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MORPHOLOGIC STUDIES

Contrasting Histoarchitecture of Calcified Leaflets From Stenotic Bicuspid Versus Stenotic Tricuspid Aortic Valves

JEFFREY M. ISNER, MD, FACC, SAURABH K. CHOKSHI, MD, FACC, ANTHCNY DeFRANCO, MD, JOHN BRAIMEN, MD, GERALYN A. SLOVENKAI, BS

Boston, Massachusetts

Preliminary findings from clinical trials of percutaneous balloon aortic valvuloplasty and intraoperative debridement of calcific deposits in patients with aortic stenosis have suggested that calcified, congenitally bicuspid aortic valves may be less amenable to these techniques than are calcified tricuspid aortic valves. Accordingly, we evaluated the histoarchitecture of calcific deposits in 30 operatively excised aortic valves. Light microscopic sections taken through the calcified aortic valve leaflets disclosed two principal types of histoarchitecture. In 11 aortic valves nodular calcific deposits were superimposed on an underlying fibrotic aortic valve leaflet (type A); in 17 valves calcific deposits were diffusely distributed throughout the body (spongiosa) of the aortic valve leaflets (type B). Two aortic valves could not be classified histologically.

These histologic subtypes were *not* randomly distributed with regard to gross valvular morphology. All 14 bicuspid valves (100%) were type B; in contrast, 11 (69%) of 16 tricuspid aortic valves were type A, and only 3 (19%) of 16 tricuspid valves were type B ($p < 0.01$). Both valves with nonclassifiable histologic features were tricuspid on the basis of gross examination.

Thus, the histoarchitectural distribution of calcific deposits is different for bicuspid than for tricuspid stenotic aortic valves. The more diffuse distribution of calcium throughout the body of calcified bicuspid aortic valve leaflets may render these valves less amenable to operative and percutaneous valvuloplasty than are calcified tricuspid aortic valve leaflets on which calcific deposits are typically superimposed in nodular form.

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Aortic stenosis characterized by extensive calcification of the aortic valve leaflets constitutes the most common form of valvular heart disease in persons older than 40 years (1). Previous studies of stenotic aortic valves examined at necropsy (2–4) or after operative excision (5,6) have indicated that congenitally bicuspid aortic valves account for most cases of aortic stenosis in adults under 70 years; in contrast, among persons beyond the sixth decade of life aortic stenosis most often results from calcification of a congenitally normal tricuspid valve.

Because of the increased mortality associated with this lesion in symptomatic patients (7–9), the onset of symptoms has generally been considered an indication for intervention. Most commonly, such patients have been recommended for aortic valve replacement. Alternatively, liabilities inherent in valvular prostheses have led to renewed interest in valvuloplasty procedures (10–20) designed to preserve the native aortic valve.

Preliminary findings from clinical trials of both percutaneous (18,21–24) and intraoperative (12,13,20) aortic valvuloplasty have suggested that calcified, congenitally bicuspid stenotic aortic valves may be less amenable to such techniques than are calcified stenotic tricuspid aortic valves. Accordingly, the present study was undertaken to investigate the hypothesis that the distribution of calcific deposits in stenotic aortic valves varies according to whether or not the valve is congenitally normal.

Methods

Selection of specimens. All operatively excised aortic valves accessioned by our laboratory were reviewed for

From the Departments of Biomedical Research, Pathology and Medicine (Cardiology), St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, Massachusetts. This study was supported in part by Grants HL32747 and HL40518 from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland; the Eleanor Naylor Dana Foundation, New York, New York; and the John and Cora Davis Foundation, Washington, D.C. This work was presented in part at the 37th Annual Scientific Session of the American College of Cardiology, Atlanta, Georgia, March 1988.

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Address for reprints: Jeffrey M. Isner, MD, St. Elizabeth's Hospital, 736 Cambridge Street, Boston, Massachusetts 02135.

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associated clinical evidence of aortic stenosis. Preoperative cardiac catheterization data were then reviewed to confirm the diagnosis of aortic valve stenosis, including a calculated valve area <0.75 cm^2 and a peak aortic gradient ≥50 mm Hg (25). To diminish the likelihood that rheumatic heart disease might have contributed to aortic valve disease, valves were excluded if there was clinical or operative evidence, or both, of coexisting mitral valve disease (4).

All such valves were then reinspected by gross examination with regard to two criteria. The first criterion required that the anatomic integrity of the valve had been sufficiently preserved during operative excision so that the valve could be classified with certainty on the basis of gross examination as congenitally bicuspid or congenitally tricuspid. The second criterion required that on radiographic examination of the excised valve, calcific deposits could be documented to involve >25% of total leaflet area. A total of 30 specimens fulfilling both of these criteria were thereby identified and selected for analysis in the present study.

Classification of valvular morphology. Aortic valves were classified as congenitally bicuspid or tricuspid according to certain features of the gross examination described previously by Roberts (1–4) and others (5,26–28). Valves were considered to be congenitally bicuspid if the excised valve consisted of two leaflets; the two leaflets were asymmetric; and the larger of the two leaflets, the so-called conjoined leaflet (29), contained an identifiable median raphe.

Preparation of specimens for light microscopic examination. After photographic and radiographic examination of each specimen, each of the two leaflets from each bicuspid valve and each of the three leaflets from each tricuspid valve were partially decalcified by using a solution of 10% formic acid and formalin for 24 to 72 h. A representative section was then removed from each leaflet for light microscopic examination; this section was arbitrarily taken through the largest apparent calcific deposit including the combined full thickness of the calcific deposit and valve leaflet, perpendicular to the broad aspect of the valve leaflet. Each section was then cleared with xylene, impregnated with and embedded in paraffin, cut at 4 μm intervals and stained with hematoxylin-eosin, Richardson's modification (30) of the elastic tissue/trichrome stain and the von Kossa stain for calcium salts.

Light microscopic examination. Each light microscopic slide, prepared as described was labeled with a lettered code so that neither the specimen from which it was taken nor the classification made on the basis of gross examination was disclosed. On completing the light microscopic examination of slides prepared from each specimen, the histoarchitecture was classified as type A, type B or as nonclassifiable. This differential histologic classification is illustrated schematically in Figure 1. In type A, nodular calcific deposits, typically enclosed by a collagen envelope, were superimposed on a fibrotic aortic valve leaflet; in type B, calcific deposits were diffusely distributed throughout the body (spongiosa) of the aortic valve leaflet from the aortic through the ventricular aspect of the leaflet. If the histoarchitecture of the valve could not be accurately classified according to either of these two types, it was considered nonclassifiable.

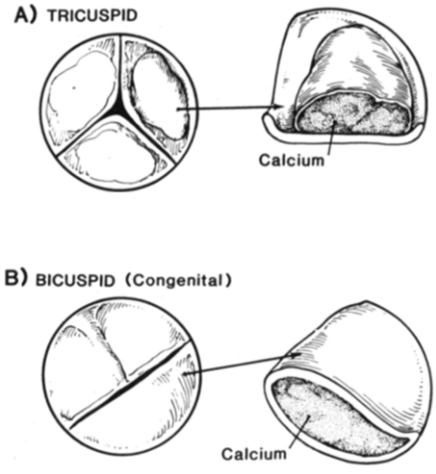

Figure 1. Classification scheme used to describe histoarchitectural distribution of calcific deposits in stenotic aortic valves. In type A, nodular calcific deposits are superimposed on a fibrotic aortic valve leaflet. In type B, calcific deposits are diffusely distributed throughout the body of the aortic valve leaflet from the aortic through the ventricular aspects.

Data analysis. On completion of the light microscopic examination, the identity and gross classification of each valve were disclosed. Chi-square analysis was then used to compare the results of morphologic classification based on gross examination with those based on light microscopic examination. Outcomes were considered statistically significant when the p value was <0.05. Values are expressed as mean values ± SEM.

Results

Clinical features. Aortic valves operatively excised from 30 patients fulfilled the criteria outlined and were submitted for further pathologic examination. The patient's ages ranged from 49 to 87 years (mean 79); 16 (53%) were women. All had documented aortic stenosis with minimal or no aortic regurgitation. Mean aortic valve gradient ranged from 24 to 88 (48 ± 12) mm Hg. Aortic valve area ranged from 0.2 to 1.1 (0.55 ± 11) cm^2.

Gross examination. On the basis of gross pathologic examination, 16 (53%) of 30 valves were classified as congenitally tricuspid valves; each contained extensive calcific deposits. The remaining 14 valves (47%) were classified as congenitally bicuspid; likewise, each contained radiographically documented extensive calcific deposits.

Light microscopic examination. In 11 (37%) of the 30 valves, nodular calcific deposits were superimposed on a fibrotic aortic valve leaflet (type A). In 17 valves (57%),

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calcific deposits were diffusely distributed throughout the body (spongiosa) of the aortic valve leaflet (type B). Two valves could not be classified histologically.

These histologic subtypes were *not* randomly distributed with regard to gross valvular morphology. In each of the 14 valves classified on gross examination as congenitally bicuspid, the distribution of calcific deposits conformed to type B, as defined. A representative example of the gross and light microscopic findings typical of a heavily calcified, stenotic, congenitally bicuspid aortic valve is illustrated in Figure 2.

In contrast, in 11 (69%) of the 16 congenitally tricuspid aortic valves, the histoarchitecture was classified as type A, and the distribution of calcific deposits conformed to type B in only 3 (19%) of these tricuspid valves. A representative example of the gross and light microscopic findings typical of a heavily calcified, stenotic, congenitally tricuspid aortic valve is illustrated in Figure 3. In two cases, a valve that was judged to be a congenitally tricuspid aortic valve did not clearly conform to either type A or type B on light microscopic examination.

The relation between gross valvular morphology and light microscopic distribution of calcific deposits is summarized in Figure 4. Chi-square analysis disclosed that the disproportion of type B histoarchitecture observed among congenitally bicuspid valves versus type A histoarchitecture observed among congenitally tricuspid valves was statistically highly significant ($p < 0.01$).

Discussion

Previous studies of valvular morphology. Congenital deformity of the aortic valve resulting in two, rather than three, leaflets is estimated to be the most frequent form of congenital heart disease, affecting as many as 2% of the population (3). It is furthermore one of the oldest recognized and most thoroughly studied cardiovascular anomalies. Osler (29) described it as the "bicuspid condition" and recognized that "clinically this is a very important congenital defect, owing to the liability of the combined valve [conjoined leaflet] to sclerotic changes." Edwards (26) subsequently attributed such sclerosis to the chronic degenerative effects associated with the aberrant opening and closing of two asymmetric leaflets. Roberts (1–4), in a series of classic studies, defined the clinical liability posed by the congenitally bicuspid aortic valve in terms of valvular stenosis, valvular insufficiency (31) and endocarditis (32).

Despite these studies, surprisingly little information has been published regarding the histologic characteristics of the congenitally bicuspid aortic valve, particularly once it has become heavily calcified. Studies are similarly limited with regard to the light microscopic features of heavily calcified, congenitally tricuspid valves.

It is perhaps understandable that little attention was paid to the histoarchitecture of a rock-like mass of poorly mobile valvular tissue obstructing left ventricular outflow in years characterized by unavailable interventions (presurgical period), ineffective interventions (before prosthetic valve replacement) or a single effective therapeutic response (valvular excision with prosthetic valve replacement).

Valvuloplasty experience. A renaissance of interest in operative (10–15) and percutaneous balloon (16–19) aortic valvuloplasty, however, redirected attention to the specific morphologic features of calcific aortic stenosis. For example, previous experiments performed in our laboratory (11) indicated that laser irradiation could be effectively used in vitro to accomplish near-total debridement of calcific deposits from congenitally tricuspid, but not congenitally bicuspid valves. More recently, experience with manual (12,13) or ultrasonic (15,20) debridement of heavily calcified aortic valves has also disclosed that congenitally bicuspid aortic valves are less amenable to this therapy than are congenitally tricuspid valves. Finally, certain clinical observations (21–24) have suggested that, in contrast to congenitally tricuspid valves, congenitally bicuspid valves are more refractory to and associated with a higher risk of complications from percutaneous balloon valvuloplasty.

Clinical implications. The contrasting histoarchitectural patterns described in this study provide a possible explanation for these previous observations. Distribution of calcific deposits according to the pattern arbitrarily designated as type A implies that a cleavage plane may be established between the calcific nodule and underlying fibrotic leaflet. Accordingly, such valves would be expected to be amenable to intraoperative manual or ultrasonic debridement. In contrast, the calcific deposits in a type B valve are distributed as such an integral part of the valve leaflet that attempts to remove the calcific deposits necessarily violate the integrity of the valve leaflet.

The fact that *all* bicuspid valves in the present study were type B suggests that few exceptions regarding debridement opportunities will be found. The distribution of type A versus type B among tricuspid valves suggests that although most tricuspid valves will prove appropriate for intraoperative debridement, there will be some in which debridement will not be possible.

A second potential implication of the present findings is the possibility that the pattern by which the calcific deposits are distributed within the valve leaflet determines whether or not such a valve will prove amenable to balloon valvuloplasty. It is possible, but as yet unproved, that fracture of calcific deposits (17,21,22) may be more readily accomplished when the calcium is deposited in nodular form on an underlying leaflet rather than distributed throughout the full thickness of the leaflet. Perhaps this explains the apparent increased refractoriness of the bicuspid valve to balloon dilation.

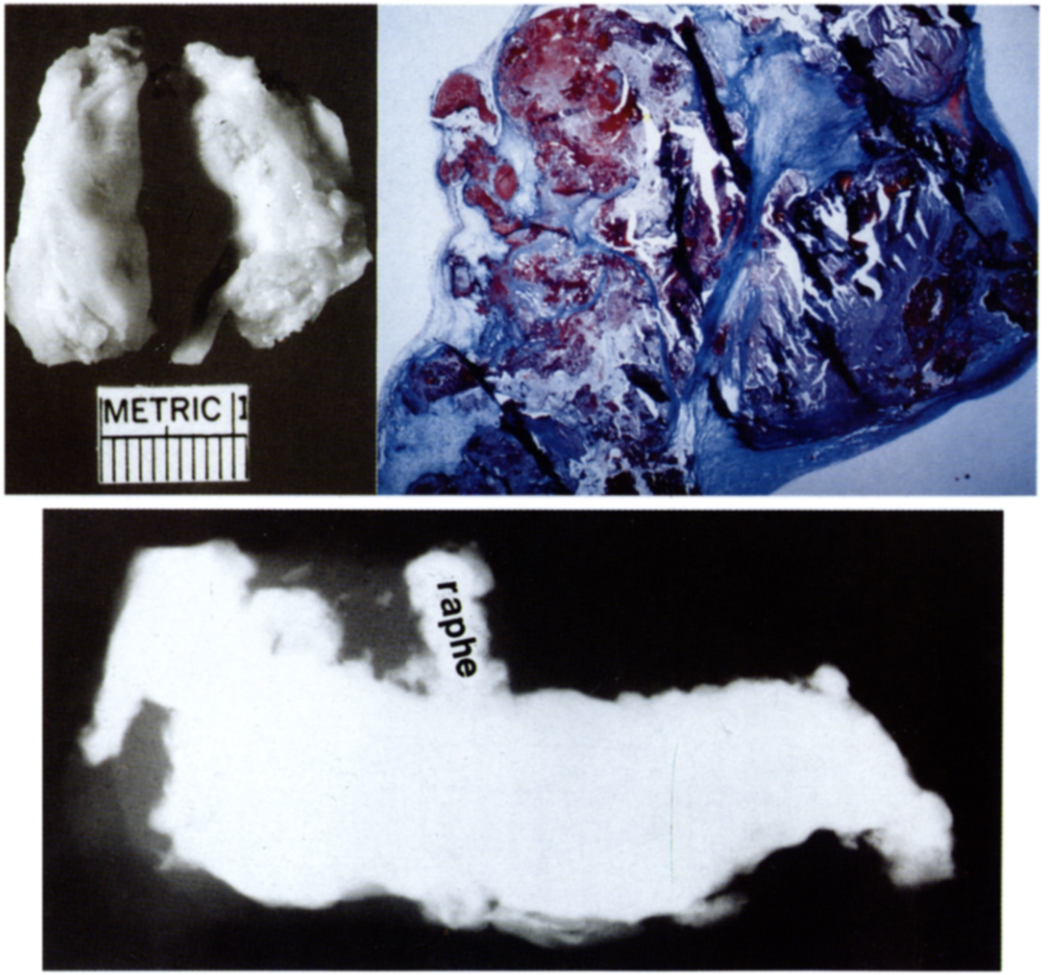

Figure 2. Gross and light microscopic findings in an operatively excised heavily calcified, congenitally bicuspid aortic valve. Gross findings: the valve consists of two asymmetric leaflets; the larger of the two includes a calcified median raphe. X-ray findings (**bottom panel**): saggital section taken through the larger of the two leaflets discloses calcific deposits from the aortic to the ventricular aspects. Histologic findings (**upper right panel**): Richardson's stain (×5) confirms presence of transleaflet calcific deposits from the aortic to the ventricular aspects.

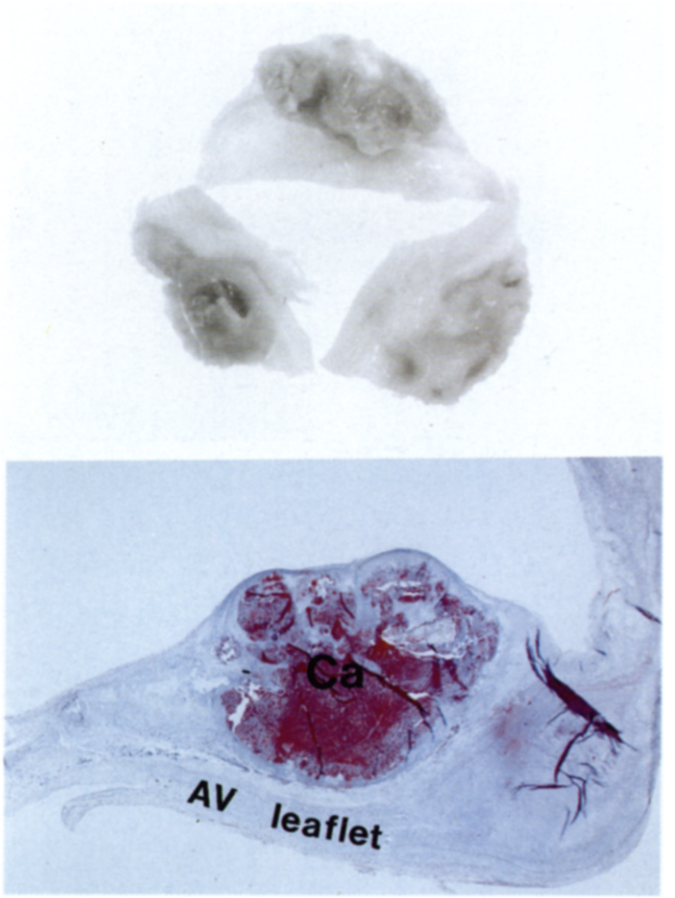

Figure 3. Gross and light microscopic findings in an operatively excised, heavily calcified, congenitally tricuspid aortic valve. Gross findings (**upper panel**): the valve consists of three leaflets, each of which contains large, nodular calcific deposits. Histologic findings (**lower panel**): hematoxylin-eosin stain (×5) confirms a nodular calcific deposit (Ca) enclosed by a collagen envelope superimposed on the underlying fibrotic aortic valve (AV) leaflet.

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GROSS EXAM	HISTOLOGIC EXAM: A	B	Neither
Bi	0	14	0
Tri	11	3	2

Figure 4. Relation of gross valvular morphology and light microscopic distribution of calcific deposits for specimens studied. Chi-square analysis disclosed that the disproportion of type B histoarchitecture observed among congenitally bicuspid (Bi) valves versus type A histoarchitecture observed among congenitally tricuspid (Tri) valves was statistically highly significant ($p < 0.01$).

Underlying basis. The basis for the differing histoarchitectural patterns observed in this study remains enigmatic. One possible explanation relates to the duration of the degenerative process. Because the bicuspid valve has been considered to be subject to accelerated degeneration as a result of stresses imposed by asymmetric leaflet motion (26), the onset of calcification at a relatively earlier age may ultimately lead to more thorough leaflet involvement than in the case of a normally functioning tricuspid valve. Alternatively, these two patterns could conceivably be related to sites of maximal stress. For example, a heteromerous bileaflet valve in the aortic position may open and close in such a manner that all aspects of the leaflet are stressed to an abnormal and equivalent degree. In a normal valve the finding of calcium distributed primarily on the aortic (versus ventricular) surface of the valve leaflet suggests that stress may be predominant along the aspect of the valve leaflet subjected over long periods to the closing pressure of the systemic circulation.

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