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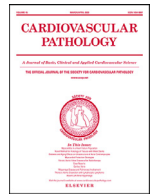
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## Original Article

Structural abnormalities in the non-dilated ascending aortic wall of bicuspid aortic valve patients<sup>☆</sup>

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## ABSTRACT

**Background:** A bicuspid aortic valve (BAV) is the most common congenital cardiac malformation. The development of the aortic valve is closely related to the development of the ascending aorta, associated with structural differences in the bicuspid aorta. Here we describe the non-dilated ascending aortic wall in bicuspid aortic valve patients.

**Methods:** BAV (n=41) and tricuspid aortic valve (TAV) (n=18) non-dilated ascending aortic wall samples were studied. We investigated the following features of the aortic wall: vessel wall thickness, endothelial cell morphology, atherosclerosis, and elastic lamellae organization. Medial pathologic features encompassing elastic fiber thinning, fragmentation and degeneration, overall medial degeneration, mucoid extracellular matrix accumulation, and smooth muscle cell nuclei loss were studied. Furthermore, we included apoptosis, periaortic inflammation, and the level of expression of differentiated vascular smooth muscle cells.

**Results:** The non-dilated BAV ascending aortic wall is characterized by a significantly thinner intimal layer, without features of atherosclerosis ( $P < .001$ ). The medial layer is significantly thicker ( $P < .001$ ) with more mucoid extracellular matrix accumulation ( $P < .001$ ). All other medial pathologic features were more prominent in the TAV ( $P < .001$ ). The media has significantly less differentiated vascular smooth muscle cells ( $P < .001$ ) between the neatly regulated elastic lamellae which are thinner in the BAV as compared to the TAV ( $P < .0001$ ).

**Conclusions:** The BAV ascending aorta without dilatation is characterized by a differentiation defect of vascular smooth muscle cells in the media and a significantly thinner intimal layer without overt pathologic features.

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## 1. Introduction

A bicuspid aortic valve (BAV) is the most common congenital cardiac anomaly, with a population prevalence of 1-2% [1]. The

clinical course of a bicuspid aortic valve is often complicated by valvular abnormalities and thoracic aortopathy [2]. In particular, the latter one forms a critical complication, as the increased risk of aortic dilatation and dissection carries a high morbidity and mortality.

Till date over 900 papers have been written on the pathophysiologic mechanisms leading to bicuspid aortic complications. Most studies have investigated bicuspid aortopathy patients to unravel the mechanisms underlying aortic dilatation and dissection. While studying bicuspid aortic complications however, one must keep in mind that the development of the aortic valve is narrowly related

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to the development of the ascending aorta [3,4]. The cardiac progenitor neural crest cells and second heart field cells are responsible for the valvulogenesis as well as for the development of many components of the vascular wall such as the elastic lamellae, the endothelial cells and the vascular smooth muscle cells (VSMCs) in the aortic root, ascending aorta and aortic arch [3,5-7]. Hence, a combined developmental defect in early embryogenesis could lead to structural differences of the ascending aortic wall associated with a bicuspidy [3,8]. When solely focusing on aortopathy in bicuspidy, these embryonic variants in the non-pathologic specimen can be misread.

This paper aims at describing the non-dilated ascending aortic wall in bicuspid aortic valve patients without thoracic aortic dilatation or dissection. These findings can serve as a reference for future studies to compare and describe bicuspid aortopathy.

## 2. Material and methods

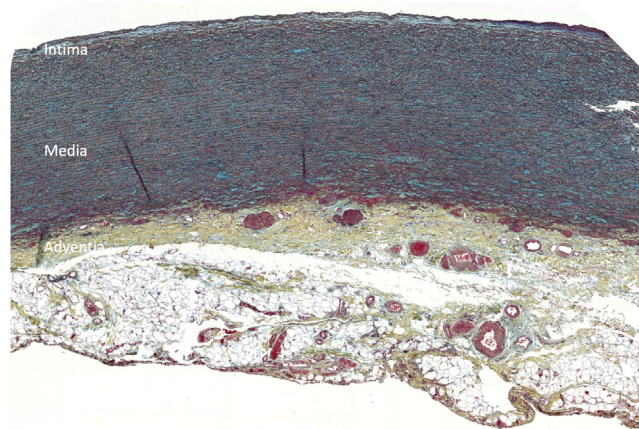
### 2.1. Aortic tissue samples and ethical agreement

Non-dilated ascending aortic wall tissue samples were collected from individuals with BAV and a tricuspid aortic valve (TAV). A non-dilated aorta was clinically defined by an ascending aortic wall diameter of <45mm, based on the cut off for concomitant aortic surgery in the current guidelines [9].

Patients included in this study were collected in the Leiden University Medical Center (LUMC), Leiden, The Netherlands and the Central Hospital, Bad Berka, Germany. The Heart Valve Bank, Thoraxcenter, Erasmus Medical Center, Rotterdam, also provided six BAV samples without aortic dilatation as these were not suitable for transplantation, as approved by their Scientific Advisory Board.

A total of 41 BAV patients were studied (mean age 56.7 ± 8.8 years, 70% male). All included BAV patients had a raphe between the right and left coronary cusp. Material from BAV patients was available from patients undergoing elective aortic valve replacement at the LUMC and the Central Hospital, Bad Berka, Germany.

A total of 18 TAV patients (mean age 63.5 ± 9.0 years, 67% male) were included, which were obtained during aortic valve surgery at the central Hospital, Bad Berka (n=7) and postmortem at the Leiden University Medical Center (n=11). All aortic samples were uniformly obtained from the aortotomy incision. As the BAV patients are generally younger at presentation of thoracic aortopathy, we intended to include patients with a higher cardiovascular risk profile in this study to observe pathological features of the as-



**Fig. 1.** Transverse histologic section (5 μm) stained with MOVAT pentachrome staining of a non-dilated ascending aortic specimen in a bicuspid aortic valve patient. Three layers of the aortic wall are denoted: intima, media and adventitia. Microscopic magnification: 5x

ending aortic wall. We know from our previous studies that BAV patients with an aortic valve stenosis have an increased cardiovascular risk profile as compared to BAV patients with a regurgitant aortic valve. We therefore included all BAV and TAV patients undergoing surgery with a severe aortic valve stenosis with either mild or no regurgitation. The BAV and TAV patients thus had comparable valve pathology and an underlying increased cardiovascular risk profile.

Patients with a proven genetic disorder (eg, Marfan disease) were excluded from the study.

For this study sample, collection and handling were carried out according to the official guidelines of the Medical Ethical Committee of the Leiden University Medical Center and the Central Hospital Bad Berka. All patients gave written informed consent.

Following excision, all specimens were fixed in formalin for 24 hours, decalcified in Kristensen's solution (a formic acid-formate buffer) for 120 hours and subsequently embedded in paraffin in a transverse orientation. Transverse sections (5 μm) were mounted on precoated Starfrost slides (Klinipath B.V., 3057-1, Duiven, Netherlands) comparing different stainings on consecutive sections.

**Table 1**  
Immunohistochemistry reagents.

Primary antibody	Vendor, order number	Concentration	Secondary antibody
Anti-αSMA	A2547, Sigma-Aldrich Chemie, Darmstadt, Germany	1:5000	RAM-PO (1:250) (DAKO p0260)
Anti-SM22α	AB10135, Abcam, Cambridge, United Kingdom	1:100	GAR (1:200) & NGS (1:66) (Vector Laboratories, USA, BA-1000 and S1000)
Anti-Smoothelin	16101, ProgenBiotechnik, Heidelberg, Germany	1:200	HAM (1:200) & NHS (1:66) (Vector Laboratories, USA, BA-2000) (Brunschwig Chemie, Switzerland, S-2000)
Anti-lamin A/C	MAB3211, Millipore, Billerica, USA		HAM & NHS
Anti-cleaved Caspase-3	9661, Cell Signaling, Beverly, United States	1:250	GAR & NGS
Anti-PECAM-1	sc-1506, Santacruz Biotechnology, California, USA	1:500	GAR & NGS
Anti-VonWillebrandFactor	A 0082, DakoCytomation, Denmark	1:500	GAR & NGS
Anti-IL-6	sc-73319, Santacruz Biotechnology, California, USA	1:400	HAM & NHS
Anti-CD68	Thermo Fisher Scientific, Massachusetts, USA	1:500	HAM & NHS

αSMA: alpha smooth muscle actin, SM22α: smooth-muscle-22-alpha, GAR: goat-anti-rabbit-biotin, NGS: normal goat serum, HAM: horse-anti-mouse-biotin, NHS: normal horse serum and RAM-PO: peroxidase-conjugated rabbit anti-mouse.

## 2.2. Routine histology and immunohistochemistry

To study the morphology of the vessel wall the sections were stained with hematoxylin-eosin (HE) and resorcin fuchsin (RF) and Movat pentachrome staining.

For the immunohistochemical stainings, antigen retrieval was performed in a microwave oven in citrate buffer (pH 6.0, 12 minutes) after deparaffinization. Sections were then treated with 0.3% H<sub>2</sub>O<sub>2</sub> 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 and PBS-T. Bound antibodies were detected using 1-hour incubation with a secondary antibody diluted in PBS-T (Table 1). Subsequently, all slides, except for alpha smooth muscle actin ( $\alpha$ SMA), 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. All slides were thereafter incubated with 400 mg/mL 3,30-diaminobenzidine tetrachloride (Sigma-Aldrich Chemie, Buchs, Switzerland; D5637) dissolved in Tris-maleate buffer to which 20 mL of H<sub>2</sub>O<sub>2</sub> 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.

## 2.3. Immunohistochemical and morphometric analyses

Sections were studied with a Leica BM5000 microscope equipped with plan achromatic objectives (Leica Microsystems, Wetzlar, Germany). Features studied were considered according to the three layers of the aortic wall, summarized in Table 2.

The maximum intimal thickness in micrometres is measured as the distance between the endothelial layer and the first major internal elastic lamella, excluding atherosclerotic areas, in the resorcin fuchsin stained sections. Endothelial cell morphology is analysed in the haematoxylin eosin, PECAM-1 and von Willebrand factor stained sections. Presence of atherosclerosis is analyzed in the MOVAT stained sections.

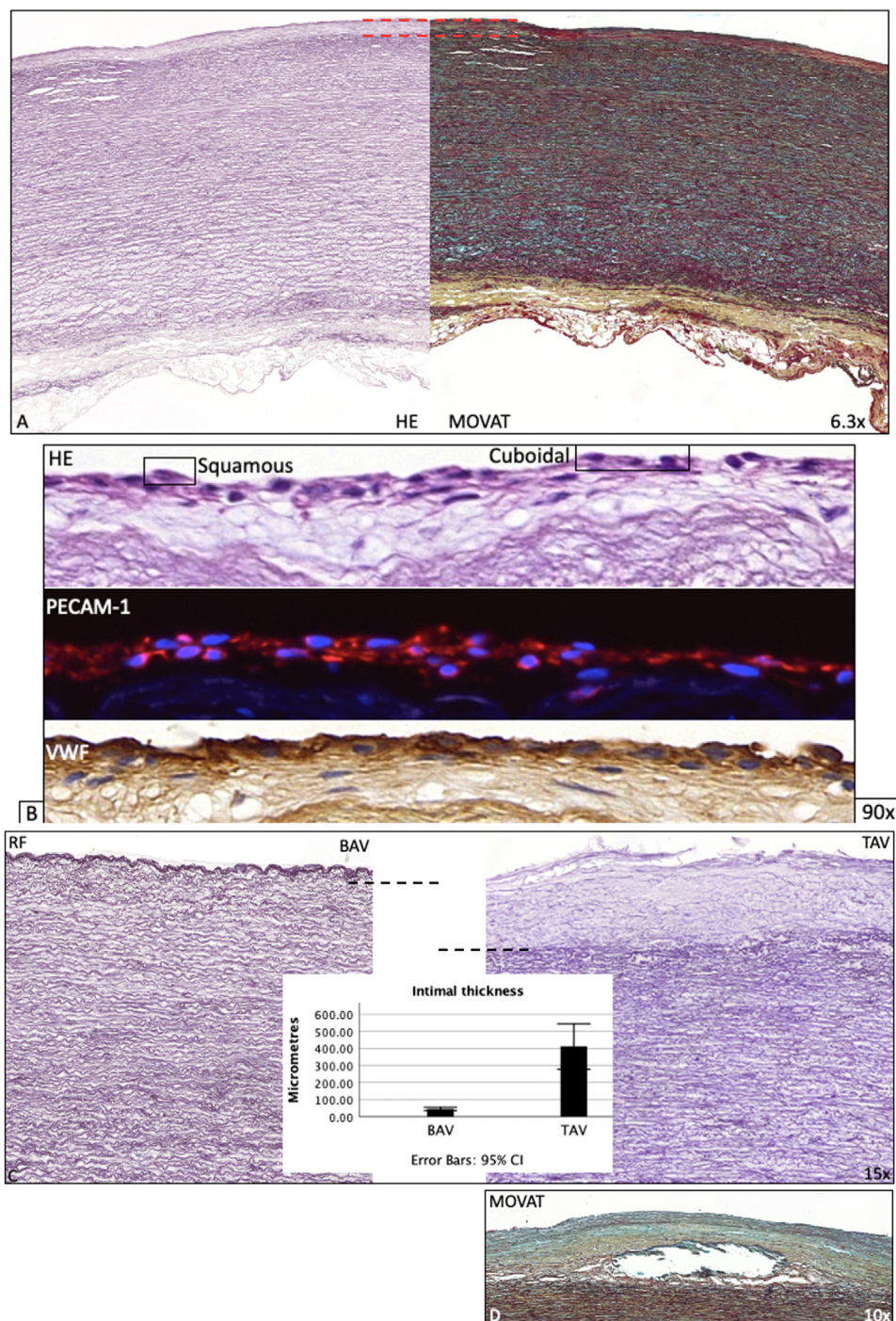
In the resorcin fuchsin stained sections the maximum medial thickness in micrometres was measured as the distance between the first and last elastic lamella on the borderline with the adventitia and the organization of the elastic content was studied qualitatively. Pathologic features of the elastic lamellae, elastic fiber fragmentation/ loss, elastic fiber thinning and elastic fiber degeneration were indexed from zero (none), one (mild), two (moderate) to three (severe) on three predetermined locations (left, middle and right) of every section, that we refer to as 'microscopic fields' maintained in evaluation of all stainings on sister sections. Overall medial degeneration and smooth muscle cell nuclei loss was graded in the haematoxylin eosin, MOVAT and VSMCs stainings  $\alpha$ SMA and smooth muscle 22 alpha (SM22 $\alpha$ ) and indexed from 0-3 on three microscopic fields. Mucoïd extracellular matrix accumulation was graded in the MOVAT stainings and indexed from zero to three. Vascular smooth muscle cell (VSMC) expression was studied on the  $\alpha$ SMA, SM22 $\alpha$  and smoothelin stained sections. Contractile vascular smooth muscle cells express multiple markers indicative of their relative state of differentiation/ maturation, but no single marker is specific. A variety of vascular smooth muscle cell markers have been described for contractile vascular smooth muscle cells. We have chosen to study a combination of markers which are expressed in contractile vascular smooth muscle cells [10]. The cytoplasmatic level of expression of  $\alpha$ SMA, SM22a and smoothelin was indexed at the three microscopic fields, from 0 (no expression in vascular smooth muscle cells), one (expression in less than one-third of all VSMC), two (expression in two-thirds of all VSMC) to three (expression in all VSMC). To determine apoptosis in the specimen with smooth muscle cell nuclei loss, caspase-3 expression was determined in a sample staining of five BAV and five TAV patients. In each stained section the number of positively stained nuclei was counted using ImageJ in the three microscopic fields. A threshold was applied to filter background noise. The total number of nuclei (positively stained and negative) was equal in all specimens. In each microscopic field, the number of lamin-positive nuclei was therefore normalized to the total number of nuclei per 100,000 mm<sup>2</sup>. The number of normalized lamin- and caspase 3-positive nuclei was averaged between the three microscopic fields.

Periaortic inflammation (presence of a cellular infiltrate in the adventitia), was analysed in the haematoxylin eosin, IL-6 and CD68 stained sections and indexed from zero (no inflammatory cells), one (a few cells), two (groups of cells) to three (large clusters of cells).

**Table 2**  
Features studied in the ascending aortic wall.

Vessel wall layer	Feature	Staining
Tunica intima	Intimal layer thickness Endothelial cell morphology Atherosclerosis	Haematoxylin eosin Resorcin fuchsin MOVAT pentachrome staining PECAM-1 Von Willebrand Factor
Tunica media	Medial layer thickness Elastic lamellae organization Medial pathologic features: EMD, EFF/L, EFT, EFD, MEMA, SMCNL Vascular smooth muscle cell expression Apoptosis	Haematoxylin eosin Resorcin fuchsin MOVAT pentachrome staining $\alpha$ SMA SM22 $\alpha$ Smoothelin Caspase-3
Tunica adventitia	Fat cells Inflammatory cells	Haematoxylin eosin Resorcin fuchsin MOVAT pentachrome staining IL-6 CD68

Abbreviations: EMD: overall medial degeneration; EFF/L: elastic fiber fragmentation and loss; EFT: elastic fiber thinning; EFD: elastic fiber disorganization; MEMA: mucoïd extra cellularmatrix accumulation; SMCNL: smooth muscle cell nuclei loss;  $\alpha$ SMA: alpha smooth muscle actin; SM22 $\alpha$ : smooth muscle 22 alpha; TGF $\beta$ : Transforming growth factor beta; phosphorylated SMAD2; IL-6: interleukin-6.



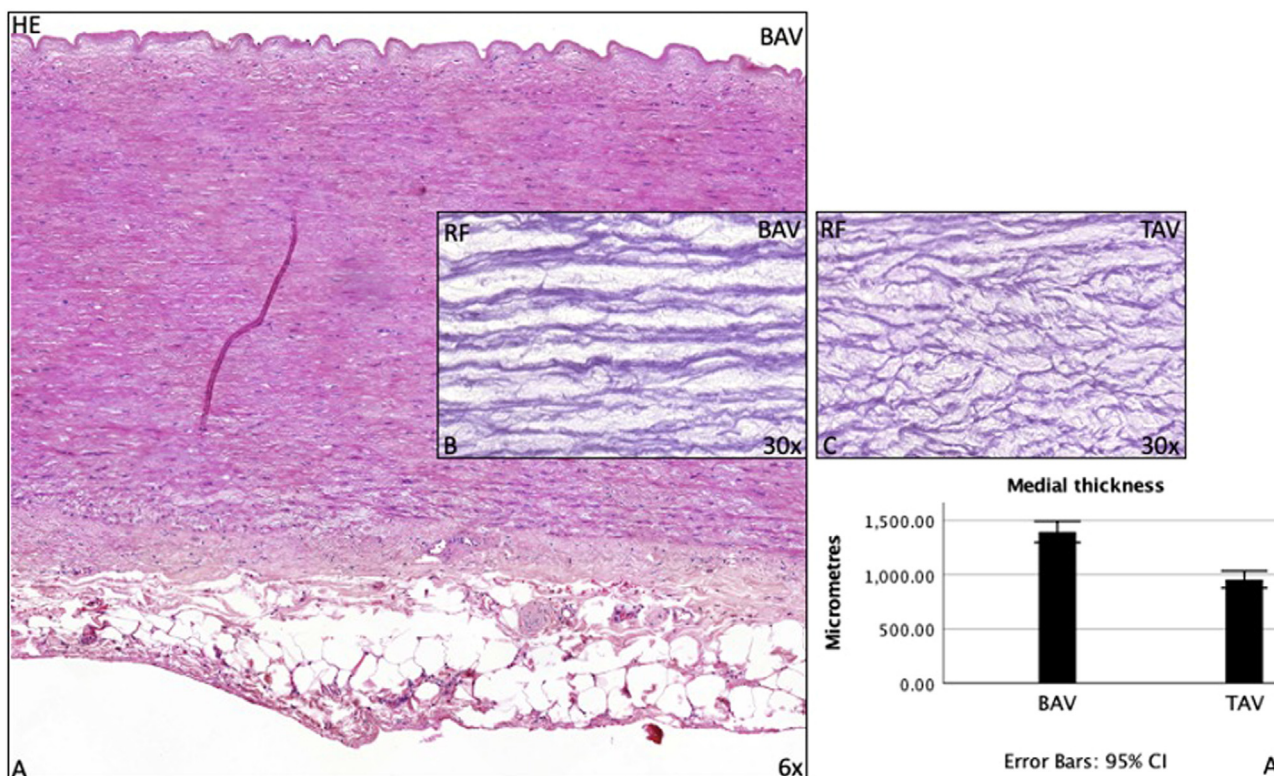
**Fig. 2.** Transverse histologic sections of a non-dilated ascending aortic specimen in a bicuspid aortic valve patient (5  $\mu$ m). 2A stained with resorcin fuchsin (left) and MOVAT pentachrome (right) staining. The intimal layer is delineated between the lamina elastica interna and the endothelial cell layer with a red dashed line. 2B: the intimal cell layer is shown in detail, squamous and cuboidal cells are found lining the luminal surface in haematoxylin eosin, PECAM-1 and Von Willebrandfactor staining. 2C The intimal layer is shown in the BAV (left) and TAV (right), the intimal layer is significantly thinner in the BAV as compared to the TAV (graph 2C). 2D: In the TAV atherosclerosis is shown in the intimal layer in MOVAT pentachrome staining. Microscopic magnification: 2A 6.3x; 2B 90x; 2C 10x. Abbreviations: HE: Haematoxylin eosin, RF: Resorcin fuchsin; VWF: Von Willebrandfactor; BAV: bicuspid aortic valve; TAV: tricuspid aortic valve.

All specimens were re-evaluated by an independent, experienced histopathologist who was blinded to the clinical data, which confirmed the findings.

#### 2.4. Statistical analyses

All numerical data are presented as mean  $\pm$  standard deviation of three fixed microscopic fields on each stained slide. Statisti-

cal differences were evaluated with the Mann–Whitney U-test for comparison between the groups. An additional 1-, 2- and 3-way analysis of variance test was performed to correct for age and gender and it was found that both factors were not confounding in this study. Significance was assumed when  $P < .05$  using the SPSS 25.0 software program (SPSS, Inc., Chicago, IL). GraphPad software (GraphPad Software, Inc, San Diego, CA) was used to create graphics of the statistical analyses.



**Fig. 3.** Transverse histologic sections of a non-dilated ascending aortic specimen in a bicuspid and tricuspid aortic valve patient (5  $\mu$ m). 3A stained with haematoxylin eosin, graph 3A demonstrates the significant difference in medial thickness between the BAV and TAV. A detail of the elastic lamellae is shown in 3B and 3C in resorcin fuchsin stained sections. BAV shows more crosslinking of the elastic lamellae as compared to the TAV (3B vs 3C). Microscopic magnification: 3A 6x; 3B and 3C 30x. Abbreviations: HE: Haematoxylin eosin, RF: Resorcin fuchsin; BAV: bicuspid aortic valve; TAV: tricuspid aortic valve.

### 3. Results

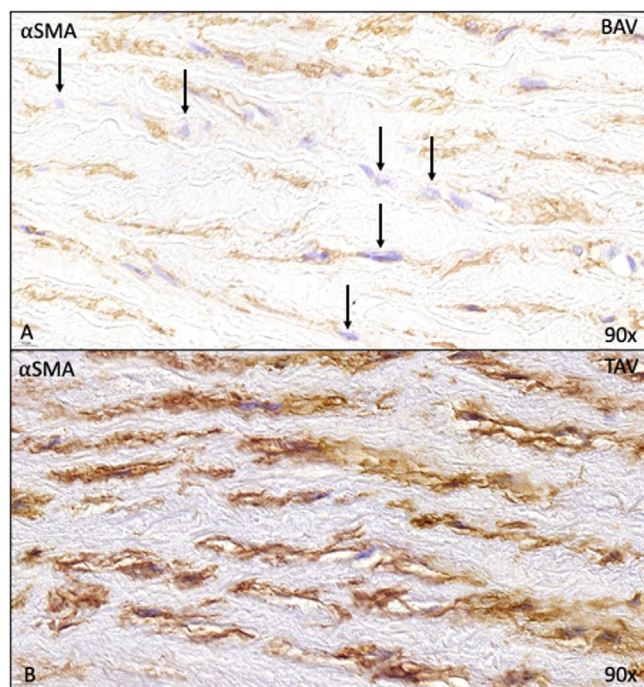
The ascending aortic wall is divided in three layers: the tunica intima, media and adventitia (Fig. 1). Each layer is separately described for the non-dilated bicuspid ascending aorta and compared to the non-dilated tricuspid ascending aorta. The findings are described in the following sections.

#### 3.1. The intimal layer

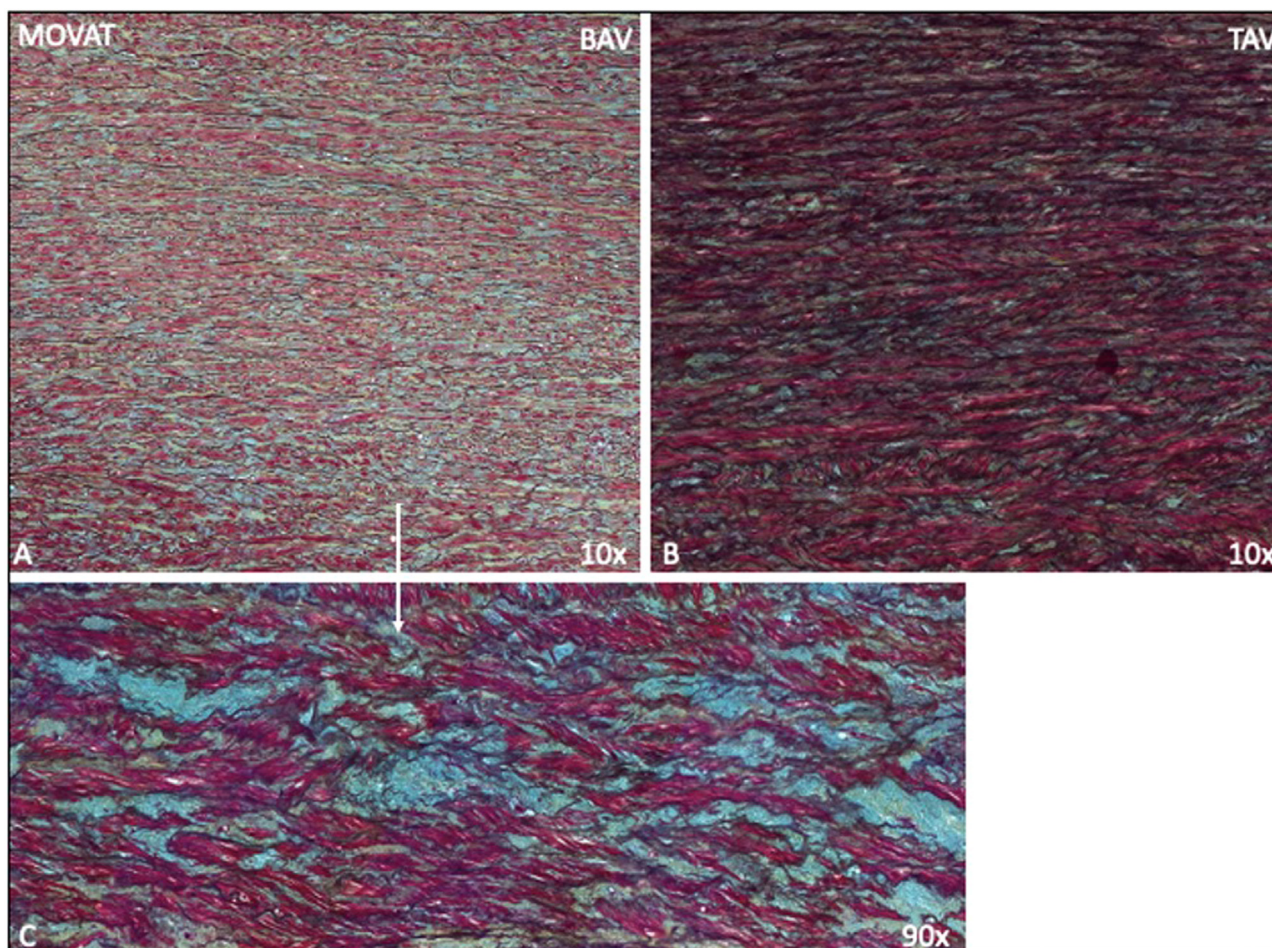
The intima is bordered by the endothelial cell layer lining the luminal surface and the lamina elastica interna (Fig. 2A). The endothelial cells had a similar morphological appearance in the BAV and TAV patients, with a predominant squamous morphology, with a few cuboidal cells in both patient groups (Fig. 2B). The absolute intimal thickness was significantly lesser in all BAV patients as compared to the TAV ( $P < .001$ ) (Fig. 2C, graph 2C). The mean intima to total vessel ratio was  $0.03 \pm 0.02$  in the BAV as compared to the  $0.28 \pm 0.15$  TAV ( $P < .001$ ). Intimal atherosclerosis was significantly less apparent in the BAV as compared to the TAV patients ( $P < .0001$ ) (Fig. 2D). Observed intimal differences in BAV and TAV were not found related to age or gender.

#### 3.2. The medial layer

The media is situated between both the lamina elastica interna and externa. The absolute medial thickness was greater in all BAV as compared to all TAV patients ( $P < .001$ ) (Fig. 3A, graph 3A). The number of elastic lamellae was not different between the BAV and TAV patients (data not shown). In both groups the elastic lamellae had a regular distribution, although in the TAV the lamellae



**Fig. 4.** Transverse histologic sections of a non-dilated ascending aortic specimen in a bicuspid and tricuspid aortic valve patient (5  $\mu$ m). Figure 4A demonstrates that individual smooth muscle cells in the BAV express less actin (noted with arrows). Microscopic magnification: 90x. Abbreviations:  $\alpha$ SMA: alpha smooth muscle actin; BAV: bicuspid aortic valve; TAV: tricuspid aortic valve.



**Fig. 5.** Transverse histologic sections of a non-dilated ascending aortic specimen in a bicuspid and tricuspid aortic valve patient (5  $\mu$ m). The ascending aortic media in the BAV shows more blue lakes of mucoïd extracellular matrix as compared to the TAV, A-C. Microscopic magnification: 5A,B: 10x and 5C: 90x. Abbreviations: BAV: bicuspid aortic valve; TAV: tricuspid aortic valve.

showed more fragmentation as compared to the BAV population (Fig. 3B and 3C).

Differences in the expression of actin-positive VSMC were noted between both groups. The BAV patients showed less aSMA, SM22 alpha and smoothelin expression as compared to the TAV ( $P < .001$ ). Less expression in BAV was not due to focal loss of vascular smooth muscle cells, as is seen in vessel wall degeneration, rather individual cells expressed less aSMA, SM22 alpha and smoothelin (Fig. 4).

Several pathologic features of the aortic media were more prominent in the TAV as compared to the BAV. These included elastic fiber fragmentation/ loss ( $P < .001$ ), elastic fiber degeneration ( $P < .001$ ), overall medial degeneration ( $P = .001$ ) and smooth muscle cell nuclei loss ( $P < .001$ ). On the other hand, elastic fiber thinning ( $P > .001$ ) and mucoïd extracellular matrix accumulation were more outspoken in the thicker medial layer of the BAV as compared to the TAV ( $P < .001$ ) (Fig. 5). To determine apoptosis in the specimen with smooth muscle cell nuclei loss, caspase-3 expression was determined in a sample staining of five BAV and five TAV patients. In this subgroup apoptosis was significantly less in the bicuspid as compared to the tricuspid patients ( $P = .05$ ).

### 3.3. The adventitial layer

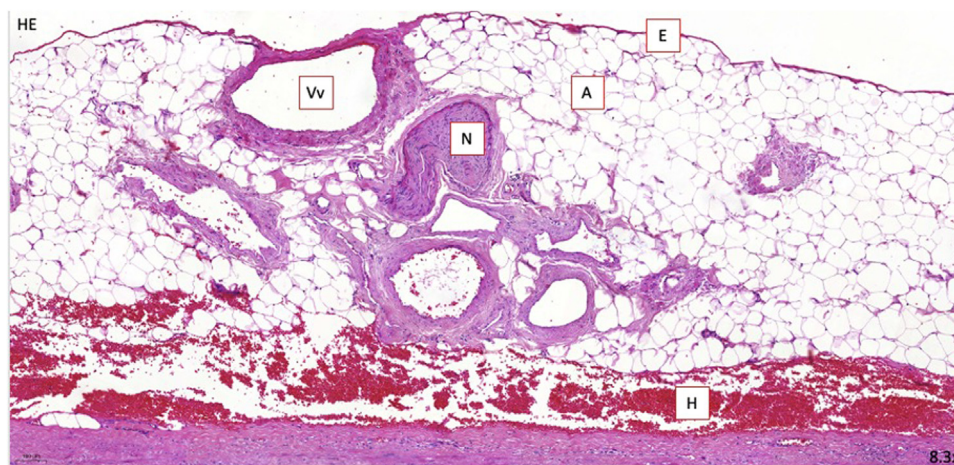
The adventitia consisted of loose fibrous tissue containing epicardium-derived cells, nerve fibers, fibroblasts, adipocytes, and vasa vasorum lined by endothelium and VSMCs (Fig. 6). No differences in number of adipocytes were noted between the groups

(data not shown). Inflammation was significantly less observed in the bicuspid group as compared to the tricuspid aortic valve patients ( $P < .001$ ).

## 4. Discussion

BAV patients have a higher prevalence of thoracic aortopathy as compared to their tricuspid counterparts [11]. During embryonic development the cardiac progenitor neural crest and second heart field cells are responsible for the valvulogenesis as well as for the development of the ascending aortic wall [3,5,6,12]. As a result, a developmental defect in of these populations during embryogenesis would not only lead to a bicuspid aortic valve but also to an associated altered development of the ascending aortic wall [8]. Till date, pathological findings in dilated or dissected bicuspid aorta have been extensively investigated to understand the underlying pathogenetic mechanisms leading to aortopathy. We hypothesize that an early embryonic defect in the ascending aortic wall formation will persist as microstructural disease in the adult aortic wall. All patients with a bicuspid aortic valve would present with these structural differences, even though approximately one third of all BAVs will never develop aortopathy during their lifetime. It is therefore particularly interesting to study the differences in characteristics which are already present in the non-dilated bicuspid versus tricuspid ascending aorta.

To gain knowledge about the structural differences in the BAV aorta, we studied the non-dilated and non-dissected ascending aor-



**Fig. 6.** Transverse histologic sections of a non-dilated bicuspid ascending aortic wall adventitial layer (5  $\mu$ m) stained with haematoxylin eosin (HE). Several features are seen in the adventitial layer: epicardial cells (E); adipocytes (A), nerve cells (N), vasa vasorum (Vv) and some haemorrhage (H). Microscopic magnification: 8.3x

tic wall in BAV patients and compared it to the non-dilated tricuspid aortic valve patients.

Tricuspid aortic aneurysm and dissection are regarded as degenerative vascular diseases [13,14]. The main pathologic features which are seen in tricuspid aortopathy are periaortic inflammation, medial degeneration, remodeling of extracellular matrix, and atherosclerosis [15]. Even before dilatation, the ascending aorta in tricuspid aortic valve already shows early signs of pathology associated with cardiovascular ageing [16]. Bicuspid aortopathy, however, is not associated with features of cardiovascular ageing [14,17]. We hypothesize that embryonic differences might underlie the decreased risk for degenerative vasculopathy.

During embryonic development, the intimal layer is just a few cell layers thick in TAV which, after birth, grows out to a significantly thicker layer [18]. After adolescence characteristics of pathology are seen in tricuspid and atherosclerotic plaques build up in the intima [18]. In the bicuspid patients in contrast, the intimal layer is thicker during the developmental phase and after birth the intimal layer regresses to being only a few cell layers thick [18]. In this study we found that the intimal layer in adult, non-dilated and non-dissected tissues is still significantly thinner in all patients with a bicuspid aortic valve as compared to the tricuspid. As expected from an embryonic point of view, the intimal layer differences in BAV and TAV were not age-dependent. The findings of similarities between the embryonic TAV and adult BAV intimal layer corroborates our hypothesis that in bicuspid a developmental defect might underlie part of the structural differences seen in the aortic wall. The thinner intimal layer in the bicuspid aortic intima further showed hardly any signs of atherosclerosis. This is in line with many clinical studies which describe less atherosclerosis in the bicuspid patients as compared to the tricuspid group [19,20]. Intimal layer formation and the development of atherosclerosis require endothelial to mesenchymal transition (endoMT) [21,22]. EndoMT is a complex biological process in which endothelial cells lose their specific endothelial cell markers and acquire a mesenchymal or myofibroblastic phenotype initiating expression of mesenchymal cell products such as  $\alpha$ SMA and SM22alpha. Recent studies have reported distorted endoMT in patients with a bicuspid aortic valve [23]. In this study, we did not find any differences in the morphological appearance of the endothelial cells in both groups, even though no functional analysis was performed. We however did observe a significant lower expression of contractile vascular smooth muscle cell markers in the BAV as compared to the TAV.

Vascular smooth muscle cells which reside in the aortic wall are not terminally differentiated. Therefore, VSMCs have the ability to undergo a phenotypic switch from a quiescent contractile to a proliferative synthetic phenotype. The contractile smooth muscle cells produce low levels of extracellular matrix proteins, but express contractile proteins including  $\alpha$  SMA, SM22 $\alpha$  and smoothelin [10]. When activated, smooth muscle cells proliferate, migrate, and produce large amounts of extracellular matrix proteins. In BAV, the majority of smooth muscle cells express synthetic markers which could partly explain the large amounts of extracellular matrix. The aortic media was also significantly thicker in the BAV, which was not due to the number of elastic lamellae. Rather the area between the lamellae was greater in the BAV, and was prominently filled with mucoid extracellular matrix. The elastic lamellae were evenly distributed in the BAV and TAV. In the TAV the lamellae were more fragmented as compared to the BAV. The BAV had more neatly organized fine lamellae, which has earlier been described [17]. These fine lamellae are similar to lamellae which are observed before birth [18].

In bicuspid aortic valve patients, an early embryonic defect leads to a defect in the development of the aortic valve as well as the structural components of the vessel wall. Consequently, all BAV patients have a structurally different ascending aortic wall as compared to TAV individuals. Importantly, 20-40% of BAV patients do not develop aortic complications, implicating that the BAV population is heterogeneous in terms of susceptibility for aortopathy. The findings described in this study are therefore not predictive for future aortopathy, rather indicate that additional hemodynamic or molecular biological processes are responsible for thoracic aortopathy in the majority of BAV patients.

Future studies should focus on the vascular wall pathology in BAV which is superimposed on the findings described in this study.

## 5. Conclusions

The BAV ascending aorta without aortopathy is characterized by a differentiation defect of VSMCs in the media and a significantly thinner intimal layer without overt pathologic features. We propose that the structural differences of the ascending aortic wall caused by early embryonic defects could explain the lack of age-dependent degenerative features in bicuspid aortopathy. In a subgroup of BAV patients with an increased susceptibility for thoracic aortopathy, additional pathologic factors are yet to be identified.



## Conflicts of interest

None.

## Acknowledgments

None.

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