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Intrinsic histological and morphological abnormalities of the pediatric thoracic aorta in bicuspid aortic valve patients are predictive for future aortopathy

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ABSTRACT

Background: Patients with a bicuspid aortic valve (BAV) have an increased risk to develop aortic complications. Many studies are pointing towards a possible embryonic explanation for the development of both a bicuspid aortic valve as well as a defective ascending aortic wall in these patients. The fetal and newborn ascending aortic wall has however scarcely been studied in bicuspid aortic valve patients. We hypothesize that early histopathological defects might already be visible in the fetal and pediatric ascending aortic wall of bicuspid aortic valve patients, indicating at an early embryonic defect.

Methods: Non-dilated BAV ascending aortic wall samples were collected (n = 40), categorized in five age groups: premature (age range *17.5 weeks + days GA till 37.6 weeks + days GA*) 2. neonate (age range *1 – 21 days*) 3. infant (age range *1 month – 4 years)* 4. adolescent (age range *12 years – 15 years)* and 5. adult (age range *41 – 72 years)*. Specimen were studied for intimal and medial histopathological features.

Results: The premature ascending aortic wall has a significantly thicker intimal and significantly thinner medial layer as compared to all other age categories ($p < 0.05$). After birth the intimal thickness decreases significantly. The medial layer increases in thickness before adulthood ($p < 0.05$) with an increasing number of elastic lamellae ($p < 0.01$) and interlamellar mucoid extracellular matrix accumulation ($p < 0.0001$). Intimal atherosclerosis was scarce and medial histopathological features such as overall medial degeneration, smooth muscle cell nuclei loss and elastic fiber fragmentation were not appreciated in the BAV ascending aortic wall of any age.

Conclusions: The main characteristics of a bicuspid ascending aortic wall are already present before adulthood, albeit not before birth. Considering the early manifestations of ascending aortic wall pathology in bicuspid aortic valve patients, the pediatric population should be considered while searching for markers predictive for future aortopathy.

1. Introduction

A bicuspid aortic valve (BAV) is characterized by an aortic valve that consists of two instead of the normal three cusps. Most BAV patients have a significantly increased risk to develop ascending aortic wall pathology [30]. To prevent the occurrence of lethal thoracic aortopathy in BAV patients, the ascending aortic dimensions are followed during their entire life. Once the aortic diameter reaches a predetermined threshold, it is recommended to prophylactically replace the aorta. It however remains difficult to determine which group of BAV individuals is at risk to develop future aortic complications and would thus benefit most of an open-heart surgery, and in which group of patients a preventive intervention is not required. These uncertainties have led to several revisions of the aortic guidelines over the past years [6,24]. Nevertheless, the current approach is still generalized and does not take the individual patient characteristics into account. Several studies have advocated a personalized treatment approach in thoracic aortopathy [20,23]. By studying the ascending aortic wall in non-dilated and dilated BAV

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Table 1
Study population.

Age category	Bicuspid aortic valve	Gender
Premature (<38 weeks gestational age)	– N = 5	Unknown
	– 17,5 WK, D (n = 1)	Unknown
	– 19 WK (n = 1)	Unknown
	– 20 WK (n = 1)	Unknown
	– 36 WK (n = 1)	Unknown
	– 37.6 WK, D (n = 1)	
Neonate (0 - <30 days)	N = 6	Unknown
	– 1 D (n = 2)	Unknown
	– 2 D (n = 1)	Unknown
	– 11 D (n = 1)	Unknown
	– 13 D (n = 1)	Unknown
	– 21 D (n = 1)	
Infant (1 month – < 4 years)	N = 8	Unknown
	– 1 M (n = 1)	Male
	– 3 M (n = 2)	Unknown
	– 4 M (n = 1)	Unknown
	– 5.5 M (n = 1)	Unknown
	– 11 M (n = 1)	Unknown
	– 2 Y (n = 1)	Unknown
	– 4 Y (n = 1)	
Adolescent (12 – < 18 years)	N = 4	Male
	– 12 Y (n = 2)	Female
	– 13 Y (n = 1)	Female
	– 15 Y (n = 1)	
Adult (> 18 years)	N = 17	Female
	– 41 Y (n = 1)	Male
	– 42 Y (n = 1)	Male and Female
	– 47 Y (n = 2)	Male
	– 49 Y (n = 1)	Female
	– 50 Y (n = 1)	Male
	– 51 Y (n = 1)	Male
	– 52 Y (n = 1)	Male
	– 54 Y (n = 1)	Female
	– 56 Y (n = 1)	Female
	– 58 Y (n = 1)	Male
	– 62 Y (n = 1)	Male
	– 64 Y (n = 1)	Male
	– 65 Y (n = 1)	Male
	– 70 Y (n = 2)	Male
	– 72 Y (n = 1)	

specimens, potential markers for future aortic complications have been defined [9,15]. Comparison of adult non-dilated and dilated specimens has broadened our knowledge on the pathogenesis of bicuspid aortopathy, although some primary questions remain unanswered. It has recently been shown in the literature that the development of a bicuspid aortic valve and abnormalities in the ascending aortic wall are linked to each other through an early embryonic defect [19,27,28].

Lineage tracing studies have till date focused on the early development of a bicuspid aortic valve [8,27], the ascending aortic wall has scarcely been studied in fetal and newborn BAV patients. We hypothesize that histopathological ascending aortic wall defects in BAV patients have an embryonic origin and are therefore already visible in the unborn and pediatric patients. The aim of this study is to describe early histopathological defects in the fetal and pediatric ascending aortic wall of BAV patients, predictive for future aortopathy.

2. Material and methods

2.1. Patients, ascending aortic wall tissue and ethical approval

A total of 40 non-dilated ascending aortic wall specimens from bicuspid aortic valve patients were included in this study (Table 1). Inclusion criteria were an age of 18 years or above and being scheduled for an elective surgery. Patients were excluded if they were known with a genetic or connective tissue disorder. The cohort age categories were derived from The World Health Organization classification: premature < 38 weeks gestational age; neonate 0 < 30 days of age; infant 1 month < 2 years; adolescent 12 < 18 years and adults > 18 years of age [7]. The age of the patients ranged from 17.5 weeks + days gestational age (GA) till 72 years. Serial sections of postmortem premature fetal (n = 5), neonate (n = 6) and infant (n = 8) ascending aortic tissue were selected from the Leiden Collection of (malformed) hearts obtained from autopsies (Department of Anatomy and Embryology, LUMC, Leiden, the Netherlands). Adolescent (n = 4) postmortem BAV specimens were obtained from the Pathology Department (LUMC).

Adult non-dilated (diameter <45 mm) aortic specimens were obtained during aortic surgery when the preferred stentless aortic root replacement was performed in the LUMC. In all abovementioned age-categories the aortic specimens were uniformly obtained from the aortotomy site.

Patients with a congenital cardiac anomaly besides a BAV were excluded in this study (e.g., coarctation of the aorta, ventricular septal defect, Tetralogy of Fallot).

The study was undertaken in accordance with the local ethics committee and the Dutch regulation for the proper use of human tissue for medical research purposes. Written informed consent was obtained from the adolescent and adult patients.

Premature fetal, neonatal, and infant aortic specimens which were obtained from the Leiden Collection were preserved in ethanol and glycerin dating from the 1950s in an era where no formal approval was in effect. The material was anonymized, and no privacy rules were violated. Due to the anonymization, gender of the premature, neonate and infant patients was not known to the researchers. This study was conducted in accordance with the LUMC institutional guidelines for the use of human tissue and the Declaration of Helsinki.

2.2. Sample processing and routine histology

The sectioning and staining protocols are described previously [21]. All specimens were fixed in formalin, decalcified, embedded in paraffin, and subsequently sectioned (4 μm). The sections were stained with hematoxylin-eosin (HE), resorcin fuchsin (RF), and Movat pentachrome.

Characteristic histopathologic features were studied in the ascending aortic wall of all patients. To describe the histopathological features in the aortic wall in a standardized way, we used terms from the grading system described in the aortic consensus paper and our own study [13,22]. Features described in this scoring system are overall medial degeneration (OMD), elastic fiber fragmentation and loss (EFF/L), elastic fiber thinning (EFT), elastic fiber disorganization (EFD), mucoid extra cellular matrix accumulation (MEMA), and smooth muscle cell nuclei loss (SMCNL). All features were indexed as 0 (none), 1 (mild), 2 (moderate), or 3 (severe) on three predetermined locations (left, middle, and right) of every section, which we refer to as “microscopic fields” maintained in evaluation of all stainings on sister sections. The mean index of each section was evaluated for statistical analysis.

We further studied the number of medial elastic lamellae and the intimal architecture, intimal thickness and intimal atherosclerosis. The absolute intimal thickness was defined as the area between the lamina elastica interna and the endothelial cells. The severity of atherosclerosis was graded in all patients and plaque morphology was classified according to the modified AHA classification scheme proposed by Virmani et al. [31].

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

2.3. Statistical analysis

All numerical data are presented as mean ± SD of three microscopic fields on each stained slide. For comparison between the groups, statistical differences were evaluated with the one way ANOVA test. Significance was assumed when $p < 0.05$ using Graphpad Prism 9.0 software program. Graphpad software was further used to create graphics of statistical analysis.

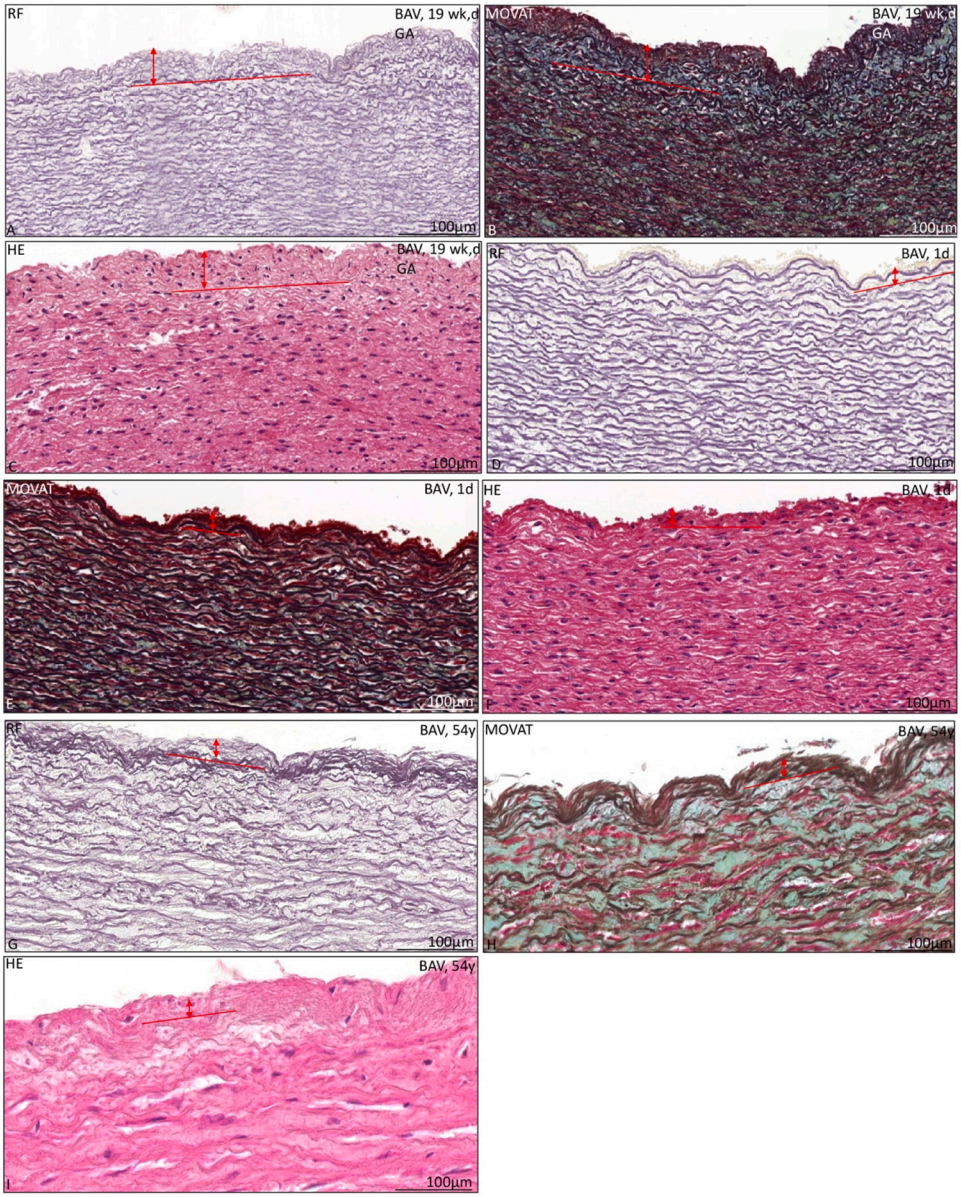

Fig. 1. Transverse histologic section of the ascending aortic wall (4 µm) in non-dilated bicuspid aortic valve patients, stained with recorsin fuchsin (A,D,G), MOVAT pentachrome staining (B,E,H) and hematoxylin eosin (C, F, I). The intimal layer thickness is significantly greater in the premature age group (A-C) as compared to the neonatal age (D-F) and the adult age group (G-I). The intimal layer thickness is indicated with the red line and arrow in all figures (A-I). Abbreviations: BAV: bicuspid aortic valve, GA: gestational age, HE: hematoxylin eosin, RF: recorsin fuchsin. Scale bars shown in the figures.

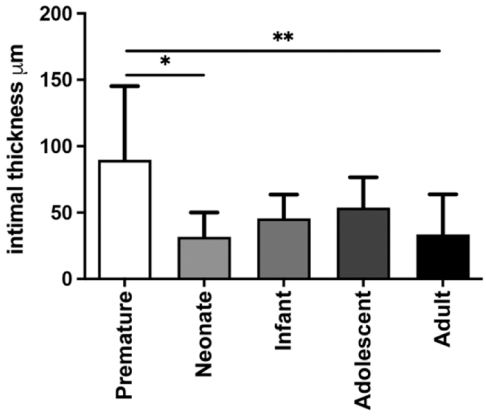

Fig. 2. The graph in Fig. 2 illustrates the differences in intimal thickness between the five age categories. The intimal layer thickness is significantly greater in the premature age group as compared to the neonatal age $p < 0.05$ and the adult age group, $p < 0.01$.The * $p < 0.05$, **$p < 0.01$.

3. Results

The ascending aortic wall specimens were divided in 5 age categories: 1. Premature (age range *17.5 weeks + days GA till 37.6 weeks + days GA*) 2. Neonate (age range *1 – 21 days*) 3. Infant (age range *1 month – 4 years)* 4. Adolescent (age range *12 years – 15 years)* and 5. Adult (age range *41 – 72 years)*.

3.1. The ascending aortic intima

The intimal layer thickness differed considerably across the age groups of the BAV patients (Fig. 1). The premature, fetal intimal layer had a mean thickness of 89.80 ± 55.37 µm (Fig. 1A-C), which was significantly greater as compared to the aortic intimal layer in the neonatal (mean thickness 31.67 ± 18.35 µm) (Fig. 1D,F,G) and adult (mean thickness 33.53 ± 30.20 µm) (Fig. 1H-J) patient group ($p < 0.05$ and $p < 0.01$ respectively) (Fig. 1).

The intimal layer was devoid of atherosclerosis in nearly all patients. Only three patients in the adult group showed moderate atherosclerotic plaques, defined as pathological intimal thickening in two patients

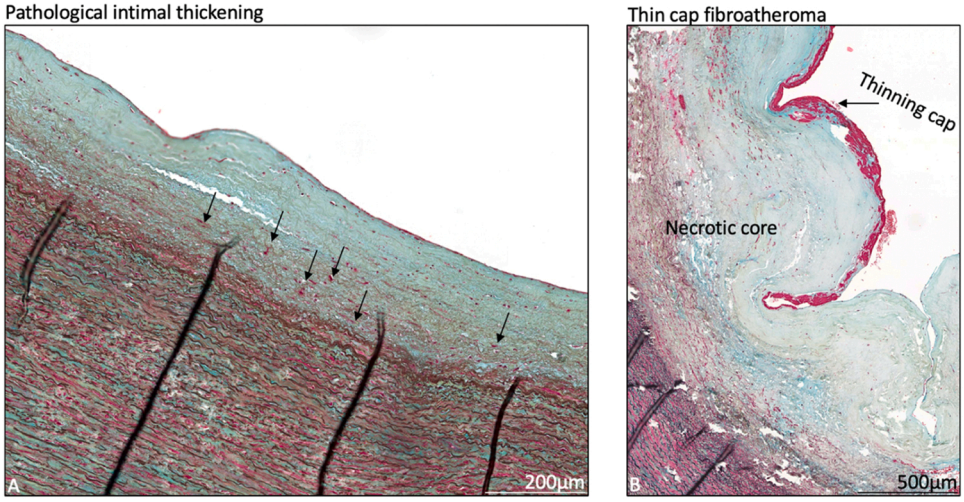

Fig. 3. Transverse histologic section of the ascending aortic wall (4 µm) in adult non-dilated bicuspid aortic valve patients, stained with MOVAT pentachrome staining. Atherosclerosis was scarcely seen in the BAV population, two patient showed pathological intimal thickening (2 A), and one patient had a thinning cap fibroatheroma. Pathological intimal thickening is characterized by the presence of lipid pools deep within the intima near the intimal medial border with overlying vascular smooth muscle cells (black arrow). Scale bars shown in the figures.

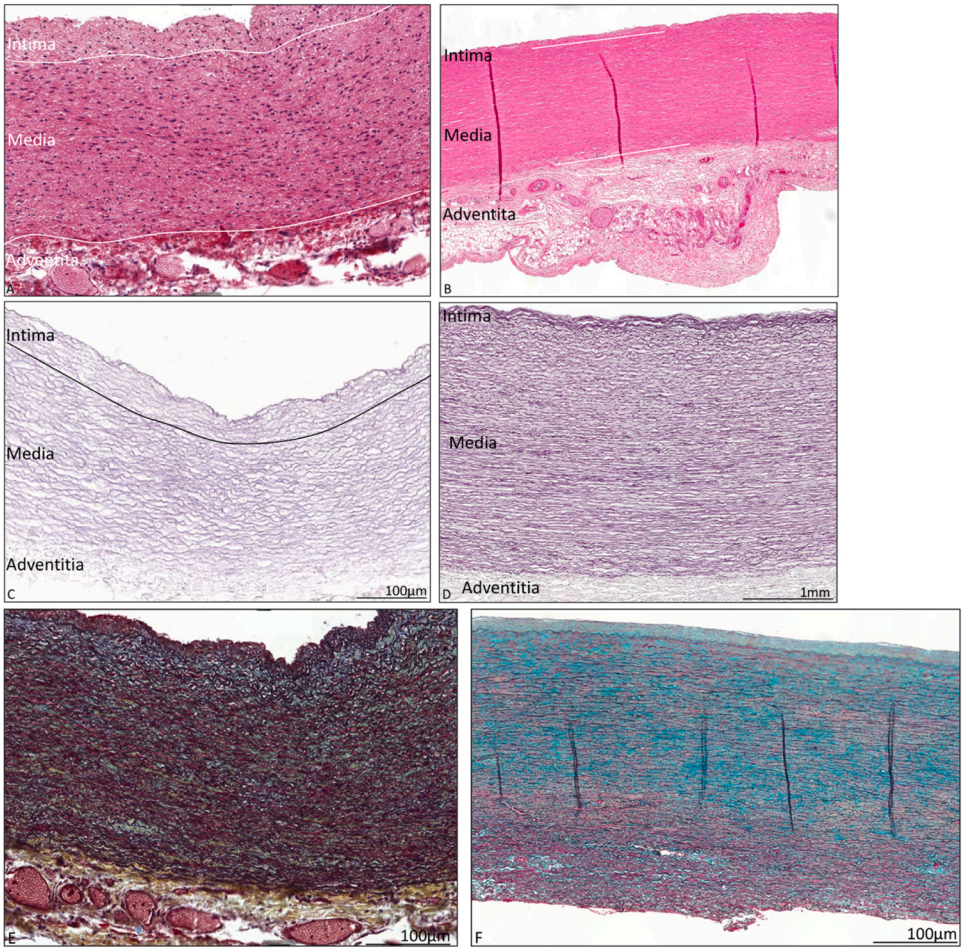

Fig. 4. Transverse histologic section of the ascending aortic wall (4 µm) in non-dilated bicuspid aortic valve patients, stained with hematoxylin eosin (3 A,B), resorcin fuchsin (3 C, D) and MOVAT pentachrome staining (3E,F). MOVAT pentachrome stains collagen and reticulin in yellow, glycosaminoglycans in light blue, muscle in red, elastic fibers in dark purple, and nuclei in black. The premature ascending aortic wall is shown in Fig. 3A, C and E, while the adult ascending aortic wall is demonstrated in Fig. 3B, D and F. The medial layer was thinnest before birth and increased significantly in thickness throughout life (Fig. 3A,B). A significant increase in the number of elastic lamellae is further observed across the age categories (Figure C,D). Mucoid extracellular matrix accumulation was almost absent in the premature ascending aortic wall (Fig. 3E, whereas a significant amount is seen in the adult aortic wall, which is revealed by the light blue MOVAT staining between the elastic lamellae (Fig. 3E,F).Scale bars shown in the figures.

(Fig. 2A) and thin cap fibroatheroma in one patient (Fig. 2B).

3.2. The ascending aortic media

In contrast to the intimal layer, the medial layer was thinnest before birth and increased significantly in thickness throughout life (Fig. 3A-C). From the premature stage till infanthood a trend to increasing medial thickness is seen, albeit not significant. In adolescence the medial thickness is significantly greater as compared to the premature ($p < 0.01$) and the neonatal stage ($p < 0.05$). In the adult specimen medial thickness is greatest as compared to all other age categories ($p < 0.001$) (Fig. 3A-C). In line with the increase in medial thickness an increase in the number of elastic lamellae and amount of mucoid extra cellular matrix was observed across the age categories (Figure D-F). The mean number of elastic lamellae before and directly after birth was 45.4 $\pm$ 5.5 and 47 $\pm$ 5.5 respectively. The number increased significantly

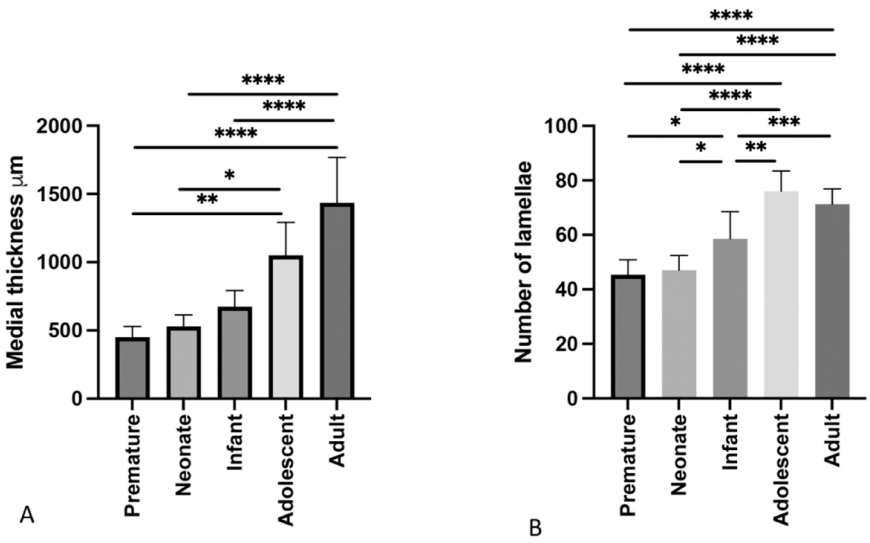

Fig. 5. The graph in Fig. 5 illustrates differences in the medial thickness (A) and the number of elastic lamellae in the medial layer (B) between the five age categories. The medial layer was thinnest before birth and increased significantly in thickness throughout life (A). A significant increase in the number of elastic lamellae is further observed across the age categories (B). The number increases significantly between every age category after the neonatal phase ($p < 0.01$) and is highest in the adolescent and adult phase (B). Abbreviations: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Scale bars shown in the figures.

between every age category after the neonatal phase ($p < 0.01$) and was highest in the adolescent and adult phase 76.0 ± 7.4 and 71 ± 5.6 respectively (Fig. 3F). The elastic fibers not only grew in numbers, a significant amount of thinning was also appreciated in adulthood ($p < 0.0001$). .

Mucoid extracellular matrix accumulation was almost absent in the first three life phases, after which a significant amount was encountered in the adolescent and adult life phase ($p < 0.001$), Fig. 3G,H).

The pathological features smooth muscle cell nuclei loss, overall medial degeneration and elastic fiber degradation, fragmentation or loss were not appreciated in any age category of the bicuspid patients.

4. Discussion

Patients with a BAV have an increased risk to develop thoracic aortopathy. Currently, risk stratification and treatment strategies are based on the aortic dimensions, which necessitates lifelong follow up with imaging modalities. A personalized approach is crucial for the adequate timing of prophylactic aortic replacement in BAV patients with an increased susceptibility for thoracic aortopathy.

Several studies have focused on markers which could distinguish the group of vulnerable BAV patients, ranging from molecular biological to hemodynamic factors. The adult ascending aortic wall has thereby been extensive studied. The pediatric ascending aortic wall has garnered less attention so far. Bicuspidy is a congenital cardiac disorder, with an embryonic defect as the most likely cause of a combination of a defective aortic valve and inherent weakness of the ascending aortic wall [16]. It is therefore interesting to study which histopathological features characteristic for BAV aortopathy are already present in the pediatric aortic wall.

The BAV ascending aortic wall hardly shows any signs of pathology in the medial layer such as smooth muscle cell nuclei loss, overall medial degeneration, elastic medial degradation, and atherosclerosis in any age category. The described less pathological features in the pediatric aortic media are in line with previously reported ascending aortic wall features of adult bicuspid aortic patients, in which specimen was compared to the tricuspid population [14,19,26]. The intimal layer is significantly thicker in the premature phase as compared to any other age group, whereas the medial layer increases in thickness throughout life with an increase in number of lamellae and interlamellar mucoid extracellular matrix. The significantly thinner intimal layer has been noted as a risk factor for future aortic complications, and is common between genetic and congenital cardiac diseases with an inherent risk for thoracic aortopathy. In adult Marfan syndrome, type A aortic dissections and bicuspid aortic valve patients the intimal layer is only a few cell layers thick [11,18,21].

Our previous study revealed a difference in the timing of intimal development in BAV and TAV patients [17]. In TAV, the expansion of the intimal layer is apparent in the neonatal phase (4 months), the subendothelial layer thereafter increases in thickness till the age of six, after which the intimal layer stabilizes for a few years and again increases in thickness during adulthood [17]. In TAV the early increase in intimal thickness is regarded as a normal physiological process, important for strengthening of the vessel wall and can be seen as a remarkable ability to adapt to injury and/or regeneration and cope with high and pulsatile blood pressure [10].

In the BAV the intimal thickening started in early gestation demonstrating an intimal thickness comparable to the newborn intima in TAV. Strikingly, after birth the intima decreased in thickness to remain as a significantly thinner layer throughout life.

It is hypothesized that the lack of intimal layer development is caused by a combination of a differentiation defect of the vascular smooth muscle cells and endothelial dysfunction.

On the one hand, the early embryonic defects could protect the bicuspid population against the development of atherosclerosis as both vascular smooth muscle cells and endothelial cells play an integral role in the development of plaques. The lack of atherosclerosis has also been shown clinically in non-dilated and dilated BAV patients [1,3–5]. On the other hand, the significantly thinner intimal layer, which also lacks the potential of physiological adaptive intimal thickening, is mechanically weaker to withstand pathological environmental stimuli such as variations in flow and wall tension [29].

Transforming growth factor beta (TGF-β) is an important factor in vessel wall maintenance and pathology. Among the known BAV hereditary genes there are a number involved in TGF-β signaling (FBN1, TGFBR2, TGFB2, SMAD6) [25]. TGF-β plays a role in endothelial to mesenchymal transition, and is a potent activator of endothelial cells and enhances intimal hyperplasia through the production of extracellular matrix proteins while it suppresses smooth muscle cell activation [2]. Furthermore, TGF-β is critical for cardiac valve formation and smooth muscle differentiation. It thus seems obvious that TGF-β plays a central role in the pathology encountered in the adult bicuspid aortic valve being a lack of intimal thickening, thick medial layer with an abundance of mucoid extracellular matrix and the earlier described smooth muscle cell differentiation defects [20]. In this study the role of hemodynamics has not been considered. Earlier studies showed no relevant effect of increased stress on the histopathology of the aortic wall, but these studies have only been conducted in adult specimen so

far [12].

We conclude that the main characteristics of a bicuspid ascending aortic wall are already present before adulthood, albeit not before birth. Directly after birth the intimal thickness layer decreases significantly and an increase in the number of elastic lamellae and mucoid extracellular matrix is seen before adulthood. Considering the early manifestations of ascending aortic wall pathology in bicuspid aortic valve patients, the pediatric population should be taken into account while searching for markers predictive for future aortopathy.

CRediT authorship contribution statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Nimrat Grewal and Robert Poelmann. The first draft of the manuscript was written by Nimrat Grewal and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

None.

Acknowledgments

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References

[1] H. Achneck, B. Modi, C. Shaw, J. Rizzo, G. Albornoz, D. Fusco, J. Elefteriades, Ascending thoracic aneurysms are associated with decreased systemic atherosclerosis, Chest 128 (2005) 1580–1586.
[2] Z. Alvandi, J. Bischoff, Endothelial-mesenchymal transition in cardiovascular disease, Arterioscler. Thromb. Vasc. Biol. 41 (2021) 2357–2369.
[3] O.B. Dolmaci, T. Ayyildiz, R.E. Poelmann, A.H.G. Driessen, D.R. Koolbergen, R.J. M. Klautz, J.H.N. Lindeman, N. Grewal, Risk for acquired coronary artery disease in genetic vs. congenital thoracic aortopathy, Front. Cardiovasc. Med. 9 (2022), 1036522.
[4] O.B. Dolmaci, A.H.G. Driessen, R.J.M. Klautz, R. Poelmann, J.H.N. Lindeman, N. Grewal, Comparative evaluation of coronary disease burden: bicuspid valve disease is not atheroprotective, Open Heart 8 (2021), e001772.
[5] O.B. Dolmaci, J. Legué, J.H.N. Lindeman, A.H.G. Driessen, R.J.M. Klautz, T.J. Van Brakel, H.J. Siebelink, B.J.A. Mertens, R.E. Poelmann, A.C. Gittenberger-de Groot, N. Grewal, Extent of coronary artery disease in patients with stenotic bicuspid versus tricuspid aortic valves, J. Am. Heart Assoc. 10 (2021), e020080.
[6] R. Erbel, V. Aboyans, C. Boileau, E. Bossone, R.D. Bartolomeo, H. Eggebrecht, A. Evangelista, V. Falk, H. Frank, O. Gaemperli, M. Grabenwoger, A. Haverich, B. Iung, A.J. Manolis, F. Meijboom, C.A. Nienaber, M. Roffi, H. Rousseau, U. Sechtem, P.A. Sirnes, R.S. Allmen, C.J. Vrints, ESC Guidelines on the diagnosis and treatment of aortic diseases: document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. the task force for the diagnosis and treatment of aortic diseases of the European Society of Cardiology (ESC), Eur. Heart J. 35 (2014) (2014) 2873–2926.
[7] L. European Medicines Agency, Reflection paper: formulations of choice for the paediatric, Population (2006).
[8] B. Fernandez, A.C. Duran, T. Fernandez-Gallego, M.C. Fernandez, M. Such, J. M. Arque, V. Sans-Coma, Bicuspid aortic valves with different spatial orientations of the leaflets are distinct etiological entities, J. Am. Coll. Cardiol. 54 (2009) 2312–2318.
[9] A. Forte, C. Bancone, G. Cobellis, M. Buonocore, G. Santarpino, T.J.M. Fischlein, M. Cipollaro, M. De Feo, A. Della Corte, A. Possible, Early biomarker for bicuspid aortopathy: circulating transforming growth factor β-1 to soluble endoglin ratio, Circ. Res. 120 (2017) 1800–1811.
[10] S. Glagov, C.K. Zarins, Is intimal hyperplasia an adaptive response or a pathologic process?—observations on the nature of nonatherosclerotic intimal thickening: research initiatives in vascular surgery *, J. Vasc. Surg. 10 (1989) 571–573.
[11] N. Grewal, R. Franken, B.J. Mulder, M.J. Goumans, J.H. Lindeman, M. R. Jongbloed, M.C. DeRuiter, R.J. Klautz, A.J. Bogers, R.E. Poelmann, A.C. Groot, Histopathology of aortic complications in bicuspid aortic valve versus Marfan syndrome: relevance for therapy, Heart Vessels 31 (2016) 795–806.
[12] N. Grewal, E. Girdauskas, M. deRuiter, M.J. Goumans, J.H. Lindeman, K. Disha, R. Wolterbeek, D. Schoor, R.J. Klautz, R. Poelmann, A. Gittenberger de Groot, The effects of hemodynamics on the inner layers of the aortic wall in patients with a bicuspid aortic valve, Integr. Mol. Med. 4 (5) (2017) 1–7.
[13] N. Grewal E. Girdauskas M. Idhrees B. Velayudhan R. Klautz A. Driessen R.E. Poelmann Structural abnormalities in the non-dilated ascending aortic wall of bicuspid aortic valve patients. cardiovascular pathology: the official journal of the Society for Cardiovasc. Pathol. 2022 107478.
[14] N. Grewal E. Girdauskas M. Idhrees B. Velayudhan R. Klautz A. Driessen R.E. Poelmann Structural abnormalities in the non-dilated ascending aortic wall of bicuspid aortic valve patients. Cardiovascular pathology: the official journal of the Society for Cardiovasc. Pathol. 62 2023 107478.
[15] N. Grewal, A.C. Gittenberger-de Groot, M.C. DeRuiter, R.J. Klautz, R.E. Poelmann, S. Duim, J.H. Lindeman, W.M. Koenraadt, M.R. Jongbloed, S.A. Mohamed, H. H. Sievers, A.J. Bogers, M.J. Goumans, Bicuspid aortic valve: phosphorylation of c-Kit and downstream targets are prognostic for future aortopathy, Eur. J. Cardio-Thorac. Surg. Off. J. Eur. Assoc. Cardio-Thorac. Surg. 46 (2014) 831–839.
[16] N. Grewal, A.C. Gittenberger-de Groot, J.H. Lindeman, A. Klautz, A. Driessen, R.J. M. Klautz, R.E. Poelmann, Normal and abnormal development of the aortic valve and ascending aortic wall: a comprehensive overview of the embryology and pathology of the bicuspid aortic valve, Ann. Cardiothorac. Surg. 11 (2022) 380–388.
[17] N. Grewal, A.C. Gittenberger-de Groot, J.V. Thusen, L.J. Wisse, M.M. Bartelings, M. C. DeRuiter, R.J.M. Klautz, R.E. Poelmann, The development of the ascending aortic wall in tricuspid and bicuspid aortic valve: a process from maturation to degeneration, J. Clin. Med. 9 (2020) 908.
[18] N. Grewal, M.J. Goumans, M. deRuiter, R.J. Klautz, R. Poelmann, B. Mulder, A. J. Bogers, A. Gittenberger de Groot, Aortopathy in bicuspid aortic valve and marfan syndrome is characterized by a lack of activation potential of the epicardium in the ascending aorta, Int. J. Pathol. Clin. Res. 3 (2017) 051.
[19] N. Grewal, A.C.G.-d Groot, J.H. Lindeman, A. Klautz, A. Driessen, R.J.M. Klautz, R. E. Poelmann, Normal and abnormal development of the aortic valve and ascending aortic wall: a comprehensive overview of the embryology and pathology of the bicuspid aortic valve, Ann. Cardiothorac. Surg. (2022) 380–388.
[20] N. Grewal, R. Klautz, R.E. Poelmann, Can transforming growth factor beta and downstream signalers distinguish bicuspid aortic valve patients susceptible for future aortic complications? Cardiovascular pathology: the official journal of the Society for, Cardiovasc. Pathol. (2022), 107498.
[21] N. Grewal, B.J.J. Velders, A.C. Gittenberger-de Groot, R. Poelmann, R.J.M. Klautz, T.J. Van Brakel, J.H.N. Lindeman, A systematic histopathologic evaluation of type-a aortic dissections implies a uniform multiple-hit causation, J. Cardiovasc. Dev. Dis. 8 (2021) 12.
[22] M.K. Halushka, A. Angelini, G. Bartoloni, C. Basso, L. Batoroeva, P. Bruneval, L. M. Buja, J. Butany, G. d'Amati, J.T. Fallon, P.J. Gallagher, A.C. Gittenberger-de Groot, R.H. Gouveia, I. Kholova, K.L. Kelly, O. Leone, S.H. Litovsky, J. J. Maleszewski, D.V. Miller, R.N. Mitchell, S.D. Preston, A. Pucci, S.J. Radio, E. R. Rodriguez, M.N. Sheppard, J.R. Stone, S.K. Suvarna, C.D. Tan, G. Thiene, J. P. Veinot, A.C. van der Wal, Consensus statement on surgical pathology of the aorta from the Society for Cardiovascular Pathology and the Association For European Cardiovascular Pathology: II. Noninflammatory degenerative diseases - nomenclature and diagnostic criteria, Cardiovasc. Pathol. Off. J. Soc. Cardiovasc. Pathol. 25 (2016) 247–257.
[23] J.S. Ikonomidis, C.R. Ivey, J.B. Wheeler, A.W. Akerman, A. Rice, R.K. Patel, R. E. Stroud, A.A. Shah, C.G. Hughes, G. Ferrari, R. Mukherjee, J.A. Jones, Plasma biomarkers for distinguishing etiologic subtypes of thoracic aortic aneurysm disease, J. Thorac. Cardiovasc. Surg. 145 (2013) 1326–1333.
[24] E.M. Isselbacher, O. Preventza, J.H. Black, J.G. Augoustides, A.W. Beck, M. A. Bolen, A.C. Braverman, B.E. Bray, M.M. Brown-Zimmerman, E.P. Chen, T. J. Collins, A. DeAnda, C.L. Fanola, L.N. Girardi, C.W. Hicks, D.S. Hui, W.S. Jones, V. Kalahasti, K.M. Kim, D.M. Milewicz, G.S. Oderich, L. Ogbechie, S.B. Promes, E. G. Ross, M.L. Schermerhorn, S.S. Times, E.E. Tseng, G.J. Wang, Y.J. Woo, ACC/AHA guideline for the diagnosis and management of aortic disease, J. Am. Coll. Cardiol. (2022) 0.
[25] A. Junco-Vicente, Á. Del Río-García, M. Martín, I. Rodríguez, Update in biomolecular and genetic bases of bicuspid aortopathy, Int. J. Mol. Sci. 22 (2021) 5694.
[26] J.F. Matthias Bechtel, F. Noack, F. Sayk, A.W. Erasmi, C. Bartels, H.H. Sievers, Histopathological grading of ascending aortic aneurysm: comparison of patients with bicuspid versus tricuspid aortic valve, J. Heart valve Dis. 12 (2003) 54–59, discussion 59-61.
[27] J.C. Peterson, L.J. Wisse, V. Wirokromo, T. van Herwaarden, A.M. Smits, A. C. Gittenberger-de Groot, M.T.H. Goumans, J.C. VanMunsteren, M.R.M. Jongbloed, M.C. DeRuiter, Disturbed nitric oxide signalling gives rise to congenital bicuspid aortic valve and aortopathy, Dis. Models Mech. (13) (2020), dmm044990.
[28] M.T. Soto-Navarrete, M. López-Unzu, A.C. Durán, B. Fernández, Embryonic development of bicuspid aortic valves, Prog. Cardiovasc. Dis. 63 (2020) 407–418.
[29] H.C. Stary, D.H. Blankenhorn, A.B. Chandler, S. Glagov, W. Insull Jr., M. Richardson, M.E. Rosenfeld, S.A. Schaffer, C.J. Schwartz, W.D. Wagner, et al., A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, in: Circulation, 85, American Heart Association,, 1992, pp. 391–405.
[30] N. Tzemos, J. Therrien, J. Yip, G. Thanassoulis, S. Tremblay, M.T. Jamorski, G. D. Webb, S.C. Siu, Outcomes in adults with bicuspid aortic valves, Jama 300 (2008) 1317–1325.
[31] R. Virmani, F.D. Kolodgie, A.P. Burke, A. Farb, S.M. Schwartz, Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1262–1275.