T lymphocyte infiltration in non-rheumatic aortic stenosis: a comparative descriptive study between tricuspid and bicuspid aortic valves

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Background: The two most common causes of aortic stenosis are primary "degenerative" calcification of tricuspid aortic valves and secondary calcification of congenital bicuspid valves. T lymphocyte infiltration occurs in stenotic tricuspid aortic valves, indicating an inflammatory component, but it has not been shown whether it also occurs in stenotic bicuspid valves.

Objective: To compare non-rheumatic tricuspid and bicuspid stenotic aortic valves for the presence and distribution of T lymphocytes.

Setting: University hospital.

Patients and design: Valve specimens were obtained from 29 patients (15 women, 14 men, mean age 69 years (range 52–81 years)), referred to the hospital for aortic valve replacement because of symptomatic aortic valve stenosis. There were 17 tricuspid valves (from 10 women and seven men, mean age 71 years) and 12 bicuspid valves (from five women and seven men, mean age 67 years). To identify mononuclear inflammatory cells, sections were stained with antibodies for CD3 (pan-T cell antigen, Dako 1:400) and then graded histologically according to the degree of T cell infiltrate.

Results: T lymphocyte infiltration was present in both tricuspid and bicuspid stenotic aortic valves, without any significant differences in extent or localisation.

Conclusions: Stenotic bicuspid aortic valves show the same degree of T lymphocyte infiltration as degenerative tricuspid aortic valves. Inflammation needs to be considered in the pathogenesis of acquired aortic stenosis, irrespective of the primary valve anomaly.

Aortic stenosis is the most common form of valvar heart disease, with a prevalence of 2% in the age group over 65 years.¹ When symptomatic, aortic stenosis carries a poor prognosis unless treated surgically.²

During the past few decades a striking change in the aetiology of aortic stenosis has occurred in the western world.³ There has been a notable decline in the incidence and prevalence of postinflammatory aortic stenosis caused by rheumatic fever.⁴ In parallel with this, degenerative calcification of tricuspid aortic valves and calcification of congenitally bicuspid aortic valves have emerged as the most common causes of significant aortic stenosis.⁵ Stenosis in congenital bicuspid valves develops faster than "degenerative" stenosis in trileaflet valves,⁶ but the molecular mechanisms involved in the pathogenesis of either condition are largely unknown.

Bicuspid aortic valves occur in 1–2% of the population.^{6–9} The less effective stress sharing properties of the bicuspid valve are thought to be the main reason for calcification of the valve. There is lack of agreement on the natural history of the congenitally bicuspid aortic valve. Some authorities believe that bicuspid aortic valves only retain normal function over an entire lifetime in exceptional cases,⁹ while others claim that many bicuspid valves maintain normal function into the eighth or ninth decade.^{7–10} Irrespective of these inconsistent claims, the fact that some individuals with congenitally bicuspid aortic valves do not develop stenosis indicates that factors other than stress may be implicated in the development of stenotic change in these valves.

During the 1990s, T lymphocyte infiltration was found in stenotic tricuspid aortic valves,^{11–13} suggesting that there was an inflammatory component to degenerative aortic stenosis. However, it has not been shown whether stenotic bicuspid valves differ in this respect. Furthermore there has been no attempt to quantify the T lymphocyte infiltration in stenotic

aortic valves. Our aim in this study was therefore to compare specimens from non-rheumatic stenotic tricuspid and bicuspid aortic valves for the presence, degree, and localisation of T lymphocytes.

METHODS

Materials

Thirty nine valve specimens were received for histopathological investigation from 51 consecutive patients referred to Linköping University hospital for aortic valve replacement because of symptomatic aortic stenosis. The diagnosis was made by preoperative Doppler echocardiography. For technical and logistic reasons specimens were not obtained from 12 patients. The clinical characteristics of the patients are given in table 1. There were no significant differences between the two groups in these variables.

Ethical considerations

The study was approved by the ethics committee of Linköping University Hospital. All patients gave informed consent.

Immunohistochemical studies

After fixation in 10% formalin, the aortic valves were measured, examined macroscopically as described by Schoen,¹⁴ and a sample representative of the calcification and fibrous thickening in the valve was taken from each cusp. In valves appearing macroscopically normal, the samples were always taken from the centre of the cusp. After decalcification in 10% formic acid solution for 24 hours the tissue was processed and cut into 4 µm sections. From the representative sample, three sections were used for the histological and immunohistochemical analysis. One reviewer, blinded as to whether the valve was bicuspid or tricuspid, was used throughout all the analysis.

Table 1 Clinical characteristics of patients undergoing valve replacement because of non-rheumatic bicuspid or tricuspid aortic valve stenosis

Characteristic	Bicuspid stenosis (n=12)	Tricuspid stenosis (n=17)
Mean age (years)	67	71
Sex (M/F)	7/5	7/10
Hypertension	3	4
Smoker	6	3
Diabetes mellitus	1	1
Cerebrovascular disease	1	0
Peripheral vascular disease	0	2
Myocardial infarction	0	1
Coronary artery disease/coronary angiograms	4/12	6/17

No significant differences between the groups.

Table 2 Histological grading of mononuclear cell infiltrate* in aortic valve sections

Grade 0	No inflammatory cells present
Grade +	Occasional scattered cells or one group of 20 cells or more in a cusp section
Grade ++	Several groups of 20 cells or more in a cusp section
Grade +++	Many groups of more than 20 cells or one group of 100 cells or more in a cusp section

*After Stratford *et al.*¹⁵

For histological analysis, sections were stained with haematoxylin-eosin and Van Gieson stains. For determination of the type of mononuclear inflammatory cells, additional sections were stained with antibodies for CD3 (pan-T cell antigen, Dako 1:400) and L26 (C20) (pan-B cell antigen, Dako, 1:400) (Dako Corp, Carpinteria, California, USA). The sections were graded histologically according to the degree of mononuclear inflammatory infiltrate, as described by Stratford and colleagues¹⁵ (table 2).

The aortic valves were considered to be of rheumatic origin if there was macroscopic evidence of commissural fusion and fibrous thickening of the apposition (closing) area only, with or without calcification.¹⁴ Microscopic criteria for rheumatic origin included destruction of the cusp architecture and neovascularisation in more than the basal third of the cusp.^{14,16} On the basis of these criteria, 29 patients of the 39 with stenotic aortic valves were considered to have non-rheumatic disease.

The non-rheumatic stenotic aortic valves were divided into tricuspid (n = 17) and bicuspid (n = 12). Ten of the patients in the tricuspid group were women and seven were men (mean age 71 years, range 59–81 years). In the bicuspid group there were five women and seven men (mean age 67 years, range 52–77 years).

The valves in each group were investigated for the presence and localisation of a mononuclear cell infiltrate. The degree of infiltration was determined semiquantitatively (table 1).¹⁵ The extent of valvar calcification was also categorised semiquantitatively as absent (0), mild (+), moderate (++), or severe (+++). In addition we investigated the localisation of the calcification, the degree of cusp thickening, and the localisation of fibrosis.

Statistical analysis

Comparisons between groups were performed by the exact p test, based on the method used by StatXact version 3, Cytel Software Corporation.¹⁷ The data were considered significant at a probability value of $p < 0.05$.

Table 3 Localisation of calcification in stenotic bicuspid aortic valves compared with stenotic tricuspid valves

	Localisation of cusp calcification		
	Base	Apposition	Diffuse
Bicuspid aortic stenosis (n=12)*	1	2	9
Tricuspid aortic stenosis (n=17)*	15	0	2

*Difference between groups: $p < 0.00005$.

Table 4 Degree of mononuclear cell infiltration in stenotic bicuspid aortic valves compared with stenotic tricuspid valves

	Degree of mononuclear cell infiltration			
	0	+	++	+++
Bicuspid aortic stenosis (n=12)*	0	5	4	3
Tricuspid aortic stenosis (n=17)*	2	5	6	4

*No significant difference between the groups ($p = 0.77$).

RESULTS

Calcification, fibrosis, and cusp thickening

Both tricuspid and bicuspid aortic valves showed a moderate to severe degree of calcification, but the bicuspid valves were significantly more affected ($p = 0.03$). While 15 of 17 tricuspid valves had calcification at the base of the cusps, in most of the bicuspid valves (9/12) the distribution of calcification was diffuse (table 3). All valves showed an increase in cusp thickness, but the bicuspid cusps were significantly thicker than the tricuspid ($p = 0.02$). In all the valves except one (a bicuspid valve), fibrosis was generalised.

Mononuclear cell infiltration

There were mononuclear cells in the specimens from all but two of the tricuspid valves and in all the bicuspid valves. There was no significant difference between the tricuspid and the bicuspid valves in the degree of lymphocyte infiltration (table 4). Twelve of the 15 tricuspid valves showed pure lymphocyte infiltration and the three remaining tricuspid valves also contained small numbers of plasma cells. Among the bicuspid valves there were six with pure lymphocyte infiltration and six with a combination of lymphocytes and scanty plasma cells. All the valves contained lymphocytes that stained positively for the CD-3 pan-T cell antigen, but eight of the 15 tricuspid valves and seven of 12 bicuspid valves also contained lymphocytes that stained positively for the CD-20 pan-B cell antigen. However, in 14 of the 15 tricuspid valves with mononuclear cell infiltration and in all the bicuspid valves, there was a dominance of CD-3 pan-T antigen positive cells. Two examples of the histological sections are shown in figs 1 and 2.

A majority of both tricuspid and bicuspid aortic valves showed a general, diffuse mononuclear cell infiltration (16/29). In addition to the generalised distribution, six valves also contained mononuclear cells localised in pericardification or perivascular locations. Only one valve had a mononuclear cell infiltrate exclusively localised to the calcified area, two valves had an exclusively perivascular cell infiltrate, and two valves contained inflammatory cells adjacent to both pericardification and perivascular locations.

Figure 1 Grade 3 lymphocyte infiltration. Photomicrograph of valve specimen from 54 year old man with bicuspid aortic valve stenosis (original magnification $\times 200$). One group of more than 100 lymphocytes (L3+), scattered lymphocytes (L), ventricularis layer (Vl), and spongiosa layer (Sl).

Figure 2 Grade 1 lymphocyte infiltration. Photomicrograph of valve specimen from 67 year old man with tricuspid aortic valve stenosis (original magnification $\times 200$). Group of less than 20 lymphocytes (L1+), calcification (Ca), fibrosis (Fi), normal blood vessel (Bv), fibroblast nuclei (Fn), and cutting artefact (Af).

DISCUSSION

In this study, histological examination of 29 excised stenotic aortic valves and semiquantitative grading of the mononuclear cell infiltrate showed T lymphocyte infiltration in both tricuspid and bicuspid valves, with no significant differences between the two in the extent and localisation of the infiltrate.

Acquired aortic stenosis is caused by rheumatic (post-inflammatory) fusion of the commissures, degenerative calcification of tricuspid aortic valves, and secondary calcification of congenital bicuspid valves.³ With the decline in the prevalence of rheumatic heart disease and the longer survival of the population in general, the spectrum of significant aortic stenosis has changed. Previously, rheumatic heart disease was the main cause of aortic valve replacement. However, among the 154 pure stenotic aortic valves excised at the Mayo clinic in 1990 there were 51% degenerative, 36% bicuspid, and only 9% rheumatic valves⁵—even fewer than in our sample, in which 10 of the 39 cases (26%) were postinflammatory.

Although the prevalence of degenerative aortic stenosis increases with age it is unlikely that age itself constitutes the major risk factor for developing this disease, and other possible mechanisms should be sought instead.

Mechanical stress has often been discussed as an initiating factor. In support of this, it is proposed that the less favourable stress sharing properties of a bicuspid valve give rise to an earlier onset and more rapid progression of stenosis. However, most patients with tricuspid aortic valves show some

inequalities in the individual cusp size.¹⁸ Differences in mechanical stress caused by inequality in cusp size, or by the presence of a bicuspid aortic valve, could contribute to the pathogenesis of the stenosis by accelerating the aging process.¹⁸

Atherosclerosis has also been discussed as a possible factor in the pathogenesis.¹⁹ In the cardiovascular health study, multiple regression analysis showed that age, sex, lipoprotein Lp(a), hypertension, smoking, and low density lipoprotein cholesterol were significant independent risk factors for aortic stenosis.¹ From the same study, Otto and colleagues found that the presence of aortic valve sclerosis without a significant reduction in blood flow through the aortic valve was associated with a 50% increase in cardiovascular death and myocardial infarction.²⁰ Recently Palta and colleagues have shown that the presence of smoking, hypercholesterolaemia, and raised serum creatinine and calcium concentrations increase disease progression.²¹ Most of these risk factors correspond well with the established risk factors for atherosclerosis, even though there is no definite association between aortic stenosis and coronary artery disease. Furthermore, the presence of lipoprotein accumulation in stenotic aortic valves supports the theory that lipoprotein contributes to the pathogenesis of aortic stenosis, and there is a connection between the presence of lipoproteins and calcium deposits or bone formation in the aortic valve.^{22–23} Mohler has identified valvar interstitial cells with osteoblast-like characteristics *in vitro*²⁴; the properties of these cells are stimulated by transforming growth factor $\beta 1$ and 25-hydroxycholesterol.

Presence of T lymphocytes in atherosclerotic lesions has been reported by several investigators,¹⁵ and evidence has accumulated of an inflammatory or even infectious pathogenesis.^{25–26} During the 1990s different groups demonstrated the presence of T lymphocytes in stenotic tricuspid aortic valves,^{11–13} giving rise to the question of a primary or secondary inflammatory component in the pathogenesis of degenerative aortic valve stenosis. By demonstrating the presence of interleukin-2 receptor positive cells in similar locations to T lymphocytes, it was also shown that the T lymphocytes were partly in an active state.¹¹ The presence of lymphocytes in stenotic aortic valves had already been shown in the 1940s and 1950s, but only in association with larger calcium deposits.^{27–28} Histological descriptions of normal aortic valves exist from the same period, and both the normal tricuspid valve and the normal bicuspid valve show a total absence of inflammatory cells and lymphocytes.^{29–30} However, in published reports histological descriptions of the bicuspid stenotic aortic valve are lacking.

In our present study the T lymphocytes showed a general distribution in nine of 17 tricuspid valves and in seven of 12 bicuspid valves. In only one of 29 valves (a bicuspid valve) was the localisation exclusively in calcific deposits. In the remaining valves—both tricuspid and bicuspid—the lymphocytes were localised adjacent to calcification, around vessels, or had a general distribution. This contradicts the assumption that T lymphocytes in stenotic valves constitute an inflammatory response to calcium deposits.

Thus stenotic bicuspid aortic valves show a similar histological picture to stenotic tricuspid valves with respect to T lymphocyte infiltration, but differ in the degree of cusp thickening and in the degree and localisation of calcification. These differences in the localisation of calcification between tricuspid and bicuspid stenotic aortic valves were described by Isner and colleagues in 1990.³¹ One possible conclusion from these results could be that primary degenerative calcification of normal tricuspid aortic valves and secondary calcification of congenital bicuspid valves are not two separate entities. Perhaps the greater degree of cusp anomaly in the bicuspid valve explains the increased cusp calcification and cusp thickening in that situation. The presence of T lymphocytes indicates that inflammation may be involved in the pathogenesis of aortic stenosis. Because of their diffuse distribution, the

T cells might constitute a primary pathogenic factor—that is, a primary autoimmune process leading to disease progression. Another possibility is that the T lymphocytes represent a secondary inflammatory response to other initiating factors—for example, infectious agents^{25,26} or atherosclerotic risk factors. This hypothesis needs to be tested in future research. Considering that bicuspid aortic valves occur in 1–2% of the population and that bicuspid valves constitute 41% of the non-rheumatic stenotic aortic valves in this study, the impact of the bicuspid anomaly should be substantial. However, according to Roberts⁷ and Fenoglio and colleagues,¹⁰ some individuals born with bicuspid aortic valves do not develop aortic valve stenosis or regurgitation. Thus aortic valve dysfunction is not an inevitable consequence of the bicuspid anomaly.

Study limitations

This study was a descriptive comparative study between tricuspid and bicuspid stenotic aortic valves. The finding of a similar degree of T lymphocyte infiltration in these two conditions does not provide any mechanistic information about the pathogenesis of non-rheumatic aortic stenosis. The study cannot determine whether the T lymphocytes play a pathogenic role in development of aortic stenosis, or if they represent a purely reactive change. Previously Olsson and colleagues have shown presence of interleukin-2 receptors in a fraction of T lymphocytes in non-rheumatic tricuspid aortic stenotic valves, possibly indicating that the T lymphocytes represent a specific antigen dependent immune response.¹¹ Though we did not investigate interleukin-2 receptors in our study, the results obtained by Olsson and colleagues suggest that the T lymphocyte infiltration in both bicuspid and tricuspid stenotic valves might constitute a specific immune related inflammatory response.

Our histological and immunohistochemical analyses were made on only a few sections from a representative portion of the valve. We cannot exclude the possibility that false negative results occurred because not all the valve tissue was sectioned.

Conclusions

The pathogenesis of aortic stenosis is still unknown. The congenital bicuspid anomaly and varying degrees of inequality in tricuspid valves may be a morphological substrate on which the disease process rests, but this is not the only factor. The fact that some individuals with bicuspid aortic valves do not develop aortic stenosis indicates that predisposing factors are required—for example, a tendency to develop autoimmune inflammation. Further studies are needed to evaluate this hypothesis.

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REFERENCES

- Stewart BF, Siscovick D, Lind BK, *et al*. Clinical factors associated with calcific aortic valve disease. Cardiovascular health study. *J Am Coll Cardiol* 1997;**29**:630–4.
- Horstkotte D, Loogen F. The natural history of aortic valve stenosis. *Eur Heart J* 1988;**9**(suppl E):57–64.
- Selzer A. Changing aspects of the natural history of valvular aortic stenosis. *N Engl J Med* 1987;**317**:91–8.
- Stollerman GH. Rheumatic fever and other rheumatic diseases of the heart. In: Braunwald E, ed. *Heart disease: a textbook of cardiovascular medicine*, 4th ed. Philadelphia: WB Saunders, 1992:1721–41.
- Dare AJ, Veinot JP, Edwards WD, *et al*. New observations on the etiology of aortic valve disease: a surgical pathologic study of 236 cases from 1990. *Hum Pathol* 1993;**24**:1330–8.
- Ward C. Clinical significance of the bicuspid aortic valve. *Heart* 2000;**83**:81–5.
- Roberts WC. The congenitally bicuspid aortic valve. *Am J Cardiol* 1970;**26**:72–83.
- Larson EW, Edwards WD. Risk factors for aortic dissection: a necropsy study of 161 cases. *Am J Cardiol* 1984;**53**:849–55.
- Sabet HY, Edwards WD, Tazelaar HD, *et al*. Congenitally bicuspid aortic valves: a surgical pathology study of 542 cases (1991 through 1996) and a literature review of 2715 additional cases. *Mayo Clin Proc* 1999;**74**:14–26.
- Fenoglio JJ, McAllister HA, DeCastro CM, *et al*. Congenital bicuspid aortic valve after age 20. *Am J Cardiol* 1977;**39**:164–9.
- Olsson M, Dalsgaard CJ, Haegerstrand A, *et al*. Accumulation of T lymphocytes and expression of interleukin-2 receptors in nonrheumatic stenotic aortic valves. *J Am Coll Cardiol* 1994;**23**:1162–70.
- Otto CM, Kuusisto J, Reichenbach DD, *et al*. Characterization of the early lesions of “degenerative” valvular aortic stenosis. *Circulation* 1994;**90**:844–53.
- Mohler ER, Gannon F, Reynolds C, *et al*. Bone formation and inflammation in cardiac valves. *Circulation* 2001;**103**:1522–8.
- Schoen FJ. Surgical pathology of removed natural and prosthetic heart valves. *Hum Pathol* 1987;**18**:558–67.
- Stratford N, Britten K, Gallagher P. Inflammatory infiltrates in human coronary atherosclerosis. *Atherosclerosis* 1986;**59**:271–6.
- Gallo P, Castelvetero M, Papalia U, *et al*. Postinflammatory scarring of cardiac valves of rheumatic and nonrheumatic etiology. *Am J Cardiovasc Pathol* 1990;**3**:101–5.
- CYTTEL Software Corporation. StatXact 3. *Statistical software for exact nonparametric inference. User manual*. Cambridge, MA: Cytel Software Corp, 1997.
- Vollebergh FE, Becker AE. Minor congenital variations of cusp size in tricuspid aortic valves. Possible link with isolated aortic stenosis. *Br Heart J* 1977;**39**:1006–11.
- Mohler ER. Are atherosclerotic processes involved in aortic-valve calcification? *Lancet* 2000;**356**:524–5.
- Otto CM, Lind BK, Kitzman DW, *et al*. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. *N Engl J Med* 1999;**341**:142–7.
- Palta S, Pai AM, Gill KS, *et al*. New insights into the progression of aortic stenosis: implications for secondary prevention. *Circulation* 2000;**101**:2497–502.
- O'Brien KD, Reichenbach DD, Marcovina SM, *et al*. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of “degenerative” valvular aortic stenosis. *Arterioscler Thromb Vasc Biol* 1996;**16**:523–32.
- Olsson M, Thyberg J, Nilsson J. Presence of oxidized low density lipoprotein in nonrheumatic stenotic aortic valves. *Arterioscler Thromb Vasc Biol* 1999;**19**:1218–22.
- Mohler ER, Chawla MK, Chang AW, *et al*. Identification and characterization of calcifying valve cells from human and canine aortic valves. *J Heart Valve Dis* 1999;**8**:254–60.
- Barron HV, Cannon CP, Murphy SA, *et al*. Association between white blood cell count, epicardial blood flow, myocardial perfusion, and clinical outcomes in the setting of acute myocardial infarction: a Thrombolysis in Myocardial Infarction 10 substudy. *Circulation* 2000;**102**:2329–34.
- Siscovick DS, Schwartz SM, Corey L, *et al*. Chlamydia pneumoniae, herpes simplex virus type 1, and cytomegalovirus and incident myocardial infarction and coronary heart disease death in older adults: the cardiovascular health study. *Circulation* 2000;**102**:2335–40.
- Margolis H, Ziellessen FO, Barnes AR. Calcareous aortic valvular disease. *Am Heart J* 1930;**6**:349–74.
- Sohval AR, Gross L. Calcific sclerosis of the aortic valve (Mönckeberg type). *Arch Pathol* 1936;**22**:477–94.
- Gross L, Kügel MA. Topographic anatomy and histology of the valves in the human heart. *Am J Pathol* 1931;**7**:445–73.
- Koletsky S. Congenital bicuspid aortic valves. *Arch Intern Med* 1941;**67**:129–56.
- Isner JM, Chokshi SK, DeFranco A, *et al*. Contrasting histoarchitecture of calcified leaflets from stenotic bicuspid versus stenotic tricuspid aortic valves. *J Am Coll Cardiol* 1990;**15**:1104–8.