Ascending aorta dilation in association with bicuspid aortic valve: A maturation defect of the aortic wall

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Objective: Patients with a bicuspid aortic valve have increased susceptibility to the development of ascending aortic dilation and dissection compared with persons with a tricuspid valve. To unravel a possible different mechanism underlying dilation in bicuspidy and tricuspidy, a comparison of the structure of the aortic wall was made.

Methods: Ascending aortic wall biopsies were divided into 4 groups: bicuspid (n = 36) and tricuspid (n = 23) without and with dilation. The expression of vascular smooth muscle cell maturation markers including lamin A/C, which plays a pivotal role in smooth muscle cell differentiation, and its splicing variant progerin indicative of aging, were studied immunohistochemically. Attention was also paid to the inflammatory status.

Results: There is a significant difference in the structure and maturation of the aortic wall in bicuspidy, persisting in the dilated aortic wall, presenting with a thinner intima, lower expression of α smooth muscle actin, smooth muscle 22α, calponin, and almost absent expression of smoothelin. We show for the first time significantly lowered lamin A/C expression in bicuspidy. Progerin was found to be significantly increased in the media of the dilated wall in tricuspidy, also showing increased periaortic inflammation.

Conclusions: The structure of the nondilated and dilated aortic wall in bicuspidy and tricuspidy are intrinsically different, with the latter having more aspects of aging. In bicuspidy there is a defective smooth muscle cell differentiation possibly linked to lowered lamin A/C expression. Based on this vessel wall immaturity and increased susceptibility to dilation, different diagnostic and therapeutic approaches are warranted. (J Thorac Cardiovasc Surg 2014;148:1583-90)

CHD

A bicuspid aortic valve (BAV) is characterized by an aortic valve with 2 semilunar leaflets. BAV is the most common congenital cardiovascular malformation with a prevalence of 0.5% to 2%.[1] The incidence of thoracic aortic dilation or aneurysm formation and dissection in patients with BAV is considered to be 50% to 70%.[2] Compared with patients with a tricuspid aortic valve (TAV), patients with BAV have larger aortic root dimensions and a higher progression rate of dilation,[3,4] suggesting that the process of dilation of the thoracic aorta is different in BAV compared with TAV. The terms aneurysm and dilation for the aortopathy are used interchangeably. We have chosen to use the term dilation for clarity. Turbulent flow, as a result of asymmetric movement of valve leaflets in BAV, has been postulated as an essential determinant for the development of aortic dilation.[5] An alternative hypothesis, that aortic dilation in BAV is mainly based on the intrinsic structure of the aortic wall,[6] is supported by a high incidence of dilation in asymptomatic patients with BAV as well as dilation observed after aortic valve replacement.[7] The latter hypothesis is further supported by reported altered molecular and/or metabolic characteristics in the aortic wall and valve leaflets in BAV, differences in elastic lamellae, loose attachment of vascular smooth muscle cells (VSMCs) to their surrounding elastic lamellae, and precocious VSMC apoptosis.[8-17] Most studies have focused on the differences between the dilated TAV and BAV wall. The exact mechanisms underlying the development and progression of an aorta with normal dimensions into dilation in patients with TAV versus BAV, however, have not been delineated. In this study, we used a unique opportunity to compare nondilated ascending aortic wall specimens from patients with BAV, representative of a specific architecture and possible early lesions rather than of end-stage disease, with the normal aortic wall in TAV.

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Abbreviations and Acronyms

αSMA = α smooth muscle actin
BA = bicuspid aortic valve without dilation
BAD = bicuspid aortic valve with dilation
BAV = bicuspid aortic valve
BSA = bovine serum albumin
DAB = diaminobenzidine tetrachloride
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
LUMC = Leiden University Medical Center
MF = microscopic field
PBS = phosphate buffered saline
PBS-T = phosphate buffered saline with 0.05% Tween-20
SM22α = smooth muscle 22α
TA = tricuspid aortic valve without dilation
TAD = tricuspid aortic valve with dilation
TAV = tricuspid aortic valve
VSMC = vascular smooth muscle cell

Furthermore, these data could be compared with the histopathology of the dilated vessel wall in BAV and TAV. Histologic procedures using hematoxylin-eosin and resorcin fuchsin were applied to assess the general vessel wall architecture, that is, inflammation, vessel wall thickness, elastic lamellae, and cystic medial necrosis (focal loss of VSMC nuclei in the media). Expression of markers of differentiated VSMCs was investigated to determine differences in vessel wall maturation or differentiation between patients with BAV and TAV. Smooth muscle 22α (SM22α), smoothelin, and calponin were used as markers for fully differentiated contractile VSMCs[18] and α smooth muscle actin (αSMA) was used as a marker for differentiation of VSMCs and myofibroblasts.[19] Lamin A/C, was investigated to explain possible differences between VSMC differentiation in BAV and TAV, because it has a pivotal role in the differentiation of myoblasts.[20] Progerin, a splice variant of lamin A/C, was studied to further elucidate differences in the pathogenesis of aortopathy between the 2 valve types, because it has been suggested that progerin not only plays a role in Hutchinson-Gilford progeria syndrome but also in cardiovascular aging.[21-23] We hypothesize that the BAV vessel wall has a maturation defect that underlies a more aggressive form of dilation.

MATERIALS AND METHODS

Ethical Approval

Sample collection and handling was carried out according to the official guidelines of the Medical Ethical Committee of Leiden University Medical Center (LUMC), Leiden, and the code of conduct of the Dutch Federation of Biomedical Scientific Societies (www.FMWV.nl). Six cryopreserved bicuspid human aortic valves were obtained from the Heart Valve Bank in Rotterdam (Erasmus University Medical Center, Rotterdam) originating from postmortem donors. These valves were declared unfit for implantation because of the bicuspid nature of the valves. The Advisory Board of the Heart Valve Bank allowed these valves to be included in the present project because the research was in line with the permission of the donation.

Tissue Samples

Samples of the ascending aorta were collected from individuals with BAV and TAV, with and without dilation. Material from patients with BAV without dilation was available in cases of stentless root replacement, the preferred technique for stentless valve implantation in LUMC. Dilation was clinically defined by reaching an ascending aortic wall diameter of 45 mm or more.[24] The patients were divided into 4 groups: (1) TAV without dilation (TA, n = 11, mean age 64.5 ± 9.0 years) obtained post mortem; (2) TAV with dilation (TAD, n = 12, mean age 72.3 ± 11.2 years) collected during elective repair; (3) BAV without dilation (BA, n = 17, mean age 55.8 ± 9.8 years); (4) BAV with dilation (BAD, n = 19, mean age 60.7 ± 7.8 years) obtained during elective repair. Patients with a proven genetic disorder (eg, Marfan disease) were excluded.

After excision, specimens were fixed in 4% formalin (24 hours), decalcified in a formic acid-formate buffer (120 hours), and embedded in paraffin. Transverse sections (5 μm) were mounted on precoated Starfrost slides (Klinipath BV, Duiven, The Netherlands).

Histologic Parameters

Sections stained with hematoxylin-eosin and resorcin fuchsin were analyzed quantitatively for (1) periaortic inflammation (presence of a cellular infiltrate in the adventitia), indexed from 0 (no inflammatory cells), 2 (a few cells), 4 (groups of cells) to 6 (large clusters of cells); (2) maximum intimal thickness in micrometers (the distance between the endothelial layer and the first major internal elastic lamella, excluding atherosclerotic areas; (3) maximum medial thickness in micrometers (the distance between the first and last elastic lamella on the borderline with the adventitia. Furthermore, the organization of the elastic content was studied qualitatively. All specimens were reevaluated by an independent, experienced histopathologist who was blinded to the clinical data.

Immunohistochemistry

After deparaffinization, antigen retrieval was performed in a microwave oven in citrate buffer (pH 6.0, 12 minutes), followed by treatment with 0.3% H_2O_2 in phosphate buffered saline (PBS, pH 7.3, 20 minutes) to extinguish endogenous peroxidase activity. Subsequently, sections were rinsed briefly twice in PBS and once in PBS with 0.05% Tween-20 (PBS-T). Sections were incubated overnight at room temperature with the primary antibodies diluted in PBS-T and 1% bovine serum albumin (BSA, Sigma, St. Louis, Mo) (Table 1). Between the incubation steps, the slides were rinsed in PBS (2×) and PBS-T (1×). Bound antibodies were detected using 1-hour incubation with a secondary antibody diluted in PBS-T (Table 1). Subsequently, all slides, except for αSMA, were incubated with ABC reagent (Vector Laboratories, Burlingame, Calif; PK 6100) for 45 minutes. Control stainings were performed using PBS-T and BSA as the first incubation step. Slides were incubated with 400 μg/mL 3,3′-diaminobenzidine tetrachloride (Sigma-Aldrich Chemie, Buchs, Switzerland; D5637) dissolved in Tris-maleate buffer to which 20 μL of H_2O_2 were added (pH 7.6, 10 minutes). After rinsing, counterstaining was performed with 0.1% hematoxylin (Merck, Darmstadt, Germany) (5 seconds), followed by rinsing in tap water (10 minutes). After dehydration, sections were mounted in Entellan (Merck, Darmstadt, Germany). Sections used for (semi)quantitative and morphometric analysis were stained in the same batch.

Double Immunofluorescence Staining

Deparaffinization, rehydration, and antigen retrieval were performed in the manner described earlier. Sections were incubated with the primary