SURGERY FOR ACQUIRED CARDIOVASCULAR DISEASE

HISTOLOGIC ABNORMALITIES OF THE ASCENDING AORTA AND PULMONARY TRUNK IN PATIENTS WITH BICUSPID AORTIC VALVE DISEASE: CLINICAL RELEVANCE TO THE ROSS PROCEDURE

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Objective: Bicuspid aortic valve disease is often associated with dilation of the aortic root and ascending aorta. This study examines the histologic features of the ascending aorta and main pulmonary artery of patients with and without aortic valve disease. **Methods:** Samples from ascending aorta and main pulmonary artery were obtained at the time of the operation from 20 patients with bicuspid aortic valve and 11 patients with tricuspid aortic valve disease. In addition, samples were also obtained from autopsy cases with normal aortic valve. The histologic changes were graded from 1 to 3 according to severity of degenerative changes. **Results:** In the ascending aorta, the severity of cystic medial necrosis ($P = .001$), elastic fragmentation ($P = .002$), and changes in the smooth muscle cell orientation ($P = .002$) were significantly more severe in patients with bicuspid than in those with tricuspid aortic valve disease. In the pulmonary trunk specimens, those 3 histologic features were also significantly more severe in patients with bicuspid than those with tricuspid valves ($P = .001$, $P = .01$, and $P = .04$, respectively). Seventy-five percent of patients with bicuspid aortic valve disease had grade 3 degenerative changes, whereas only 14% of those patients with tricuspid aortic valve disease had similar degrees of degenerative changes. **Conclusion:** Patients with bicuspid aortic valve disease have more severe degenerative changes in the media of the ascending aorta and main pulmonary artery than patients with tricuspid aortic valve disease. These findings may explain root and ascending aortic dilation in patients with bicuspid aortic valve disease and pulmonary autograft dilation in certain patients after the Ross procedure. (J Thorac Cardiovasc Surg 1999;118:588-96)

Bicuspid aortic valve is the most common congenital heart valve abnormality, occurring in 1% to 2% of the population.^{1,2} In surgical pathologic studies, con-

genital bicuspid aortic valve is also the most common cause of aortic valve disease in patients under 70 years of age in North America.³⁻⁵

Previous reports have documented increased prevalence of coarctation of the aorta,⁶ aortic dissection,⁷ annuloaortic ectasia,^{8,9} and spontaneous innominate, carotid, and vertebral arterial dissections¹⁰ in patients with congenital bicuspid aortic valve disease. In 1972 McKusick¹¹ reported an association between congenital bicuspid aortic valve and cystic medial necrosis of the aorta. Although some authors have claimed that aortic wall changes are mainly the consequence of hydraulic laws,^{12,13} others showed that the histologic changes in patients with congenital bicuspid aortic valve also occur in the absence of valve stenosis or incompetence.¹⁴ The latter view supports the hypothesis that a common

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Financial support was provided to Mauro de Sa by Medtronic, Inc, during his research fellowship.

Read at the Seventy-ninth Annual Meeting of The American Association for Thoracic Surgery, New Orleans, La, April 18-21, 1999.

Received for publication March 19, 1999; revisions requested May 6, 1999; revisions received June 7, 1999; accepted for publication June 7, 1999.

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0022-5223/99 \$8.00 + 0 12/6/100610

developmental error is responsible for the association between bicuspid aortic valve and aortic wall abnormalities.¹¹

If this hypothesis is correct, one would have to assume that the pulmonary root is also involved in this complex abnormality because the aortic and the pulmonary roots have the same embryologic origin, the conotruncus. Interestingly, we have recently observed that the pulmonary autograft tends to dilate in some patients with bicuspid aortic valve who have dilated aortic root before the performance of the Ross procedure.¹⁵ The purpose of this study was to evaluate the histologic changes in the ascending aorta and pulmonary trunk of patients with congenital bicuspid aortic valve.

Material and methods

After appropriate informed consent, 31 samples of ascending aorta wall and 16 pulmonary trunk wall were collected from patients who underwent aortic valve replacement. Twenty patients had congenital bicuspid aortic valve disease (BAV group), and 11 patients had trileaflet aortic valve disease (TAV group). In addition, 11 aortic and 10 pulmonary artery samples were obtained from 15 control subjects who had normal trileaflet aortic valve and died of a noncardiovascular illness. The control samples were included in the TAV group because the histologic findings were very similar to those of patients with TAV disease.

Tissue samples were obtained approximately 1.0 cm distal to the sinotubular junction in the anterior aspect of the ascending aorta and/or the main pulmonary artery. They were fixed in 10% buffered formalin solution. The tissue was processed for light microscopy, embedded in paraffin blocks, and sectioned out at 4 microns thickness from each specimen. Sections were stained with hematoxylin-eosin, elastic-trichrome (modified elastic Van Gieson), and the Movat pentachrome stains. All histologic specimens were graded with Movat pentachrome. Each of the sections was evaluated histologically by 2 observers, 1 of them without any knowledge of the nature of the case. In 5 instances of discrepancy, the specimens were re-evaluated by both observers and consensus was attained. The histologic evaluation of the media layer of the arterial wall was based on the presence and degree of the 5 variables: (1) fibrosis, (2) medionecrosis, (3) cystic medial necrosis (mucoid material accumulation), (4) changes in smooth muscle cells orientation, and (5) elastic fragmentation. Each variable was graded from 1 to 3 according to the severity of the process when examined at a magnification of 100 or 200 times, with a Leica DMRB microscope (Leica Mikroskopi & Systeme, Wetz/Ar, Germany). The grades were determined on the basis of the worst area observed in each specimen. The criteria for the histologic grading are summarized in the appendix. Those criteria were modified from Schlattman and Becker¹⁶ to allow evaluation of the pulmonary trunk as well. Because previous studies suggested that these

histologic changes can be age related,^{16,17} the specimens were also analyzed according to age groups: group I, 18 to 35 years of age (mean, 27 years), 13 aortas and 10 pulmonary arteries; group II, 36 to 45 years of age (mean, 40 years), 10 aortas and 7 pulmonary arteries; group III, 46 to 55 years of age (mean, 51 years), 8 aortas and 4 pulmonary arteries; and group IV, 56 to 78 years of age (mean, 69 years), 11 aortas and 5 pulmonary arteries.

The mean age of patients with BAV and TAV disease was 43 ± 12 years and 51 ± 20 years ($P = .14$) respectively. Of 20 patients with BAV disease, 16 patients had aortic stenosis (mean gradient, 67 ± 18 mm Hg), and 4 patients had aortic insufficiency. Of 11 patients with TAV disease, 7 patients had aortic stenosis (mean gradient, 62 ± 22 mm Hg), and 4 patients had aortic insufficiency.

It has been suggested that hypertension may induce histologic changes both in the ascending aorta¹⁷ and in the pulmonary artery.^{18,19} For this reason, specimens were considered to be from hypertensive subjects when at least 1 of the following conditions was fulfilled: (1) history of drug treatment for hypertension; (2) recorded diastolic pressures greater than 100 mm Hg; and (3) unexplained cardiomegaly with left ventricle hypertrophy, cardiac weight of more than 350 g in female and 400 g in male autopsy patients,²⁰ with no observed cardiac or pulmonary abnormality. According to these criteria 2 of the patients with BAV disease (10%) were found to be hypertensive, compared with 7 of the patients with TAV disease (32%; $P = .151$). None of the patients with BAV disease had pulmonary hypertension, and only 2 of the patients with TAV disease had pulmonary hypertension.

Statistical analysis. Continuous variables were presented as the mean $\pm$ SD and were evaluated by 1-way analysis of variance. Scheffé multiple range *t* test was used as a post hoc test to specify differences between groups. Categorical variables were analyzed by χ^2 or Fisher exact test when cell numbers were small.

Results

Ascending aorta histologic features. Table I shows the grades of 5 histologic features in the ascending aorta of patients with BAV and TAV disease. The severity of medionecrosis and fibrosis were similar between the 2 groups, whereas cystic medial necrosis ($P = .001$), smooth muscle cell changes ($P = .002$) and elastic fragmentation ($P = .002$) were significantly more severe in BAV than in TAV disease. In the BAV group, 9 patients (45%) had grade 3 histologic features of cystic medial necrosis, smooth muscle cell changes, or elastic fragmentation, whereas in the TAV group, only 2 patients (9%) had at least 1 grade 3 histologic change. Fig 1 shows representative samples of the ascending aorta of BAV and TAV disease.

Pulmonary trunk histologic features. Table II shows the grades of 5 histologic features in the media

Table I. Histologic features of the ascending aorta in patients with diseased bicuspid and tricuspid aortic valves

Histologic features	Grades	Bicuspid group		Tricuspid group	
		n	%	n	%
Medionecrosis	I	14	70	14	64
	II	3	15	8	36
	III	3	15	0	0
Fibrosis	I	14	70	13	59
	II	6	30	9	41
	III	0	0	0	0
Cystic medial necrosis	I	2	10	17	77
	II	13	65	3	14
	III	5	25	2	9
Cell changes orientation	I	8	40	19	86
	II	9	45	3	14
	III	3	15	0	0
Elastic fragmentation	I	5	25	16	73
	II	9	45	6	27
	III	6	30	0	0

of the pulmonary trunk in BAV and TAV groups. The severity of medionecrosis ($P = .12$) and fibrosis ($P = .12$) were similar in BAV and TAV diseases, whereas cystic medial necrosis ($P = .001$), smooth muscle cell changes ($P = .04$) and elastic fragmentation ($P = .01$) were significantly more severe in BAV disease. In the BAV group, 9 patients (75%) had grade 3 cystic medial necrosis, smooth muscle cell changes, or elastic fragmentation in the pulmonary trunk, whereas in the TAV group only 2 patients (14%) had grade 3 histologic changes. Fig 2 shows representative samples of pulmonary trunks in the BAV and TAV groups.

Correlation between age, hypertension, and histologic changes. We could not establish a correlation between age or hypertension and the 5 histologic features, either for the ascending aorta or for the pulmonary trunk, possibly because of a relatively small number of specimens.

Correlation between changes in the aorta and pulmonary artery. We could not establish a correlation between the degree of changes in the aorta with those in the pulmonary in either group of patients, except for cystic medial necrosis. In the BAV and TAV groups, 67% and 81%, respectively, had the same grade of cystic medial necrosis in the ascending aorta and pulmonary trunk.

Effect of functional status of the aortic valve. The degree of histologic changes did not correlate with the functional status of the aortic valve. All pulmonary valves were normal.

Table II. Histologic features of the pulmonary artery in patients with diseased bicuspid and tricuspid aortic valves

Histologic features	Grades	Bicuspid group		Tricuspid group	
		n	%	n	%
Medionecrosis	I	5	42	10	71
	II	7	58	4	29
	III	0	0	0	0
Fibrosis	I	5	42	10	71
	II	7	58	4	29
	III	0	0	0	0
Cystic medial necrosis	I	0	0	11	79
	II	10	83	2	14
	III	2	17	1	7
Cell changes orientation	I	2	16	6	43
	II	5	42	7	50
	III	5	42	1	7
Elastic fragmentation	I	0	0	5	36
	II	4	33	7	50
	III	8	67	2	14

Discussion

The principal finding in this study was that degenerative changes in the ascending aorta and pulmonary trunk of patients with BAV disease were more severe than in patients with TAV disease. The difference in severity was mainly related to cystic medial necrosis, elastic fragmentation, and smooth muscle cell orientation changes and not related to fibrosis or medionecrosis.

Previous studies on the histopathologic features of the ascending aorta described degenerative changes similar to those identified in the present study in patients with aortic dissection,⁷ ascending aorta aneurysms²¹ and annuloaortic ectasia.^{22,23} All those clinical entities, however, may be associated with BAV disease.²⁴ McKusick¹¹ suggested the association between BAV disease and cystic medial necrosis in the ascending aorta. Consistent with those studies, we found that cystic medial necrosis, elastic fragmentation, and smooth muscle cell orientation changes were significantly more severe in patients with BAV disease than in patients with TAV disease. Severe degenerative changes were uncommon in patients with TAV disease (9%), whereas almost one half of the patients (45%) with BAV disease had severe degenerative changes in the ascending aorta. We had the opportunity to examine the ascending aorta of 5 patients with the Marfan syndrome who had undergone an operation because of aortic root aneurysm during the conduction of the present study. We found that the severity of histologic changes in the ascending aorta of patients with BAV disease

Fig 1. A, Histologic features of a transverse section of ascending aorta, from a 54-year-old patient with a normal trileaflet aortic valve. Note the circumferential arrangement of smooth muscle cells and elastic tissue plates. Small foci of accumulation of collagen (*pale yellow white areas*) and changes in the smooth muscle cell orientation (*arrow*). There are no intimal changes. (Movat pentachrome stains; original magnification, $\times 100$.) **B,** Histologic features of the ascending aorta from a 33-year-old patient with bicuspid aortic valve disease. Note the significant medial destruction, accumulation of mucoid material (*arrow*; pool of mucopolysaccharide). There is marked fragmentation of elastic tissue, and there are large areas of loss of normal medial muscle. (Movat pentachrome stains; original magnification, $\times 100$.)

was somewhere between those of patients with TAV disease and those with the Marfan syndrome. The latter ones are at the extreme in the spectrum of degenerative changes in the ascending aorta.

Controversy exists regarding the impact of aging on the histologic changes in the ascending aorta. Schlatman and Becker¹⁶ reported on a correlation between the severity of all histologic changes and aging. However, of 6 autopsy cases with grade 3 cystic medial necrosis, 5 cases were in subjects younger than 40 years of age.¹⁶ Those investigators made no comment on the morphologic condition of the aortic valve.¹⁶ Savunen and Aho²² did not find a correlation between age and histologic changes either in annuloaortic ectasia or in normal subjects, although they

reported a slight tendency towards more severe changes in the aortas of older patients. In a morphologic study of ascending aortic aneurysms, Klima and colleagues²¹ described that cystic medial necrosis and elastic fragmentation were inversely related to age and arterial hypertension. Other investigators reported on a correlation between severity of medial changes with age and hypertension.¹⁷ We could not demonstrate such correlation either in the BAV or the TAV groups, even for fibrosis or medionecrosis. In our study, the BAV group was younger than the TAV group; therefore our aortic wall findings cannot be attributed simply to the aging process. We believe some of the discrepancies between various studies are due to the differences in patient population, that is, autopsy versus surgical studies. In

Fig 2. **A**, Normal histologic structure of pulmonary trunk from a 42-year-old patient with tricuspid aortic valve disease. The medial layer shows a greater degree of interruption and fragmentation of elastic tissue plates than is seen in the aorta. Elastic tissue and smooth muscle cells run circumferentially. Small scattered areas of collagen (pale yellow) can be seen. The medial thickness is about 50% to 60% of that of a normal aorta. (Movat pentachrome; original magnification, $\times 100$.) **B**, Histologic structure of pulmonary trunk from a 40-year-old patient with bicuspid aortic valve disease. Note disorganized media, with large areas of pools of mucopolysaccharides (arrow; pale blue) and of collagen deposition (pale yellow). There is also smooth muscle cell disorientation and fragmentation of elastic tissue. (Movat pentachrome; original magnification, $\times 100$.)

studies like ours, the more severe the histopathologic changes, the earlier the patient is subjected to surgical treatment.

Except for 1 case reported by McKusick¹¹ in 1972, the possibility that the pulmonary trunk itself might also be pathologically involved in patients with BAV disease has not been raised. In our study we showed that the pulmonary trunk was more severely affected in patients with BAV disease than in patients with TAV disease. We found that only 14% of the subjects with TAV disease had severe pulmonary trunk degenerative changes, whereas this occurred in 75% of the patients with BAV disease. Interestingly, the 2 TAV subjects with grade 3 changes in the pulmonary artery had advanced age. This may suggest that the severe

changes that occur in relatively young patients with BAV disease may also occur with the aging process in patients with TAV disease.

We were puzzled with our findings in the pulmonary arterial wall. We believe the explanation may be found on the fact that both the aortic and the pulmonary roots have the same embryologic origin, the conotruncus. Anomalous migration of cells from the neural crest may be responsible for the combined occurrence of anomalies of the aortic arch and the media of the ascending aorta. Previous studies suggested that anomalies that occur at different phases of the migration of the neural crest may account for isolated or combined defects of the aortic arch, the wall of the ascending aorta, and the aortic valve.^{25,26} Therefore it is possible

that the pulmonary trunk may also be affected by this process.

The fact that in our study only 2 patients in the BAV group had arterial hypertension and none had pulmonary hypertension would exclude hypertension as an underlying mechanism for the morphologic changes in the aorta and pulmonary trunk, a pathophysiologic mechanism that has been proposed by some investigators.¹⁷⁻¹⁹ The control group with TAV disease who had no aortic valve disease had similar histologic changes to those with diseased TAV. This finding suggests that the hemodynamic disturbances in the blood flow did not play a major pathophysiologic role as previously suggested.²⁷ The similarity of the aortic wall histologic changes in the BAV and Marfan groups suggests that the mechanism of aortic and pulmonary artery wall changes in BAV disease could be a primary one and possibly related to a microfibrillar protein (eg, fibrillin-1 disorder or extracellular matrix).

Clinical implications. This study shows that patients with BAV disease are more likely to experience the development of degenerative changes in the aorta and in the pulmonary trunk than patients with TAV disease. These degenerative changes may be as severe as those seen in some patients with the Marfan syndrome. It is probable that these changes greatly reduce the cohesive and the tensile strength of the media.²⁸ These findings explain the relatively high incidence of dilation of the aortic root and ascending aorta and aortic dissections in patients with BAV disease.⁷⁻⁹ They also justify a surgically more aggressive approach when treating patients with BAV disease and dilated aortic root/ascending aorta.

We have recently detected an increased incidence of pulmonary autograft dilation in patients with BAV disease who underwent the Ross procedure.¹⁵ We believe that the dilation of the pulmonary autograft is related to the histopathologic nature and possibly degenerative changes in the pulmonary trunk as described in this study. We proposed that pulmonary autograft should be implanted with the use of the aortic root technique instead of aortic root replacement in patients with BAV disease. In addition, both the aortic anulus and the sinotubular junction should be fixed with a strip of Dacron fabric.

Limitations of this study. The principal limitation of this study is its sample size. Degenerative changes in the arterial wall of the aorta and pulmonary artery are complex and variable process. With the current techniques to assess degenerative changes, a prohibitively high number of arterial wall samples is required to determine the extent of this problem and its correlation with various clinical entities. Another limitation is that

we examined histologically only a small segment of ascending aorta and pulmonary artery.

REFERENCES

1. Roberts WC. The congenitally bicuspid aortic valve: a study of 85 autopsy cases. *Am J Cardiol* 1970;26:72-83.
2. Campbell M. Calcific aortic stenosis and congenital bicuspid aortic valves. *Br Heart J* 1968;30:606-16.
3. Passik CS, Ackerman DM, Pluth JR, Edwards WD. Temporal changes in the causes of aortic stenosis: a surgical pathologic study of 646 cases. *Mayo Clin Proc* 1987;62:119-23.
4. Stephan PJ, Henry AC 3rd, Hebel RF Jr, Whiddon L, Roberts WC. Comparison of age, gender, number of aortic cusps, concomitant coronary artery bypass grafting, and magnitude of left ventricular-systemic arterial peak systolic gradient in adults having aortic valve replacement for isolated valve stenosis. *Am J Cardiol* 1997;79:166-72.
5. Peterson MD, Roach RM, Edwards JE. Types of aortic stenosis in surgically removed valves. *Arch Pathol Lab Med* 1985;109:829-33.
6. Abbott ME. Coarctation of the aorta of the adult type. *Am Heart J* 1928;3:381-421.
7. Gore I. Dissecting aneurysms of the aorta in persons under forty years of age. *Arch Pathol* 1953;55:1-13.
8. Pachulski RT, Weinberg AL, Chan KL. Aortic aneurysm in patients with functionally normal or minimally stenotic bicuspid aortic valve. *Am J Cardiol* 1991;67:781-2.
9. Hahn RT, Roman MJ, Mogtader AH, Devereux RB. Association of aortic root dilation with regurgitant, stenotic and functionally normal bicuspid aortic valves. *J Am Coll Cardiol* 1992;19:283-8.
10. Schievink WI, Mokri MB. Familial aorto-cervicocephalic arterial dissection and congenitally bicuspid aortic valve. *Stroke* 1995;26:1935-40.
11. McKusick VA. Association of congenital bicuspid aortic valve and Erdheim's cystic medial necrosis. *Lancet* 1972;1:1026-7.
12. Holman E. The obscure physiology of post-stenotic dilatation: its relation to the development of aneurysms. *J Thorac Surg* 1954;28:109-33.
13. Stebbins WE. Structural and architectural changes during arterial development and the role of hemodynamics. *Acta Anat* 1996;157:261-74.
14. Larson EW, Edwards WD. Risk factors for aortic dissection: a necropsy study of 161 cases. *Am J Cardiol* 1984;53:849-55.
15. David TE, de Sa M, Omran A, Sonnenberg B, Armstrong S, Ivanov J, et al. Pulmonary autograft dilation after the Ross procedure. *J Thorac Cardiovasc Surg*. In press.
16. Schlatman TJM, Becker AE. Histologic changes in the normal aging aorta: implications for dissecting aortic aneurysm. *Am J Cardiol* 1977;39:13-20.
17. Carlson RG, Lillehei CW, Edwards JE. Cystic medial necrosis of the ascending aorta in relation to age and hypertension. *Am J Cardiol* 1970;25:411-5.
18. Tredal SM, Carter JB. Cystic medial necrosis of the pulmonary artery. *Arch Pathol* 1974;97:183-6.
19. Butto F, Lucas R, Edwards J. Pulmonary arterial aneurysm: a pathologic study of five cases. *Chest* 1987;91:237-41.
20. Silver MM, Freedom RM. Gross examination and structure of the heart. In: Silver M, editor. *Cardiovascular pathology*. 2nd ed. New York: Churchill Livingstone; 1991. p. 1-42.
21. Klima T, Spjut HJ, Coelho A, Gray AG, Wukasz DC, Reul GJ,

- et al. The morphology of ascending aorta aneurysms. *Hum Pathol* 1983;14:810-7.
22. Savunen T, Aho HJ. Annulo-aortic ectasia: light and electron microscopic changes in aortic media. *Virchow Archiv A [Pathol Anat]* 1985;407:279-88.
 23. Fukunaga S, Akashi H, Tayama K, Kawano H, Kosega K, Aoyagi S. Aortic root replacement for annulo-aortic ectasia in Shprintzen-Goldberg Syndrome: a case report. *J Heart Valve Dis* 1997;6:181-3.
 24. Braveman AC. Bicuspid aortic valve and associated aortic wall abnormalities. *Curr Opin Cardiol* 1996;11:501-3.
 25. Kappetein AP, Gittenberger-de Groot AC, Zwinderman AH, Rohmer J, Poelmann RE, Huysmans HA. The neural crest as a possible pathogenetic factor in coarctation of the aorta and bicuspid aortic valve. *J Thorac Cardiovasc Surg* 1991;102:830-6.
 26. Duran AC, Frescura C, Sans-Coma V, Angelini A, Basso C, Thiene G. Bicuspid aortic valves in hearts with other congenital heart disease. *J Heart Valve Dis* 1995;4:581-90.
 27. Leonard JC, Hasleton PS. Dissecting aortic aneurysms: a clinicopathological study. *Q J Med* 1979;46:55-76.
 28. Hirst AE, Gore I. Is cystic medionecrosis the cause of dissecting aortic aneurysm? *Circulation* 1976;53:915-6.

Appendix

Criteria for histologic classification of the aorta and pulmonary artery (modified from Schlatman TJM, Becker AE. Histologic changes in the normal aging aorta: implications for dissecting aortic aneurysm. *Am J Cardiol* 1977;39:13-20)

Fibrosis. Defined as an increase in interstitial collagen. Sections were examined at a magnification of $\times 100$ times. Three grades were recognized: grade 1, an increase in collagen content in an area comprising less than one third of the total width of the media or focal accumulation of collagen spread through the section; grade 2, an increase in collagen in an area consisting of between one third and two thirds of the total width of the media or multiple small areas that together could represent the same; grade 3, an increase of collagen in an area comprising more than two thirds of the total width of the media. These areas of medial damage are large, and collagen is increased significantly. These criteria were used for both the aorta and the pulmonary artery.

Medionecrosis. Defined as a focal loss of smooth muscle cell nuclei in the media. It was graded at a magnification of $\times 100$ times. Three grades were recognized: grade 1, focal loss of nuclei in an area consisting less than one third of the total width of the media; grade 2, focal loss of nuclei in an area consisting of one and two thirds of the medial thickness; grade 3, focal loss of nuclei in an area comprising more than two thirds of the total media thickness or batched areas that together could represent the same.

Accumulation of mucoid material (cystic medial necrosis). The mucoid material or mucopolysaccharides were observed in the presence of intact elastic lamellae and in the presence of fragmented elastic fibers; no cystic wall was ever identified. Three grades of accumulation of mucoid material were defined at a magnification of $\times 100$ times: grade 1, minute foci of mucoid material (cysts) were present within a single lamel-

lar unit, not encompassing its entire width; grade 2, size or number of cysts increased, and the mucoid material covered the total width of 1 lamellar unit or equivalent areas (width) in different regions of the media; grade 3, the cysts were large and extended in area over more than 1 lamellar unit, either because of focal aggregation of small cysts with intact elastic lamellae or because of large cysts in an area with fragmented elastic fibers forming pools of mucoid material.

Changes in the smooth muscle cells orientation. Because changes in smooth muscle cell orientation are not always associated with severe elastic fragmentation, this feature was classified separately. These changes refer to different orientation of the cells so that the longitudinal aspect was not seen; instead, a transverse view of the cells was observed. Three grades were recognized at magnification of $\times 100$ times: grade 1, small foci with change in the orientation of the smooth muscle cells, which could be spread in different areas; grade 2, area with change in the smooth muscle cell orientation or several areas that together represent between one third and one half of the thickness of the media; grade 3, large area of changes in the smooth muscle cell orientation, usually associated with severe elastic fragmentation and consisting more than one half of the media thickness, giving a very disorganized appearance to the specimen that is used for both aorta and pulmonary artery.

Elastic fragmentation. Defined as focal fragmentation of elastic lamellae in the media of the aorta and pulmonary artery. Because the quantitation and the organization of the elastic tissue in the aorta differ from that in the pulmonary artery, somewhat different criteria were used for fragmentation of elastic lamellae in these vessels. Magnification of $\times 200$ was used.

For the aorta. Grade 1: fewer than 5 foci of elastic lamella fragmentation in 1 microscopic field, each focus comprising 2 to 4 neighboring elastic lamellae; interruption of 1 elastic fiber was not interpreted as fragmentation. Grade 2: 5 or more (less than 10) foci with elastic lamella fragmentation in 1 microscopic field, each focus consisting of 2 to 4 neighboring elastic lamellae; the foci could be confluent or scattered throughout the media of the aorta. Grade 3: presence of foci with elastic fragmentation in 10 or more neighboring elastic lamellae, irrespective of the number of foci per microscopic field; smooth muscle cells of the media usually showed alteration in orientation.

For the pulmonary trunk. At any age, in an otherwise normal pulmonary artery, the elastic tissue is not a continuous plate as it is in the aorta, even though it is easy to recognize the units comprised of parallel elastic fibers, with smooth muscle cells, collagen, and ground substance in between. Grade 1: A small area with fragmented elastic tissue can be seen and the smooth muscle cells do not show significant change in the orientation. Grade 2: Some areas, comprising between one third and one half of the medial thickness of the pulmonary artery were unusually fragmented, which shows an black point aspect of the elastic tissue or loss of parallelism of the lamellar components. It is frequently associated with focal orientation changes of the muscle cells. Grade 3:

The elastin lamellae was severely fragmented, with the black point aspect spread in the media thickness, and the identification of the lamellar unit was not possible because of the severe disorganization of the media.

Discussion

Dr Francis Robicsek (*Charlotte, NC*). This is a very interesting and important paper which provides additional proof that the aortic valve is not composed from leaflets alone but from leaflets and adjoining sinuses. Although our observations are similar to those of the authors, we went 1 step further. In our *in vitro* experiments, we have mounted normal human aortic roots in a pulse duplicator. Using 500 frames per second cinematography, as expected, we documented beautiful aortic valve function, symmetrical and synchronous leaflet opening and closure.

However, when we made the aortic wall stiff by spraying it from the outside with plastic adhesive, aortic valve function become highly irregular, asynchronous and asymmetric, and the computer-measured stress overload on the leaflet became highly significant.

Do you think that the phenomenon you observed (ie, simultaneous changes in the aortic wall and leaflet) is not just random occurrence but that the stiffening of the aortic wall itself would cause secondary leaflet anomalies?

Dr de Sa. We believe that changes in the ascending aorta wall are not a consequence of the hemodynamic changes alone. There are previous studies that show increased prevalence of aortic root dilation and aneurysm of the ascending aorta in patients with normal function or mild dysfunction of the bicuspid aortic valve. The turbulent flow causes degenerative changes in the aortic wall, but we have seen that, in patients with bicuspid aortic valve disease, these degenerative processes are worse. It means there is likely also an intrinsic defect involving the ascending aorta and possibly the pulmonary arterial walls. This may explain why these patients come to surgical treatment earlier.

Dr David. As a coauthor, I should not be discussing this paper, but I would like to make a couple of comments. The histologic appearance of those pulmonary arteries was scary for a surgeon who performs the Ross procedure using the full root replacement technique. Dr de Sa found severe degenerative changes in the pulmonary arterial wall of patients with bicuspid and tricuspid aortic valves. The second remark I would like to make is related to Dr Robicsek's comments on the interactions between leaflets and sinuses. The pulmonary valves examined by Dr de Sa were entirely normal macroscopically, and yet their arterial wall had severe degenerative changes.

Dr Timothy J. Gardner (*Philadelphia, Pa*). Do you think that you should be able to find a specific gene defect?

Dr David. Absolutely. I believe bicuspid aortic valve is an inherited disorder and that the same gene is likely responsible for the leaflets and the arterial wall abnormalities.

Dr Ronald C. Elkins (*Oklahoma City, Okla*). One of the aspects of the study that we need to look at concerns patients who have bicuspid aortic valve disease grouped into about 3

different groups of patients. There are those who have pure aortic insufficiency with a bicuspid aortic valve and that entity is frequently associated, or more frequently associated, with marked dilatation of their ascending aorta at the time they come to surgical intervention because of problems associated with their left ventricle. There is another group of patients who have aortic stenosis who infrequently have marked dilatation of their ascending aorta. And then, there is a third group of patients who have no valvular heart disease, other than a bicuspid aortic valve, who wind up with ascending aortic disease on occasions. The same is true for patients with tricuspid valve.

One of the things that we do not have in this article, which would be very helpful, is any correlation between the clinical presentation of the patient and the factors that are involved. We have been very interested for a long period in trying to document possible abnormalities in fibrillin and in elastin in the pulmonary artery, particularly in those patients who have dilatation of the ascending aorta; and it has taken us about 3 years to really get to the point that we could consistently use a monoclonal antibody stain to identify fibrillin and elastin in a specific fashion.

We have now stained 39 ascending aortas that were associated with significant dilatation or aneurysm formation, and we have stained 9 pulmonary arteries in these same patients. In those 39 patients, our data identifies 3 distinct groups of patients. There are those patients who have marked abnormalities in the elastin expression; there is a group of patients that have essentially normal fibrillin and elastin expression, and then there is a small group of patients that is an intermediary group. We have only identified 1 patient in the series who had significant elastin expression abnormalities in the pulmonary artery.

I agree with Dr David that there are patients who will have significant abnormalities in their pulmonary artery. Is it as prevalent, as he would say, and what is the significance of the findings that they have demonstrated to us in terms of clinical presentation? Because we all know that irrespective of what valve one puts in the aorta, many times the ascending aorta goes on to considerable dilatation, and even to dissection, after an aortic valve replacement. What we need to identify is who are those patients and how do they differ from those patients who have no abnormality in their ascending aorta or pulmonary artery.

Dr de Sa. We are also measuring fibrillin-1 in the ascending aorta and pulmonary artery in this group of patients. But we have found that the histologic aspect in light microscopy does not necessarily correlate with the molecular findings. This means that, even in patients with significant cystic medial necrosis and elastic fragmentation, the molecular analysis of fibrillin-1 may not differ from controls. This is described in previous studies about quantitative analyses in Marfan disease. This microfibrillar protein is not quantitatively reduced in all cultures from Marfan specimens.

Unfortunately, these degenerative changes are part of a variable and complex process, and many clinical factors (such as age, hypertension, type of lesion, and metabolic dis-

eases) could influence in these histologic findings. We have not found a correlation between type of lesion, stenosis, or regurgitation, but we have to recognize that the sample size is very small and that we cannot generalize.

Dr Gino Gerosa (*Padova, Italy*). I enjoyed your presentation. After the Wheat studies of the late 1960s, we extensively reviewed the histologic appearance of the pulmonary artery wall in comparison with the aortic counterpart. We know that, in the absence of pulmonary hypertension, the pulmonary artery wall will modify its aorta-like structure during fetal life, which has elastic fibers that are long, uniform, and parallel, to the so-called pulmonary-like configuration with elastic fibers that are not anymore long, uniform, and parallel.

Did you compare the histologic findings of the pulmonary artery wall and the aortic wall from the same heart?

Do you agree with the concept that probably the best indication for the Ross procedure, irrespective of the bicuspid aortic valve presence, is for neonates and infants before the modification of the pulmonary artery histologic configuration will occur?

Dr de Sa. We collected both aorta and pulmonary artery

walls from 12 patients with bicuspid aortic valve disease and from 9 patients with trileaflet aortic valve disease. Sixty-seven percent of the patients in the bicuspid group and 81% of patients in the trileaflet group had the same grade of cystic medial necrosis in the ascending aorta and pulmonary trunk, whereas the others had only 1 grade difference between the 2 vessels. This was the only histologic finding in which we have found a correlation.

The oxygen saturation may be related in some way to the histologic changes, and we may keep the pulmonary artery histologic features if we expose this tissue to a very high oxygenation. But these degenerative processes are correlated with other factors like pressure, flow, metabolic factors, and genetic factors. I cannot answer whether a child with other congenital malformation would have a normal molecular and histologic formation of these vessels. Theoretically, after the birth, the pulmonary trunk wall, which is thicker and more organized, may have a more appropriate function as a systemic valve or conduit, but we do not have experience with Ross procedure in infants or neonates.