identified for each patient using Gaussian mixture modelling (Supplementary Figure 2). This method ascertains the range of Hounsfield units for each scan in the blood pool and applies this to the aortic valve fibrotic and calcific tissue, enabling specific tissue types to be identified. The analysis was performed by two experienced observers (KPP and AL) and overseen by two experienced mentors (KG and DD).

To adjust for differences in patient size and annulus dimensions, the tissue volume was indexed to the aortic valve annular area, to provide the fibrotic and calcific scores (Supplementary Figure 1). This indexing step allows for an objective comparison between bicuspid and tricuspid valves which differ in size and has been described before (17,18). In a secondary analysis, we employed a second alternative method to correct for valve size, instead indexing the fibrotic and calcific valve volumes to the average sinus of Valsalva diameter. The average sinus of Valsalva diameter was obtained using three cusp-to-commissure diameters for all valves except type 0 bicuspid valves which had two measurements: cusp-to-cusp and commissure-to-commissure diameters (Supplementary Figure 2).

The sum of the fibrotic and calcific scores provided the fibro-calcific score. The fibrotic score divided by the calcific score provided the fibro-calcific ratio. Our previous study has demonstrated excellent reproducibility for valve composition assessment using FUSIONQUANT software (11).

Echocardiography

Transthoracic echocardiography was performed by British Society of Echocardiography-accredited physiologists prior to TAVI and within 3 months of CT angiography. As per British Society of Echocardiography guidelines (19), measurements of peak aortic jet velocity, mean gradient and aortic valve area were performed for each patient. Where possible, left ventricular ejection fraction was calculated using Simpson biplane method. All patients went through a multi-disciplinary team meeting to confirm the severity of aortic stenosis and decide on the optimal management strategy.

Statistical analysis

All patients with bicuspid valves were propensity score matched 1:1 to patients with tricuspid valves using matching factors determined a priori (age, sex and cardiovascular risk factors) and a greedy nearest neighbor algorithm without replacement were determined and entered into a logistic regression. Normality of continuous variables was evaluated using the Shapiro-Wilk test and presented using the mean ± standard deviation for normally distributed variables and median [interquartile range] for non-normally distributed variables. Frequencies are presented as number (percentage). Baseline characteristics, including demographics, comorbidities, CT, and echocardiography variables were compared between bicuspid and tricuspid patients. Aortic valve scores were compared between all bicuspid and tricuspid patients, with further sub-analyses performed according to sex. Inter-observer variability of valve tissue composition measurements was independently assessed in a random sample of 10 patients by two observers (KP and AL) (Supplementary Results). A 2-sided p value of <0.05 was deemed statistically significant. All analysis were performed using SPSS version 28.0 (SPSS, Chicago IL, United States).

Results

Overall, 1,874 patients were registered in the study database. Seventy-four patients with bicuspid aortic valves were identified, of whom four were excluded due to insufficient image quality. Twenty-three patients had a type 0 valve (no raphe) and 41 patients a type 1 valve (single raphe). Only 1 patient had a type 2 valve (two raphe) Further bicuspid classifications are provided in the supplementary results. These 70 patients with bicuspid valves were propensity matched to 70 patients with tricuspid valves. The study population were predominantly elderly men with severe aortic stenosis. As expected, bicuspid valves had larger aortic root dimensions compared to tricuspid valves (Table 1).

The analysis time per scan ranged from 3-6 min. Measurements of tissue volumes showed excellent inter-observer repeatability with interclass correlation coefficients of 0.928 (0.718-0.982) for fibrotic tissue volume, 0.999 (0.997-1.000) for calcified tissue volume and 0.985 (0.939-0.996) for fibro-calcific tissue volume, with no fixed or proportional biases and very good the limits of agreements (Supplementary Figure 3).

Tissue composition by valve subtype

Compared to those with tricuspid valves, patients with bicuspid valves (n=70) had higher fibrotic scores (144 [99-208] versus 204 [118-267] mm^3/cm^2, p=0.006) and higher fibrocalcific scores (326 [249-416] versus 389 [273-516] mm^3/cm^2, p=0.015) but similar calcific scores (152 [100-230] versus 172 [91-267] mm^3/cm^2, p=0.614) (Figure 2). The fibro-calcific ratio was similar between tricuspid and bicuspid valves: 1.03 [0.56-1.70] versus 1.32 [0.56-2.23], p=0.191. Consistent findings were observed when the alternative method for indexing was used and when assessing non-indexed fibrotic, calcific and fibro-calcific volumes (Figure 2).

There were no demonstrable differences in calcific, fibrotic or fibro-calcific scores between patients with Type 0 and Type 1 valves although there was an apparent trend for higher calcific scores in patients with a Type 0 valve (216 [171-284] versus 139 [63-210] mm^3/cm^2, p=0.051; Table 2).

Valve tissue composition by sex

Women had greater fibrotic scores than men in bicuspid (224 [181-307] versus 169 [109-247]mm^3/cm^2; p=0.042) but not tricuspid valves (184 [94-253] versus 133 [99-187] mm^3/cm^2, p=0.232). Men had greater calcific scores than women in both bicuspid (203 [124-355] versus 130 [70-182] mm^3/cm^2; p=0.008) and tricuspid valves (177 [136-249] versus 100 [62-150] mm^3/cm^2; p=0.004). Among both valve types, women had greater fibro-calcific ratio compared to men (tricuspid 1.86 [0.94-2.56] versus 0.86 [0.54-1.24], p=0.001 and bicuspid 1.78 [1.21-2.90] versus 0.74 [0.44-1.53], p=0.001) (Table 3). Similar results were obtained when fibrotic volumes were indexed to sinus of Valsalva diameters (Supplementary Table 1).

Discussion

To our knowledge, this is the first contrast-enhanced CT angiography study to evaluate quantitative fibro-calcific valve composition in patients with severe aortic stenosis according to valve morphology. Our primary findings are that bicuspid valves have more fibrotic and total tissue, but a similar extent of calcification compared to tricuspid valves. We also confirm prior findings that women have more fibrotic tissue and men have more calcific tissue, but have further demonstrated that this holds true regardless of valve morphology. This has implications for the evaluation of aortic valve disease severity.

Inflammation of the aortic valve promotes the differentiation of valve interstitial cells into activated fibroblasts and thence osteoblasts that drive progressive fibrosis and calcification of the valve respectively (1). Whilst the calcification process appears similar in tricuspid and bicuspid valves, fibrotic tissue is greater in bicuspid than tricuspid valves for a similar hemodynamic severity of disease. Bicuspid valves have more macrophages, T cells and neovascularization (20,21), contributing to a greater inflammatory burden. This in turn probably drives increased fibrosis of the valve leaflets and a disorganized protein-rich extracellular matrix (22).

The influence of sex on aortic valve calcification is well established in tricuspid valves (4,9). Here, we extend these findings to bicuspid valves, demonstrating that women have more fibrosis than men whilst men have more calcification than women. The sex differences in the fibro-calcific ratio were similar regardless of valve morphology. Sex-related differences in the pathophysiology of aortic stenosis therefore appear to apply to bicuspid valves just as they do to tricuspid valves and suggest that different pharmacological treatment strategies may be required in men and women.

Previous studies using the Agatston calcium score have included very few (7% of study population) (4) or no (9) patients with bicuspid valves. Amongst bicuspid valves, correlation between Agatston calcium score and severity of aortic stenosis is strongly dependent on age, with correlations less apparent in younger patients (8). Valve fibrosis has previously been measured using histology of explanted bicuspid valves rather than *in vivo* imaging. These pathological studies have reported discrepant findings, with one demonstrating no differences (10) and another showing more fibrosis among bicuspid compared to tricuspid valves (23). Differences in the prevalence of comorbidities (such as hypertension and coronary artery disease), severity of aortic stenosis and age between the two studies are likely to have accounted for this. To our knowledge, our study is the first to account systematically for these confounders and to use non-invasive CT angiography, which is currently used for TAVI planning and follow-up. Therefore, evaluation of quantitative aortic valve composition from standard CT angiography could potentially be widely applicable.

Two previous studies have compared calcification between bicuspid and tricuspid valves with discrepant results (8,10). One study showed higher calcification with bicuspid compared to tricuspid valves, although aortic stenosis severity was also higher in the bicuspid cohort (10). Another study showed more calcification in tricuspid compared to bicuspid valves. However, patients with bicuspid valves were over 20 years younger and had less comorbidities including diabetes mellitus, hypertension, coronary artery disease and dyslipidemia (8). To our knowledge this is the first study to account for the confounding imposed by aortic stenosis severity, patient demographics, and presence of co-morbidities. We confirmed that fibrosis is indeed greater in bicuspid than tricuspid

valves in both men and women. Fibro-calcific scores have been shown to correlate better with aortic stenosis severity compared to Agatston score in a population with 93% tricuspid valves (17). Among bicuspid valves where fibrotic content is greater, fibro-calcific scores may play an important role in tracking aortic stenosis severity.

Bicuspid valves have larger aortic roots and annuli compared to tricuspid valves (24). Similarly, men have large valves than women. For both of these reasons, it is important to index fibrotic and calcific volumes for the valve annular area – a consistent measurement that is now made to guide valve sizing in all patients undergoing TAVI. The resulting fibrotic and calcific scores subsequently allow comparisons across different patient populations, although many of the observations we made in this study also held true for the unadjusted fibrotic and calcific volumes and when indexing was perfomed based on sinus of valsalva diameters.

Limitations

We acknowledge limitations in our single center study. We could only include elderly patients undergoing TAVI who were at intermediate-to-high surgical risk. Patients undergoing surgical aortic valve replacement were not included as they do not undergo routine planning CT angiography. Thus, our findings may not be generalizable to younger and lower surgical risk populations with aortic stenosis. Finally, we do not have histological validation for our CT angiography-derived valve tissue volumes, although this has been successfully demonstrated in previous studies investigating the fibrocalcific score (17).

Conclusions

In patients with severe aortic stenosis, bicuspid valves had higher quantitative measures of fibrosis with similar measures for calcification when compared to tricuspid valves. There are important sex-specific differences in valve composition, with men having more calcific tissue and women more fibrotic tissue, regardless of valve morphology. Differences therefore exist in the pathophysiology of bicuspid valve disease that may have important implications for detecting earlier disease, grading severity of aortic stenosis and the development of novel pharmacological interventions.

Data availability statement

Data for this study is not available for external review due to ethical restrictions

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Tables

Table 1: Baseline characteristics of the propensity matched study population.

Variables	Tricuspid Aortic Valve (n=70)	Bicuspid Aortic Valve (n=70)	p value
Demographics			
Age (years)	77.0 [69.8- 83.3]	76.5 [70.0-83.3]	0.965
Male sex (%)	45 (64.3%)	42 (60.0%)	0.601
Body surface area (m^2)	1.81 [1.56-2.05]	1.80 [1.60-1.97]	0.759
Co-morbidities			
Logistic Euroscore	9.0 [5.2-14.1]	7.5 [5.3-12.1]	0.522
Estimated glomerular filtration rate (ml/min/1.73m^2)	62 ± 24	60 ± 20	0.705
Diabetes Mellitus	14 (20.0%)	15 (21.4%)	0.835
Chronic kidney disease	33 (47.1%)	32 (45.7%)	0.865
Previous myocardial infarction	8 (11.4%)	10 (14.2%)	0.614
Pulmonary disease	15 (21.4%)	16 (22.9%)	0.839
Previous stroke	4 (5.7%)	8 (11.4%)	0.227
Hypertension	51 (72.9%)	48 (68.6%)	0.577
Multivessel coronary artery disease	17 (24.3%)	10 (14.3%)	0.134
Frailty	7 (10.0%)	9 (13.0%)	0.574
Computed tomography measures			
Average aortic annulus diameter (cm)	2.5 [2.3-2.7]	2.5 [2.2-2.8]	0.633
Average sinotubular junction diameter (cm)	**2.9 ± 0.4**	**3.3 ± 0.5**	**<0.001**

Average annular area (cm^2)	4.7 [4.0-5.3]	4.9 [3.8-5.9]	0.149
Average sinus of Valsalva diameter (cm)	**3.2 [3.0-3.5]**	**3.5 [3.1-3.9]**	**0.007**
Eccentricity index	0.22 ± 0.06	0.20 ± 0.09	0.103
Average ascending aortic diameter (cm)	**3.4 [3.1-3.7]**	**3.9 [3.6-4.3]**	**<0.001**
Maximum ascending aortic diameter (cm)	**3.4 [3.2-3.8]**	**4.0 [3.7-4.4]**	**<0.001**
Valve Agatston score (AU)	2654 [1814-4172]	3019 [1789-4259]	0.803
Echocardiographic parameters			
Left ventricular diameter in diastole (cm)	4.7 ± 0.8	4.9 ± 0.8	0.219
Left ventricular diameter in systole (cm)	3.1 [2.7-3.8]	3.2 [2.8-3.7]	0.591
Anteroseptal wall thickness (cm)	**1.3 [1.1-1.5]**	**1.2 [1.0-1.4]**	**0.015**
Inferolateral wall thickness (cm)	1.1 [0.9-1.3]	1.1 [0.9-1.2]	0.062
Left ventricular ejection fraction (%)	55 [48-59]	55 [40-58]	0.238
Left ventricular stroke volume indexed (ml/m^2)	38.4 ± 12.3	37.1 ± 11.4	0.612
TAPSE (cm)	1.9 ± 0.5	2.0 ± 0.6	0.282
Peak velocity (m/s)	4.2 [3.6-4.6]	4.1 [3.7-4.5]	0.401
Peak gradient (mmHg)	72 [54-85]	68 [54-81]	0.467
Mean gradient (mmHg)	43 [32-54]	40 [32-51]	0.649
Aortic valve area (cm^2)	0.70 [0.54-0.88]	0.64 [0.60-0.90]	0.746

TAPSE- tricuspid annular planar systolic excursion. Data are presented as number (percentage), median [interquartile range] or mean ± standard deviation

Table 2: Valve composition according to bicuspid subtype.

Variable	Type 0 (n=23)	Type 1 (n=41)	p value		Type 2 (n=1)
Fibrotic volume (mm^3)	847 [672-1343]	1015 [607-1308]	0.978		867
Fibrotic score (mm^3/cm^2)	206 [169-292]	204 [121-268]	0.812		225
Calcified volume (mm^3)	1063 [549-1560]	664 [310-1229]	0.236		660
Calcific score (mm^3/cm^2)	216 [171-284]	139 [63-210]	0.051		171
Fibro-calcific volume (mm^3)	2254 [1516-2837]	1718 [1257-2560]	0.481		1527
Fibro-calcific score (mm^3/cm^2)	446 [379-553]	339 [258-519]	0.181		396

The p value denotes to the comparison between type 0 and type 1 bicuspid valve. Type 2 bicuspid valve was not included in this comparison as our study population had only one such patient. Data are presented as median [interquartile range].

Table 3: Valve composition among bicuspid and tricuspid valves by sex.

Variables	Tricuspid Aortic Valve			Bicuspid Aortic Valve		
	Women (n=25)	**Men (n=45)**	**P value**	**Women (n=28)**	**Men (n=42)**	**P value**
Fibrotic volume (mm^3)	720 [325-941]	664 [478-981]	0.745	857 [635-1271]	912 [564-1361]	0.623
Fibrotic score (mm^3/cm^2)	184 [94-253]	133 [99-187]	0.232	**224 [181-307]**	**169 [109-247]**	**0.042**
Calcified volume (mm^3)	**359 [243-587]**	**880 [684-1163]**	**<0.001**	**505 [258-705]**	**1145 [719-2201]**	**<0.001**
Calcific score (mm^3/cm^2)	**100 [62-150]**	**177 [136-249]**	**0.004**	**130 [70-182]**	**203 [124-355]**	**0.008**
Fibro-calcific volume (mm^3)	**1185 [612-1561]**	**1572 [1178-2090]**	**0.003**	**1435 [1020-1962]**	**2293 [1517-3410]**	**<0.001**
Fibro-calcific score (mm^3/cm^2)	307 [177-414]	331 [273-416]	0.58	359 [262-499]	404 [285-554]	0.346
Fibro-calcific ratio	**1.86 [0.94-2.56]**	**0.86 [0.54-1.24]**	**0.001**	**1.78 [1.21-2.90]**	**0.74 [0.44-1.53]**	**0.001**

Data are presented as median [interquartile range].

Figure legends

Figure 1

Assessment of fibrosis and calcification in a patient with tricuspid (left) and bicuspid (right) valves. Based on tailored Hounsfield unit thresholds, fibrosis (red) and calcification (yellow) are quantified, and a 3D representation of the valve tissue composition created (bottom row).

Figure 2

Comparison of tissue volumes and scores according to valve morphology.

Graphical abstract

Influence of sex and bicuspid valve on valve composition in patients with severe aortic stenosis