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Bicuspid aortic valve disease. Morphology matters

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Bicuspid aortic valve disease

Morphology matters

Wilke MC Koenraadt

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Bicuspid aortic valve disease

Morphology matters

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
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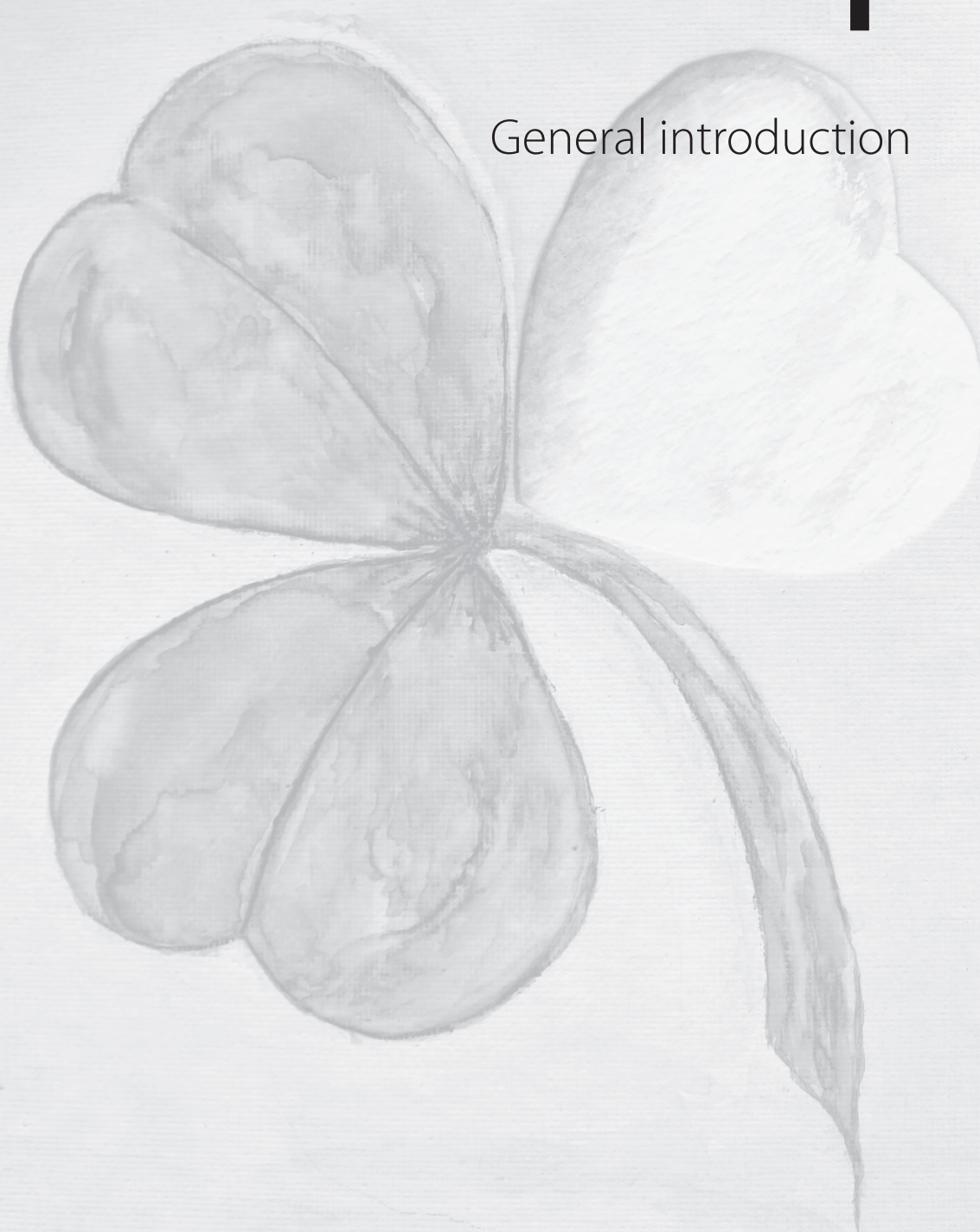
Voor mijn ouders en Marijn, Lara & Ise

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General introduction



GENERAL INTRODUCTION

Bicuspid aortic valve disease (BAV) is the most common congenital anomaly, with an estimated prevalence of 0.5-2% in the general population. In some forms of congenital heart disease, such as aortic coarctation, a prevalence of up to 60% has been described^{1,2}. BAV is also associated with chromosomal abnormalities, such as Turner syndrome (Monosomy X or XO mosaic), then it is related to a female genotype, the occurrence of BAV, however, generally has a male preponderance.

In BAV, the aortic valve consists of 2 functional leaflets, instead of the normal tricuspid morphology. Within the subgroup of BAV patients, several morphological subtypes can be recognised, with regard to the presence or absence and location of the supposed site of “fusion” of the valvular leaflets, the so-called raphe. The developmental mechanism of a bicuspid aortic valve is not yet fully known.

The early embryonic heart is the first functional organ that forms. The heart starts out as a linear tube that will undergo a process of looping and remodelling during development. In order to achieve a functional 4 chambered heart, the basis for the mature circulation, several septation processes need to occur. A complex series of morphogenetic interactions, involving cells from several embryonic origins, will contribute to all cardiac structures, such as cardiac chambers, septa, valves, coronary arteries and the cardiac conduction system³.

The cardiac valves develop from the so-called endocardial cushions, that are present at the atrioventricular junction and in the outflow tract of the heart. The cardiac outflow tract is initially connected to a common arterial vessel. The unseptated cardiac outflow tract is lined by a septal and parietal endocardial cushion. Migration of cardiac neural crest cells (CNCs) from the neural tube to the heart induces the formation of the aorto-pulmonary septum, which divides the common cardiovascular outflow tract into an aortic and pulmonary part. This separation will occur at the level of the cardiac outflow tract, valves and great arteries, after which a separate aorta and pulmonary trunk, connected to a left and right ventricle, can be recognised. The parietal cushion eventually gives rise to the right facing leaflets of the aortic and pulmonary valve, while the septal cushion will develop into the left facing aortic and pulmonary valve leaflets. The origin of the non-facing leaflets (non-coronary leaflet of the aortic valve and anterior leaflet of the pulmonary valve) is as yet unresolved. They may either develop as separate structures or may originate as part of the septal or parietal cushions. Recognition of the developmental origin of the non-facing/non-coronary aortic valve leaflet would provide insight in the developmental mechanism of BAV, being either a pathological fusion or a lack of proper separation of valvular leaflets.

Formation of a coronary arterial circulation originating from the aortic root is a relatively late event during embryonic development⁴. By the beginning of the third week, a vascular system begins to form over the epicardial surface of the myocardium⁵. Bogers et al. showed that the major coronary arteries could be identified in the walls of the aortic sinuses before the emergence of coronary arterial orifices, suggesting ingrowth rather than outgrowth of the aorta⁶. In the subepicardial space, an endothelial network is formed that grows into the myocardium, connects to the aortic root and finally enters the anterior part of the right atrium. After these connections have been established, arteriogenesis follows with formation of a media and an adventitia⁷, that receive contributions from epicardium derived cells (reviewed in ⁸).

In BAV, apart from the aortic valve itself, variations in coronary anatomy can occur, as well as abnormalities in other valves.

These morphological variations, and the potential implications to outcome, are the subject of this thesis.

Complications of BAV

Although some patients with isolated BAV remain asymptomatic throughout their lifetime, others develop severe cardiac complications from an early age onwards, such as aortic valve stenosis, aortic insufficiency and/or endocarditis. However, the first presentation can also be a clinically relevant aortic wall abnormality, including ascending aortic dilation (reported to occur in 45% of patients⁹), aneurysm formation or rupture of the ascending aorta.

Identifying patients who are prone to develop complications is a major challenge. Several studies have shown that different BAV phenotypes have a different clinical outcome, regarding valvular dysfunction and aortopathy^{10, 11}.

BAV morphology

In BAV, several morphological phenotypes are distinguishable based on the presence and number of raphe, as classified by Sievers et al¹². Most BAVs consist of one free leaflet and two leaflets that are conjoined (or have failed to separate during embryonic development). The term 'raphe' defines the conjoined area of the two underdeveloped leaflets turning into a malformed commissure between both leaflets¹². When no raphe is present, the valve is called strictly bicuspid.

As from a developmental point of view a morphological spectrum may exist in which the extent of the raphe can be regarded as a continuum, in the current thesis the major valve morphologies observed in BAV patients are described as 3 different types (Type 1A, 2A and 3A, based on valve leaflet orientation), in which the extent of the raphe can

vary (Figure 1, modified after Schaeffer et al.¹¹). "Fusion" of the right and left coronary leaflet is defined as type 1A BAV, fusion of the right and non-coronary leaflet as type 2A BAV and fusion of the left and non-coronary leaflet as type 3A BAV. Strictly bicuspid valves (i.e. a valves without a raphe) are defined as being either type 1, 2 or 3, based on the orientation of their leaflets, and referred as a subgroup B.

Although several studies have focused on the correlation of BAV morphology with regard to which valvular leaflets are fused (i.e. type 1, 2 or 3), less attention has been paid to the relevance of the presence and absence of a raphe (i.e. type A or B). This issue is addressed in Chapter 2 of this thesis.

Coronary anatomy associated with BAV and other congenital heart disease

In case of the normal, tricuspid, aortic valve, the aortic leaflet facing the pulmonary valve (i.e the left and right aortic leaflet) carry the coronary arteries, although many variations exist¹³.

Variations in coronary anatomy have also been described in BAV patients, including increased incidence of separate ostia of the left anterior descending coronary artery (LAD) and left circumflex coronary artery (LCX), and of a left dominant coronary system¹⁴⁻¹⁶. The incidence of left coronary dominance in patients with BAV was reported to be 25-29%¹⁵⁻¹⁸, as compared to 10-12% in the general population with normal aortic valves^{15, 16}. Also differences in coronary ostial positions as related to the aortic sinus have been noted¹⁶⁻²⁰. Coronary ostia can be located a few millimetres above the sinotubular (ST) junction in 5-22% percent of normal hearts, although literature is scarce^{21, 22}. Coronary ostial take-off well above the ST junction, referred to as high coronary take-off, is rare, occurring in less than 1% of the normal population^{23, 24}. Data concerning coronary height in BAV patients are scarce and not consistent, mostly confined to case reports^{19, 20}. Lerer et al. reported the left coronary artery (LCA) in patients with isolated BAV above the aortic ST junction in 29% of cases, whereas the right coronary artery (RCA) is located too high in 11%¹⁷. The reason why these coronary patterns occur more often in BAV patients, is to date unclear. In addition, variations in coronary anatomy may correlate to BAV morphology, which had not been studied to date. This is addressed in Chapter 4 and 5 of this thesis.

Knowledge of coronary anatomy is relevant, particularly when operation necessitating perfusion cardioplegia is required. In the eighties, a coronary classification system was introduced, providing a means to describe coronary anatomy regardless of position of the great arteries²⁵. This system, named the Leiden Convention, was, however, not suitable for application in BAV anatomy. The introduction of a modification of the Leiden Convention is provided in Chapter 3.

BAV and Turner syndrome

BAV is common in Turner syndrome (TS), described in 12-30% of TS patients²⁶⁻²⁸, versus 1-2% in the general population^{9,29}. Variations in coronary anatomy have been described in patients with BAV, but also in Turner syndrome coronary anomalies are also more frequent, 20% versus 5% in the general population³⁰. Absence of the left main coronary artery seems to be the most common anomaly with a reported incidence of 14%. A higher prevalence of left coronary dominance is also noted in patients with Turner syndrome³⁰. The etiology of coronary arterial anomalies in TS, and whether this is related to the frequent occurrence of BAV, remains unclear. In addition, coronary anatomy has never been related to aortic valve morphology (BAV versus TAV) in TS patients. Therefore, it is unknown whether the coronary anomalies observed in Turner syndrome are related to BAV, and if so, whether this can be attributed to a specific BAV subtype. This is the subject of Chapter 6 of this thesis.

BAV and pulmonary valves

Given the common developmental origin of aorta and pulmonary valves^{31,32} as described above, the question arises whether pulmonary valve morphology is affected in specific subgroups of BAV patients. Bicuspid pulmonary valves are rare with an incidence of 0.1%³². They are usually associated with other congenital heart disease such as tetralogy of Fallot or transposition of the great arteries^{33, 34}. Bilateral bicuspid semilunar valves seem to be rare and are diagnosed mainly during surgery or post-mortem^{32, 35}. The morphology of the pulmonary valve in BAV disease is described in Chapter 7.

In summary, this thesis addresses the morphology of the bicuspid aortic valve in relation to coronary anatomy, pulmonary valve and outcome.

OUTLINE THESIS

1

In **Chapter 2**, the presence and extent of a raphe and its association with the degree of aortopathy and valvular dysfunction in BAV patients, was studied.

In **Chapter 3** coronary artery anatomy as related to BAV morphology was evaluated and related to outcome in BAV patients.

Chapter 4 describes coronary anatomy in patients with BAV and associated complex congenital heart diseases.

In **Chapter 5** coronary anatomy in patients with Turner Syndrome with and without BAV was compared with coronary anatomy in patients with isolated BAV. These results are correlated to BAV morphology.

Chapter 6 reports on pulmonary valve morphology in post-mortem hearts with BAV associated with complex congenital heart disease.

In **Chapter 7** a classification system for coronary anatomy is described, the modified Leiden Convention, which is applicable in hearts with normally related great arteries, as well as in hearts with an altered great artery relationship. This modified classification system is also extended to allow its use in BAV patients.

Finally, the last chapters provide a general summary, conclusions and future perspectives in English and Dutch.

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2

The extent of the raphe in bicuspid aortic valves is associated with aortic regurgitation and aortic root dilatation

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Neth Heart J. 2016 Feb;24(2):127-33

ABSTRACT

Background

The clinical course of bicuspid aortic valves (BAVs) is variable. Data on predictors of aortopathy and valvular dysfunction mainly focuses on valve morphology.

Aim

To Determine whether presence and extent of the raphe (fusion site of valve leaflets) is associated with degree of aortopathy and valvular dysfunction in patients with isolated BAV and associated aortic coarctation (CoA).

Methods

Valve morphology and aortic dimensions of 255 BAV patients were evaluated retrospectively by echocardiography.

Results

BAVs with complete raphe had significantly higher prevalence of valve dysfunction (especially aortic regurgitation) than BAVs with incomplete raphe (82.9% vs. 66.7%, $p=0.01$). Type 1A BAVs (fusion of right and left coronary leaflets) and complete raphe had larger aortic sinus diameters compared to the rest of the population (37.74 vs. 36.01, $p=0.031$). Patients with CoA and type 1A BAV had significantly less valve regurgitation (13.6% vs. 55.8%, $p<0.001$) and smaller diameters of the ascending aorta (33.7mm vs. 37.8mm, $p<0.001$) and aortic arch (25.8mm vs. 30.2mm, $p<0.001$) than patients with isolated BAV.

Conclusion

Type 1A BAV with complete raphe is associated with more aortic regurgitation and root dilatation. The majority of CoA patients has an incomplete raphe, associated with smaller aortic root diameters and less valve regurgitation.

INTRODUCTION

Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation, with a clear male predominance and an estimated prevalence of 0.5-2%¹⁻³.

BAV can occur in an isolated form or in association with other congenital malformations, such as coarctation of the aorta (CoA). The prevalence of BAV in CoA patients is reported to be as high as 60%^{4,5}.

Although some patients with isolated BAV remain asymptomatic throughout their lifetime, others develop severe cardiac complications from an early age onwards, such as aortic valve stenosis, aortic insufficiency and/or endocarditis. However, the first presentation can also be a clinically relevant aortic wall abnormality, including ascending aortic dilation (reported to occur in 45% of patients⁶), aneurysm formation or rupture of the ascending aorta. Identifying patients who are prone to develop complications is a major challenge⁷.

It is now recognized that BAV should not be considered as one single entity, but that distinct morphological phenotypes are distinguishable based on the presence and number of raphe, as classified by Sievers et al.⁸. Most BAVs consist of one free leaflet and two leaflets that are conjoined (or have failed to separate during embryonic development). The term 'raphe' defines the conjoined area of the two underdeveloped leaflets turning into a malformed commissure between both leaflets⁸. When no raphe is present, the valve is called strictly bicuspid. Schaefer et al. identified three BAV morphologies: type 1 with fusion of right and left coronary cusp; type 2 with right and non-coronary fusion and the rare type 3 with left and non-coronary fusion⁹. In this study, this classification was followed, with addition of A (for valves with a raphe) or B (for strictly bicuspid valves, figure 1).

Several studies have shown before that different BAV phenotypes have a different clinical outcome, regarding valvular dysfunction and aortopathy⁹⁻¹⁴.

To our knowledge however, the extent of the raphe (i.e. the extent to which the leaflets are conjoined) has never been taken into account.

Therefore, the purpose of this study was to determine whether the extent of the raphe is associated with the degree of aortopathy and valvular dysfunction.

As stated before, the most common associated congenital malformation of BAV is coarctation of the aorta (CoA). Whereas in isolated BAV the most common morphology is a fusion of right and left coronary cusp (Type 1A in 80% of patients⁹), data on the distribution of BAV morphology in CoA patients is limited¹⁵. Therefore, a sub analysis was performed in patients with BAV and CoA to see if there was a difference in BAV morphology and extent of the raphe.

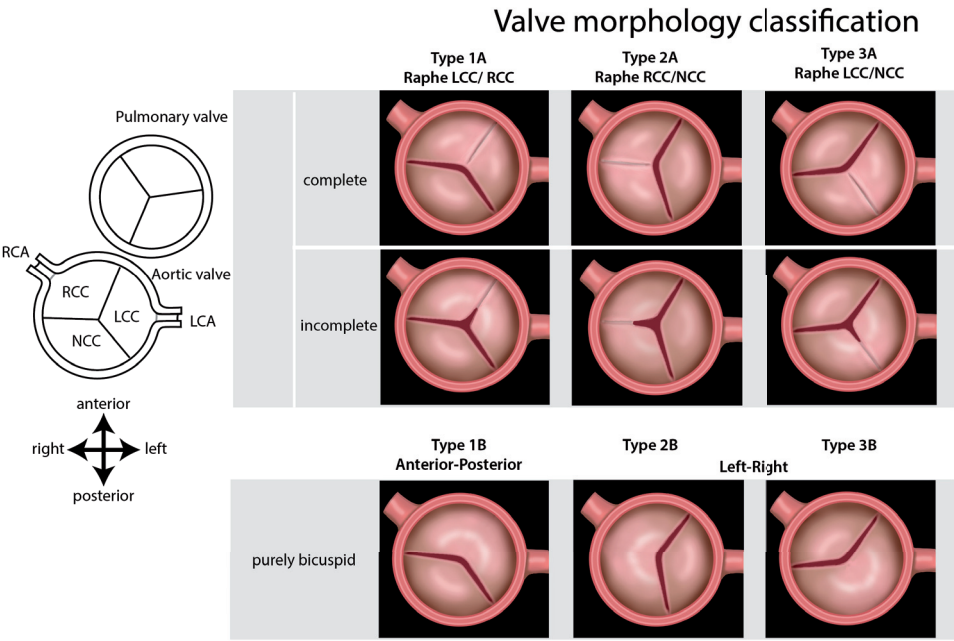


Figure 1. Schematic overview of bicuspid aortic valve (BAV) morphologies. The drawings are oriented in an echocardiographic view of the aortic valve. Upper panel: The three major valve morphologies observed in BAV patients are described as three different types based on the valve leaflet orientation: type 1,2 and 3. The extent of the raphe is indicated as analyzed in this study (incomplete or complete). Lower panel: Strictly bicuspid valves (without a raphe) are defined as a subgroup B. Modified after Schaefer et al.⁹

METHODS

Study population

All patients who had undergone transthoracic echocardiography (TTE) between 2005 and 2010 and had been diagnosed with BAV disease were identified from the echocardiography database. A total of 263 BAV patients were selected, in 8 patients the diagnosis of BAV was not correct¹⁶, thus 255 patients remained. Clinical and echocardiographic data were collected and analyzed from the departmental Cardiology Information System (EPD-Vision®, LUMC) and the echocardiography database respectively. For this analysis of clinically acquired data, the Institutional Review Board waived the need of patient written informed consent.

Classification of valve morphology

As from a developmental point of view a morphological spectrum may exist in which the extent of the raphe can be regarded as a continuum, in the current study we chose to describe the major valve morphologies observed in BAV patients as 3 different types (Type 1A, 2A and 3A, based on valve leaflet orientation, modified after Schaefer et al.⁹), in which the extent of the raphe can vary (Figure 1). In this study, fusion of the right and left coronary cusp is defined as type 1A BAV, fusion of the right and non-coronary cusp as type 2A BAV and fusion of the left and non-coronary cusp as type 3A BAV. Strictly bicuspid valves (i.e. a valves without a raphe) were defined as being either type 1, 2 or 3, based on the orientation of their leaflets, and referred as a subgroup B (Figure 1).

Echocardiography

Transthoracic echocardiography was performed using a GE Vivid7 or E9 (GE-Vingmed, Horten, Norway) ultrasound machine with standard views from the parasternal, subcostal, suprasternal and apical windows. The aortic valve was examined in the two dimensional parasternal short-axis view and classified as bicuspid when two cusps could be clearly identified and the typical 'fish-mouth opening' of the valve was observed (Figure 2). Diameters of the aortic root and ascending aorta were determined in the parasternal long axis view.

Echocardiographic analysis

All echocardiograms were evaluated by an experienced observer, using GE Medical Systems's EchoPac, Version 7 (110.0.0, GE-Vingmed, Horten, Norway). The aortic valve was evaluated in a cross-sectional view for the presence and extent of a raphe. For valves where a raphe could be distinguished (subgroup A), distinction was made between a complete raphe and an incomplete raphe. Cases where no raphe was detected (subgroup B) were defined as strictly bicuspid valves. Diameters of aortic sinus, ascending aorta and aortic arch were measured from leading edge to leading edge in end-diastole according to the European Association of Echocardiography recommendations¹⁷. Aortic annular diameter was measured from inner edge to inner edge during systole. All measurements were made in mm, rounded to 2 significant figures. The ascending aorta was considered dilated at a diameter of >38mm.

Valvulardysfunction was defined as aortic stenosis or regurgitation. EA recommendations were used for determining severity of aortic stenosis and regurgitation, grading from mild to severe^{18,19}.

Subgroup analysis was performed in patients with a history of CoA, the same protocol was followed in this group.

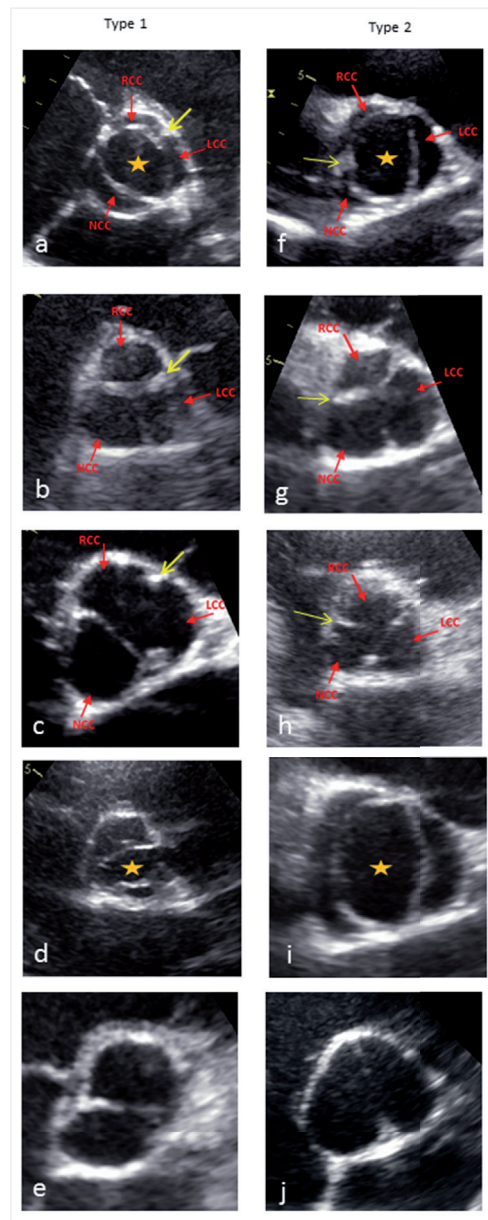


Figure 2. Representative examples of different bicuspid aortic valve (BAV) types as seen from the short-axis parasternal view on echocardiography. The *yellow arrow* indicates the raphe. The *orange star* indicates the typical fish mouth opening of the valve. a Type 1A in open position. b Type 1A with complete raphe in closed position. c Type 1A with incomplete raphe in closed position. d Type 1B in open position. e Type 1B in closed position. f Type 2A in open position. g Type 2A with complete raphe in closed position. h Type 2A with incomplete raphe in closed position. i Type 2B in open position. j Type 2B in closed position

STATISTICAL ANALYSIS

All collected data were registered in a Microsoft Office Access 2003 database. The database was exported into IBM SPSS Statistics Version 20 for computing variables and statistical analysis. Independent samples T-tests were used to compare means of numerical data in 2 categories. One-way ANOVA tests were used for comparing numerical data in more than 2 categories. Cross-tabulations were made for binary categorical data, on which chi-square goodness-of-fit-tests were performed to test for independence. For sets of independent numerical data linear regression analysis was used to evaluate trends. Similarly, trends for binary categories were evaluated with binary logistic regression to correct for possible confounding factors such as age and gender. All statistical analyses were two-tailed and considered significant if $p < 0.05$.

RESULTS

Patient characteristics

A total of 255 patients with BAV (age 18-85 years, mean 48 ± 15 years) were identified and analyzed. Of these, 179 were male (70.2%) and 76 female (29.8%). Patient characteristics and echocardiographic data are summarized in Table 1. Baseline characteristics were not significantly different between the BAV subtypes (Table 2). The distribution of valve morphology is shown in figure 3.

More valvular dysfunction in BAVs with complete raphe

A total of 120 patients were diagnosed with aortic stenosis, the same number of patients showed aortic regurgitation (Table 1). Mean age was not significantly different in the different

BAV subgroups ($p = 0.515$, Table 2) or in patients with incomplete and complete raphes ($p = 0.357$). There were no significant differences in valvular function between the different morphological subtypes. However, BAVs with a complete raphe had a significantly higher prevalence of valvular dysfunction than BAVs with an incomplete raphe (82.9% vs. 66.7%, $p = 0.01$, table 3). When specifying this for type of valvular dysfunction, this difference remained significant in patients with aortic regurgitation (56.8% vs. 42.3%, $p = 0.05$), whereas in patients with aortic stenosis there was no difference between patients with a complete or incomplete raphe (49.5% vs 43.6%, $p = 0.419$, table 3). In type 1A BAVs with a complete raphe the difference in prevalence of aortic regurgitation was even more outspoken compared to the rest of the study population (57.6% vs. 41.8% $p = 0.017$).

Table 1. Patient Characteristics

Variable	n (%)
Gender	
Male	179 (70.2)
Female	76 (29.8)
Bicuspid aortic valve morphology	
Type 1A	151 (59.2)
Type 2A	37 (14.5)
Type 3A	1 (0.4)
Strictly bicuspid (type B)	66 (25.9)
Type 1B	42 (63.6)
Type 2B	24 (36.4)
Extent of the raphe	
Complete	111 (43.5)
Incomplete	78 (30.6)
Valve Surgery	66 (25.9)
Mitral valve replacement	1 (0.39)
Mitral valve repair	2 (0.78)
Aortic valve replacement	50 (19.6)
Aortic valve repair	10 (3.9)
Bentall	22 (8.6)
Tricuspid valve repair	1 (0.39)
Pulmonary valve replacement	1 (0.39)
Congenital Defects	51 (20.0)
Aortic coarctation	39 (15.3)
Atrial septal defect	1 (0.39)
Ventricular septal defect	11 (4.3)
Marfan	2 (0.78)
Common truncus	1 (0.39)
Patent ductus arteriosus	5 (2.0)
Valvular Dysfunction	189 (74.1)
Aortic valve stenosis	120 (47.1)
Aortic valve regurgitation	120 (47.1)
Mitral valve regurgitation	16 (6.3)
Tricuspid valve regurgitation	8 (3.1)
Pulmonary valve stenosis	1 (0.39)
Pulmonary valve regurgitation	1 (0.39)

Table 1. Continued

Variable	<i>n</i> (%)
Arrhythmias	64 (25.1)
Atrial fibrillation	39 (15.3)
Supraventricular tachycardia	19 (7.5)
Ventricular tachycardia	14 (5.5)
Pacemaker	6 (2.4)
Implantable Cardioverter Defibrillator	15 (5.9)
Secondary prevention	10 (66.7)
Primary prevention	5 (33.3)
Medication	140 (54.9)
Angiotensin converting enzyme inhibitors	91 (35.7)
Statins	61 (23.9)
Carbasalate calcium	45 (17.6)
Oral anticoagulants	43 (16.9)
Antiarrhythmic drugs	33 (12.9)
Risk Factors	117 (45.9)
Hypertension	65 (25.5)
Smoking	54 (21.2)
Diabetes	11 (4.3)
Cerebrovascular accident	14 (5.5)
Hypercholesterolemia	33 (12.9)
Peripheral arterial disease	7 (2.7)

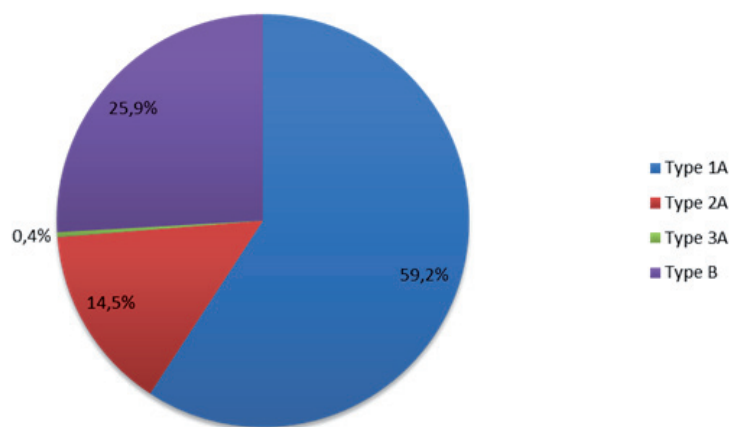


Figure 3. Schematic overview of the distribution of valve morphologies in the study population

Table 2. Patient characteristics

	Total, n=255	Type 1A, n=151	Type 2A, n=37	Type 3A, n=1	Type B, n=66	P-value
Gender (male)	179 (70%)	110 (73%)	23 (62%)	0 (0%)	46 (70%)	0.260
Age (years)	48 ± 15	49 ± 14	46 ± 17	42 ± 25	47 ± 15	0.515
Risk factors						
Diabetes	11 (4%)	8 (5%)	2 (5%)	0 (0%)	1 (2%)	0.609
Hypertension	65 (26%)	44 (29%)	8 (22%)	0 (0%)	13 (20%)	0.414
Hypercholesterolemia	33 (13%)	23 (15%)	3 (8%)	0 (0%)	7 (11%)	0.585
Smoking	51 (20%)	31 (21%)	8 (22%)	0 (0%)	12 (18%)	0.925
Medication						
Carbasalate calcium	45 (18%)	19 (13%)	8 (22%)	1 (100%)	17 (26%)	0.013
Statins	59 (23%)	31 (21%)	9 (24%)	1 (100%)	18 (27%)	0.207
Angiotensin converting enzyme inhibitor	89 (35%)	59 (39%)	12 (32%)	0 (0%)	18 (28%)	0.352
Oral anticoagulants	42 (17%)	28 (19%)	6 (16%)	0 (0%)	0 (0%)	0.068

Table 3. Valve morphology as related to dysfunction

Valve dysfunction	Complete raphe	Incomplete raphe	P value
Valve dysfunction (n = 144)	92 (82.9%)	52 (66.7%)	0.01
Aortic regurgitation (n = 96)	63 (56.8%)	33 (42.3%)	0.05
Aortic stenosis (n = 89)	55 (49.5%)	34 (43.6%)	NS

Larger aortic diameter in type 1A BAVs and complete raphe

All type 1A BAVs, compared to type 2A BAVs, showed a significantly larger sinus (37.29 vs. 33.89mm, $p=0.001$, table 4). Valves with a complete raphe had significantly larger aortic diameters at the level of the ascending aorta, as compared to valves with an incomplete raphe (38.02 vs. 35.91, $p = 0.041$). At the level of the aortic sinus and aortic arch this difference was not significant (Table 5). Type 1A BAVs and a complete raphe also showed a significant difference in sinus diameter (37.74 vs. 36.01 $p=0.031$) compared to the rest of the study population.

Table 4. Valve morphology as related to aortic diameter; type 1A versus type 2A

Aortic diameter	Type 1A	Type 2A	P value
Sinus (mm)	37.29 ± 5.88	33.89 ± 4.84	0.001
Ascendens (mm)	37.34 ± 6.75	36.33 ± 7.03	NS
Arch (mm)	29.30 ± 5.45	30.94 ± 4.70	NS

Table 5. Valve morphology as related to aortic diameter in all types BAV with complete raphe versus all types BAV with incomplete raphe

Aortic diameter	Complete raphe	Incomplete raphe	P value
Ascendens (mm)	38.02 ± 6.53	35.91 ± 7.00	0.041
Sinus (mm)	36.91 ± 5.82	36.13 ± 5.81	NS
Arch (mm)	30.07 ± 5.16	29.21 ± 5.66	NS

More incomplete raphes in CoA patients

Patients with CoA constituted the largest group of patients with associated congenital cardiac malformations (n=39). Patients with a history of CoA were 9 years younger than those without (40.7 vs. 49.3 years old, $p<0.001$) and there was no statistical difference in gender ($p=0.47$).

Distribution of BAV morphology in patients with CoA was identical to the rest of the study population, the majority having a type 1A BAV (56.4% in patients with versus 59.7% in patients without CoA, $p=0.321$). However, in the majority (72.7%) of CoA patients and type 1A BAV an incomplete raphe was seen as compared to only 38.8% in the population without CoA (Table 6).

Patients with CoA and type 1A BAV had significantly less valve regurgitation (13.6% vs. 55.8%, $p<0.001$) and significantly smaller diameters of the ascending aorta (33.7mm vs. 37.8mm, $p<0.001$) and aortic arch (25.8mm vs. 30.2mm, $p<0.001$) than type 1A patients with isolated BAV (Table 6).

Table 6. Characteristics of patients with type 1A bicuspid aortic valves (BAVs) with coarctation of the aorta (CoA) versus without CoA

Variable	CoA	No CoA	P value
Type 1A BAV, n (%)	22 (56.4%)	129 (59.7%)	NS
Raphe			0.003
incomplete, n (%)	16 (72.7%)	50 (38.8%)	
complete, n (%)	6 (27.3%)	79 (61.2%)	
Ascendens (mm)	33.3 ± 6.06	37.8 ± 6.78	<0.001
Arch (mm)	25.5 ± 4.77	30.2 ± 4.98	<0.001
Valve regurgitation	3 (13.6%)	72 (55.8%)	< 0.001
Valve stenosis	10 (45.5%)	56 (43.4%)	NS

DISCUSSION

Key findings of this study are 1) Patients with type 1A BAV and a complete raphe show more aortic regurgitation and root dilatation as compared with the rest of the study population; 2) The majority of CoA patients has an incomplete raphe and smaller aortic root diameters and less valve regurgitation

Extent of the raphe as predictor of outcome

An association has been described between aortic stenosis and type 2 BAVs^{12,20}; however, in those studies no distinction was made between the presence or not of a raphe. In the current study, the same trend was seen, but this finding was not significant ($p = 0.252$). Also a relation has been shown between type 1A BAVs and aortic regurgitation²¹, which is in line with the current study. The most important findings of the current study, however, were related to the *extent* of the raphe. A complete raphe predisposed for larger aortic diameters and more valve regurgitation. To our knowledge, the extent of a raphe in BAV disease has not been studied previously as a prognostic factor. The worse outcome observed in patients with a complete raphe is possibly due to the fact that BAVs with incomplete raphes have a more physiological, tricuspid-like opening and therefore function better. BAVs with complete raphes seem to have more unevenly sized leaflets and smaller openings which may predispose to valve dysfunction. Type 1A BAVs have been related to aortic sinus dilatation -which is in line with the current study- and type 2A BAVs have been associated with dilatation of the ascending aorta^{9,11,13}. However, none of these studies take into account the extent of the raphe. The current study showed a significant difference in ascending aorta diameter between BAVs with a complete versus incomplete raphe. Differences in dilation might therefore be explained by the extent of the raphe, e.g. due to altered flow, although this remains speculative at this point.

Patients with type 1A BAVs and a complete raphe showed significantly more regurgitation and root dilation as compared to the rest of the study population. Therefore, type 1A BAVs can be regarded as the valve orientation with the highest risk, which is in line with previous studies^{11,22,23}. This indicates that type 1A BAVs that also have a complete raphe should even be monitored more closely for valve regurgitation and aortopathy.

Effect of CoA on BAV morphology and outcome

Subgroup analysis of the CoA group revealed that these patients are on average 9 years younger than the rest of the study population, which may be explained by the fact that these patients usually show symptoms earlier and are often referred from the pediatric cardiologist as soon as they reach adulthood. The prevalence of BAV in CoA

patients is an estimated 60%^{4,5}. The majority of patients in the current study had type 1A BAV, which corresponds to reports in literature¹⁵. CoA patients had smaller aortic root diameters and less valve regurgitation, which might be explained by the fact that less CoA patients had a BAV with complete raphe. The prevalence of stenosis was similar in the CoA group compared with the rest of the study population. This in contrast to earlier research which found an association between CoA and valve dysfunction⁵, although in this study the extent of raphe was not taken into account.

Study limitations

This was a retrospective analysis of clinically obtained patient data derived from a single centre. A retrospective analysis is subjected to selection bias, as the investigator self-selects the cases. All patients were followed in a tertiary referral centre, which may have led to a selection bias of more severely affected patients. Both biases were minimised by including consecutive BAV patients who underwent echocardiography between 2005 and 2010.

Conclusions and clinical implications

This study shows that the extent of the raphe is of clinical importance, as a complete raphe predisposes for more valvular dysfunction and aortopathy. Moreover, patients with type 1A BAV and a complete raphe show more aortic regurgitation and root dilatation as compared with the rest of the study population. This could indicate that this group of patients needs closer monitoring and more regular follow up.

On the other hand, this study shows that patients with a bicuspid aortic valve and coarctation of the aorta have smaller aortic root diameters and less valve regurgitation, possibly due to the fact that in this group of patients the majority has an incomplete raphe.

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3

Coronary anatomy as related to bicuspid aortic valve morphology

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ABSTRACT

Background

Variable coronary anatomy has been described in patients with bicuspid aortic valves (BAV). This was never specified to BAV morphology and prognostic relevance of coronary vessel dominance in this patient group is unclear. The purpose of this study was to evaluate valve morphology in relation to coronary artery anatomy and outcome in patients with isolated BAV and with associated aortic coarctation (CoA).

Methods

Coronary anatomy was evaluated in 186 BAV patients (141 men (79%), 51 ± 14 years) by computed tomography and invasive coronary angiography. Correlation of coronary anatomy was made with BAV morphology and coronary events.

Results

Strictly bicuspid valves (without raphe) with left-right cusp fusion (Type 1B) had more left dominant coronary systems as compared to BAVs with left-right cusp fusion with a raphe (Type 1A) (48% vs 26%, $p = 0.047$) and showed more separate ostia (28% vs 9%, $p = 0.016$). Type 1B BAVs had more coronary artery disease than patients with type 1A BAV (36% vs 19%, $p = 0.047$). More left dominance was seen in BAV patients with CoA than in patients without (65% vs 24%, $p < 0.05$).

Conclusion

The incidence of a left dominant coronary artery system and separate ostia was significantly related to BAVs with left-right fusion without a raphe (Type 1B). These patients more often had significant coronary artery disease. In patients with BAV and CoA, left dominance is more common.

INTRODUCTION

Bicuspid aortic valve (BAV) is the most common congenital heart defect, affecting 1-2% of the general population^{1,2}. While BAV can be an isolated disorder, it is often associated with other congenital cardiac abnormalities. The most common associated congenital malformation is coarctation of the aorta (CoA), with a prevalence ranging from 1 to 34%^{3,4}. Conversely a bicuspid aortic valve is seen in about 60% of patients with aortic coarctation^{5,6}. There are different BAV morphologies: most common are valves with fusion of the left and right coronary cusp. Valves with fusion of the right and non-coronary cusp are less common and valves with fusion of the left and non-coronary cusp are rare. When no raphe is present, the valve is called strictly bicuspid⁷.

BAV morphology correlates to outcome with respect to valve function and amount of aortic dilation^{8,9}. Differences in coronary anatomy have also been described in patients with BAV. For example, an increased incidence of both a short left main coronary artery (LMCA) and of immediate bifurcation of the two major branches of the left coronary artery (LCA) was described in patients with BAV and in patients with aortic stenosis and left dominant arterial system^{10,11}. Furthermore, an association between BAV and a left dominant coronary arterial system was described^{12,13}. The incidence of left coronary dominance in patients with BAV in one study was found to be as high as 57%¹². Other studies reported lower numbers ranging from 25-29%^{10,13,14}, which is still much higher than the reported incidence of 10-12% in the general population with normal aortic valves^{10,13}. The reason why these coronary patterns occur more often in BAV patients, is to date unclear.

During development, the aortic valve, as well as the pulmonary valve, develops from the so-called endocardial cushions, which are local swellings of mesenchymal tissue located at the outflow tract of the heart. Initially, no separate aortic cusps can be detected, however after proper development the classic tricuspid division in 3 aortic valvular cusps is recognised¹⁵. In BAV patients it is generally considered that 2 cusps are fused, although a maldevelopment or lack of proper cushion division may also be the case^{8,16}.

Apparently, signalling events during development guide the proper ingrowth of the left main stem and RCA in a regionalised fashion, dictated by the borders of the valves, although the exact mechanism is still unknown. In BAV patients, where this strict bordering of the cusps is hampered, variations in coronary anatomy might occur more often, perhaps most outspoken in patients with strictly bicuspid valves where a raphe is missing and no valvular commissures can be determined¹⁷.

In the current study, we hypothesized that variations in coronary distribution patterns correlate to BAV morphology, and that strictly bicuspid valves show more variation as

valve borders are undetermined by a raphe in these cases.

Therefore, the purpose of this study was to evaluate coronary anatomy and association with coronary events as related to BAV morphology.

METHODS

Study population

All BAV patients with transthoracic echocardiography (TTE) between 2005 and 2013 were identified from the echocardiography database of the Leiden University Medical Center and Academic Medical Center Amsterdam (together forming the Center for Congenital Heart Disease Amsterdam Leiden). The current retrospective evaluation of clinically acquired data was approved by the Institutional Review Board of the Leiden University Medical Center, who waived the need for written informed consent.

Of these patients, only those were selected who had also undergone computed tomography coronary angiography (CTA), and/or invasive coronary angiography (ICA). Patients with uninterpretable CTA or ICA examination were excluded from the analysis. From a total of 420 patients, 186 patients were selected, of whom aortic valve morphology was evaluated by echocardiography and coronary anatomy was assessed by CTA or ICA. Patients with fusion of the left and non-coronary cusps (Types 3A and 3B, 8 patients) were excluded from statistical analysis due to small numbers, leaving a total number of 178 patients included in the study.

Echocardiography

Echocardiography exams were obtained using commercially available echo systems (Vivid 7 and E9, GE Vingmed Ultrasound, Horten, Norway) with standard views from the parasternal, subcostal, suprasternal and apical windows. The aortic valve was examined in the two dimensional parasternal short-axis view and classified as bicuspid when two cusps could be clearly identified and the typical 'fish-mouth opening' of the valve was observed. All echocardiography exams were re-evaluated off-line using EchoPac version 7 software (GE Medical Systems, Horten, Norway). The aortic valve was evaluated in a cross-sectional view for the presence of a raphe. In a previous study three BAV morphologies were identified: type 1 with fusion of right and left coronary cusp; type 2 with right and non-coronary fusion and the rare type 3 with left and non-coronary fusion⁸. In this study, this classification was followed (Figure 1), with addition of A (for valves with a raphe) or B (for strictly bicuspid valves).

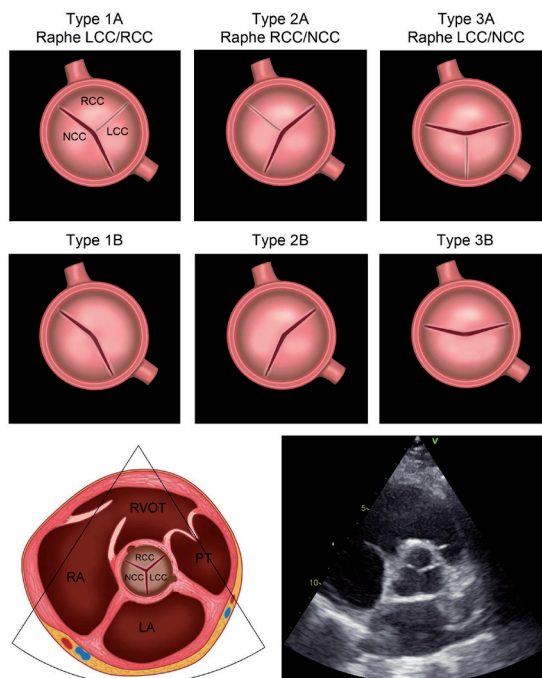


Figure 1. Schematic overview of bicuspid aortic valve (BAV) morphologies. Drawings are oriented in echocardiographic view of the aortic valve. Upper panel: the three major valve morphologies observed in patients with BAV are described as three different types based on the valve leaflet orientation: type 1 (fusion right and left coronary cusps), type 2 (fusion right and non-coronary cusps) and type 3 (fusion left and non-coronary cusps). Middle panel: strictly bicuspid valves (without a raphe) are defined as a subgroup B. Modified after Schaefer et al.⁸ Leaflet size and symmetry, as well as the position of the commissures, may vary from the schematic overview depicted here. Lower panel: schematic drawing (left) and echocardiographic view (right) of a tricuspid aortic valve, in similar orientation as the panels above.

LA, left atrium; LCC, left coronary cusp; NCC, non-coronary cusp; PT, pulmonary trunk; RA, right atrium; RCC, right coronary cusp; RVOT, right ventricular outflow tract.

Computed tomography coronary angiography data acquisition and image analysis

Patients were scanned using a 64-row CT scanner (Aquillion64, Toshiba Medical Systems, Otawara, Japan; General Electrics LightSpeed VCT, Milwaukee, WI, USA) or a 320-row CT scanner (AquillionONE, Toshiba Medical Systems, Otawara, Japan). Coronary anatomy and coronary vessel dominance were assessed in a standardized manner by dividing the coronary artery into 17 segments according to the guidelines of the American Heart Association (Austen et al, Circulation 1975).

The coronary artery system was classified as right dominant when the posterior descending artery (PDA) originated from the right coronary artery (RCA) and considered

left dominant if the PDA originated from the left circumflex artery (LCX)(Figure 2, panels A,B). The coronary artery system was classified as balanced, when the PDA originated from the RCA in combination with a large posterolateral branch originating from the LCX, reaching to or close to the posterior interventricular groove. Subsequently, the presence of a LMCA was assessed. If there was no LMCA, the LCA was designated as having separate ostia of the LAD and LCX (Figure 2, panels C,D). Finally, the presence of coronary artery disease was assessed. All 17 segments were scored as non-significant CAD (defined as < 50% luminal narrowing) or significant CAD (defined as $\geq$ 50% luminal narrowing).

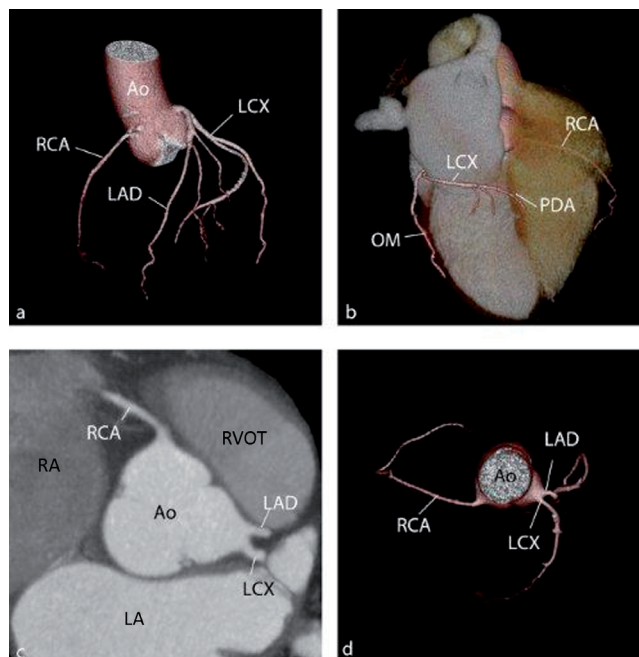


Figure 2. (A) Left dominant coronary artery system as seen on CT coronary angiography (CTA). There is a large left circumflex artery (LCX) and a relatively small right coronary artery (RCA). The valve morphology in this patient was type 1A. (B) Left dominant coronary artery system in the same patient (type 1a BAV) as seen on CTA, posterior view. The posterior descending artery (PDA) originates from the LCX. (C and D) Separate ostia of the left anterior descending artery (LAD) and LCX as seen on CTA. Valve morphology in this patient was type 1A. Ao, aorta; LA, left atrium; LCC, left coronary cusp; LV, left ventricle; NCC, non-coronary cusp; OM, obtuse marginal; RA, right atrium; RCC, right coronary cusp; RV, right ventricle.

Invasive coronary angiography acquisition and analysis

Conventional invasive coronary angiography was performed according to standard techniques¹⁸. All images were retrospectively reviewed by an experienced observer.

Coronary artery dominance, separate ostia, coronary aberrancy and severity of CAD were classified as above. Vessels were classified as having diameter stenosis of either $< 50\%$ or $\geq 50\%$ luminal narrowing, and lesions with stenosis $\geq 50\%$ were deemed significant.

Assessment of coronary events

Significant coronary artery disease was defined as stenosis causing $\geq 50\%$ luminal narrowing. Coronary events were defined as having severe coronary artery disease ($> 70\%$ luminal narrowing), having undergone revascularisation by percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) or any admission for unstable angina pectoris¹⁹.

Statistical analysis

Continuous variables are presented as mean $\pm$ standard deviation or as median and interquartile range. Categorical variables are presented as number and percentages. One-way ANOVA tests were used for comparing numerical data in more than 2 categories. Cross-tabulations were made for binary categorical data, on which chi-square goodness-of-fit-tests were performed to test for independence. For sets of independent numerical data linear regression analysis was used to evaluate trends. Similarly, trends for binary categories were evaluated with binary logistic regression to correct for possible confounding (risk) factors such as age and gender. Statistical analysis was performed using SPSS software (version 20.0, SPSS, Inc., Chicago, IL, USA). A P -value < 0.05 , by a two-sided test, was considered statistically significant.

RESULTS

Patient characteristics

The population comprised 178 patients with a mean age of 51 ± 14 years, 141 (79%) patients were male. Patient characteristics are presented in Table 1. Overall baseline characteristics were similar between the groups

No significant differences in indications for CT scan or coronary angiography were observed between the different groups (Table 2).

Coronary anatomy

Of all patients, 52 (30%) had a left dominant coronary artery system, 103 patients (60%) had a right coronary artery system and 16 patients (9%) had a balanced system. Separate ostia were seen in 20 patients (11%).

Table 1. Patient characteristics

	Total, n=178	Type 1A, n=116	Type 2A, n=27	Type 1B, n=25	Type 2B, n=10	P-value
Gender (male)	141 (79%)	95 (82%)	20 (74%)	21 (84%)	5 (50%)	0.091
Age (years)	51 ± 14	52 ± 13	49 ± 15	49 ± 15	47 ± 14	0.590
Risk factors						
Diabetes	15 (8%)	10 (9%)	3 (11%)	2 (8%)	0 (0%)	0.757
Hypertension	56 (32%)	40 (35%)	6 (22%)	9 (36%)	1 (10%)	0.269
Hypercholesterolemia	28 (18%)	20 (20%)	4 (15%)	3 (12%)	1 (10%)	0.684
Smoking	44 (25%)	30 (26%)	7 (26%)	3 (12%)	4 (40%)	0.318
Medication						
Aspirin	49 (28%)	26 (22%)	9 (33%)	8 (32%)	6 (60%)	0.057
Statins	67 (38%)	44 (38%)	9 (33%)	11 (44%)	3 (30%)	0.826
Angiotensin converting enzyme inhibitor	86 (48%)	58 (50%)	12 (44%)	13 (52%)	3 (30%)	0.621
Oral anticoagulants	58 (33%)	44 (38%)	7 (28%)	7 (28%)	0 (0%)	0.068

Table 2. Indications for CT scan and/or coronary angiogram

	Total (n (%)) n = 186	Type 1A, n = 116	Type 2A, n = 27	Type 3A, n = 4	Type 1B, n = 25	Type 2B, n = 10	Type 3B, n = 4	P-value
Preoperative	79 (43%)	49 (42%)	8 (30%)	3 (75%)	13 (52%)	4 (40%)	2 (50%)	0.466
Follow-up aortic dimensions	26 (14%)	16 (14%)	4 (15%)	1 (25%)	2 (8%)	2 (20%)	1 (25%)	0.867
Chest pain	26 (14%)	15 (13%)	7 (26%)	0 (0%)	2 (8%)	1 (10%)	1 (25%)	0.387
Suspected ACS	13 (7%)	9 (8%)	1 (4%)	0 (0%)	1 (4%)	2 (0%)	0 (0%)	0.535
Arrhythmia/pre MAZE	23 (12%)	16 (14%)	3 (11%)	0 (0%)	4 (16%)	0 (0%)	0 (0%)	0.684
Cardiac arrest	3 (2%)	1 (1%)	2 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.234
Other	8 (4%)	5 (4%)	2 (7%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	0.234
Unknown	7 (4%)	4 (3%)	0 (0%)	0 (0%)	2 (8%)	1 (10%)	0 (0%)	0.591

BAV morphology

In 143/178 patients (80%), a raphe could be identified. In 116 patients (65%), the raphe was located between the left and right coronary cusp and thus had a type 1A BAV. In 27 patients (15%), the raphe was located between the right and non-coronary cusp, designated as type 2A BAV. In 35 patients, no raphe could be identified. These strictly bicuspid valves were designated as group B BAV (Figure 1). In 25 patients (14%), valve orientation was in an anterior-posterior direction, indicated as type 1B BAV and in 10 patients (6%) valve orientation was in a left-right direction, indicated as type 2B BAV (Figure 1). Figure 3 shows the distribution of BAV morphology in the study population.

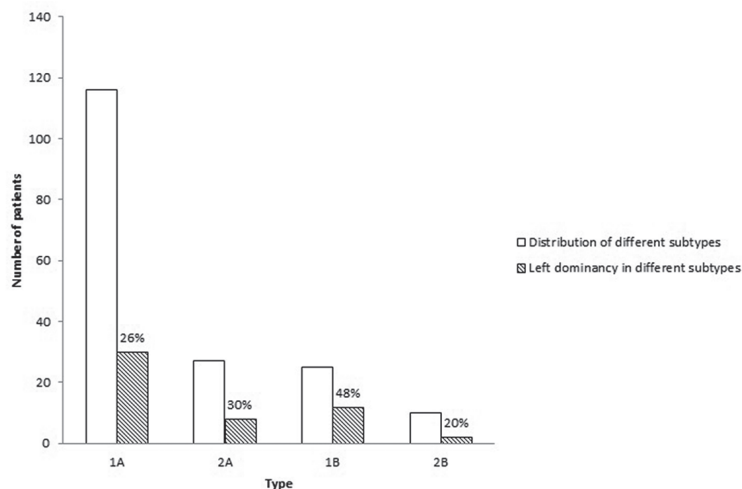


Figure 3. Schematic overview of the distribution of valve morphologies and of left dominant coronary artery systems in different valve morphologies in the study population.

Coronary anatomy and association with coronary events as related to BAV morphology

Type 1 BAVs without a raphe show more left dominance and separate ostia

As stated before, 30% of all patients had a left dominant coronary artery system.

When comparing the whole group of patients with type 1 BAV to patients with type 2 BAV, there was no significant difference in coronary vessel dominance or separate ostia (table 3).

When taking into account the presence or absence of a raphe however, patients with type 1B BAV (no raphe), had more left dominant coronary systems as compared to patients with type 1A BAV ($p = 0.047$, table 3). Type 1B BAVs also showed more separate ostia of the LAD and LCX ($p = 0.016$).

Figure 3 shows the distribution of BAV morphologies in the patients with a left dominant coronary artery system.

Type 1 BAVs without a raphe show more coronary artery disease

Next, to determine the clinical relevance of these differences in coronary anatomy, the clinical outcome in type 1A BAVs versus type 1B BAVs was studied. This showed that patients with type 1B BAV had significantly more coronary artery disease as compared to patients with type 1A BAV ($p = 0.047$, table 3). Type 1B BAVs also showed more coronary events compared to type 1A BAVs, however this was not significant. All comparisons were corrected for age and gender.

Table 3. Differences in coronary anatomy and coronary events between different subtypes

Left dominant			P value
Type 1 (n = 141) vs type 2 (n = 37)	42 (30%)	10 (27%)	NS
Type A (n = 143) vs type B (n = 35)	38 (28%)	14 (41%)	NS
Type 1A (n = 116) vs type 1B (n = 25)	30 (26%)	12 (48%)	0.047
Type 1A (n = 116) vs type 2A (n = 27)	30 (26%)	8 (30%)	NS
Type 1B (n = 25) vs type 2B (n = 10)	12 (48%)	2 (20%)	NS
Type 2A (n = 27) vs type 2B (n = 10)	8 (30%)	2 (20%)	NS
Separate ostia			
Type 1 (n = 141) vs type 2 (n = 37)	18 (13%)	2 (5%)	NS
Type A (n = 143) vs type B (n = 35)	13 (9%)	7 (20%)	NS
Type 1A (n = 116) vs type 1B (n = 25)	11 (9%)	7 (28%)	0.016
Type 1A (n = 116) vs type 2A (n = 27)	11 (9%)	2 (7%)	NS
Type 1B (n = 25) vs type 2B (n = 10)	7 (28%)	2 (20%)	NS
Type 2A (n = 27) vs type 2B (n = 10)	2 (7%)	2 (20%)	NS
Significant CAD			
Type 1 (n = 141) vs type 2 (n = 37)	31 (22%)	11 (30%)	0.023
Type A (n = 143) vs type B (n = 35)	30 (21%)	12 (34%)	0.029
Type 1A (n = 116) vs type 1B (n = 25)	22 (19%)	9 (36%)	0.047
Type 1A (n = 116) vs type 2A (n = 27)	22 (19%)	8 (30%)	0.030
Type 1B (n = 25) vs type 2B (n = 10)	9 (36%)	3 (30%)	NS
Type 2A (n = 27) vs type 2B (n = 10)	8 (30%)	3 (30%)	NS
Coronary events			
Type 1 (n = 141) vs type 2 (n = 37)	39 (28%)	12 (32%)	NS
Type A (n = 143) vs type B (n = 35)	37 (26%)	14 (40%)	0.029
Type 1A (n = 116) vs type 1B (n = 25)	29 (25%)	10 (40%)	NS
Type 1A (n = 116) vs type 2A (n = 27)	29 (25%)	8 (30%)	NS
Type 1B (n = 25) vs type 2B (n = 10)	10 (40%)	4 (40%)	NS
Type 2A (n = 27) vs type 2B (n = 10)	8 (30%)	4 (40%)	NS

Coarctation of the aorta

Out of 178 patients, 22 (12%) had a coarctation of the aorta.

Patients with CoA showed no significant difference in valve morphology, the majority (55%) having a type 1A BAV. In the CoA group more left dominance was seen as compared to the group with isolated BAV ($p < 0.05$), but there was no difference in significant coronary artery disease or events. In contrast to the isolated BAV group, most patients with CoA and a left dominant coronary artery system had a type 1A BAV (Type

1A 8/15 (53%), versus type 1B 4/15 (27%)). The CoA group was significantly younger than the patients with isolated BAV (42 ± 13 years vs 51 ± 14 years, $P < 0.001$).

DISCUSSION

The main finding of this study is that type 1 BAVs without the presence of a raphe, more often show left dominant coronary systems and separate ostia of the LAD and LCX as compared to type 1 BAVs with a raphe. In addition, more significant coronary artery disease was observed in this group.

Developmental aspects of coronary anatomy

Although many variations in coronary anatomy exist, it is striking that the posterior aortic cusp is hardly ever seen to receive an aberrant coronary artery²⁰. The same applies to the lack of ingrowth in pulmonary orifice. It is likely that signalling events during development guide the proper ingrowth of the left main stem and RCA in a regionalised fashion, dictated by the borders of the valves, however the exact mechanism is still unknown. It seems quite in line with the expectation that in BAV patients, where this strict bordering of the cusps is hampered, variations in coronary anatomy may occur more often, perhaps most outspoken in patients with strictly bicuspid valves where a raphe is missing and no valvular commissures can be determined¹⁷. Indeed, in the current study the main finding was that type 1B BAVs more often showed a left dominant coronary artery system and separate ostia of the LAD and LCX.

Coronary anatomy and association with coronary events

As described before, there is an association between BAV and the presence of a left dominant coronary artery system. The reported incidence in this study is in line with previous studies^{10, 13, 14, 21} and much higher than the reported incidence (10–12%) in patients with normal aortic valves^{10, 13}. The prognostic relevance of coronary vessel dominance is unclear, partly due to contradictory results. However, some studies report a higher incidence of CAD (obstructive and non-obstructive) in left dominant patients^{22, 23}. Also higher risk scores for significant stenosis in segments of the LAD and LCX in patients with a left dominant coronary artery system have been described²⁴. The mechanism of a potentially increased susceptibility to coronary events in patients with left dominant anatomy is to date unclear. A study screening post-mortem angiograms showed that the prevalence of a left dominant system decreased with age, suggesting a higher death rate among patients with a left dominant coronary artery system²⁵. An explanation could be the larger myocardial area at risk in case of an acute myocardial

infarction. Other possible mechanisms explaining a worse prognosis might be coronary artery length and lumen diameter. A study by Dodge *et al.*, evaluating 83 invasive coronary angiograms, demonstrated a smaller RCA diameter and a larger LCX diameter in patients with a left dominant coronary artery system²⁶. Thus, a proximal stenosis of the left coronary artery may result in more extensive ischemia and worse consequences in a left dominant system than in a right dominant system.

From a molecular point of view, hypoplasia of the coronary artery system in mouse models with bicuspid aortic valves have been reported²⁷. Whether this hypoplasia correlated to the distribution patterns of the coronary arteries has not been explored to date.

Genetic aspects

It has been shown that deficiency in endothelial nitric oxide synthase (eNOS) results in the formation of bicuspid aortic valves²⁸. In addition, mutations in the *NOTCH1* gene can lead to bicuspid aortic valves²⁹. Recently an association of Notch with CoA has been found³⁰. Interestingly, Notch signalling also plays an important role in the modulation of inflammation³¹ and the Notch pathway has been linked to coronary artery disease³². Whether Notch mutations relate to the observed variations in BAV morphology subtype (Type A versus type B) or coronary anatomy, remains to be determined.

Coarctation of the aorta

Subanalysis of the group of patients with BAV, who also had coarctation of the aorta, shows that these patients more often have left coronary system dominance, but no difference in coronary artery disease or events. A possible explanation could be that the mean age in this group was significantly lower than in the group without coarctation. Alternatively, this could be related to the fact that CoA patients with a left dominant coronary artery system more often had a type 1A BAV.

Study limitations

Only patients who underwent CT or invasive coronary angiography were included in this study, which may have led to a selection bias. However, the indications for coronary angiography were not significantly different between the groups (table 2).

Transthoracic echocardiography was the only imaging modality used for classification of BAV phenotype. Although the sensitivity of echocardiography to accurately diagnose BAV is relatively low, the specificity is quite high³³. Therefore, the selected patients most likely did have BAV. Accurate diagnosis of raphe and patterns of cusp fusion can be hampered by poor imaging quality. Therefore, patients with uninterpretable echocardiography were not included in the study.

Conclusions and clinical implications

This study shows that patients with type 1B BAVs and patients with BAV and CoA more often have a left dominant coronary artery system. Patients with type 1B BAVs also seem more at risk of developing significant coronary artery disease. The current study also shows that type 1B BAVs more often have separate ostia, which could be of clinical importance in performing coronary angiography and, more importantly, in using coronary artery perfusion as a means of myocardial protection during surgical aortic valve replacement.

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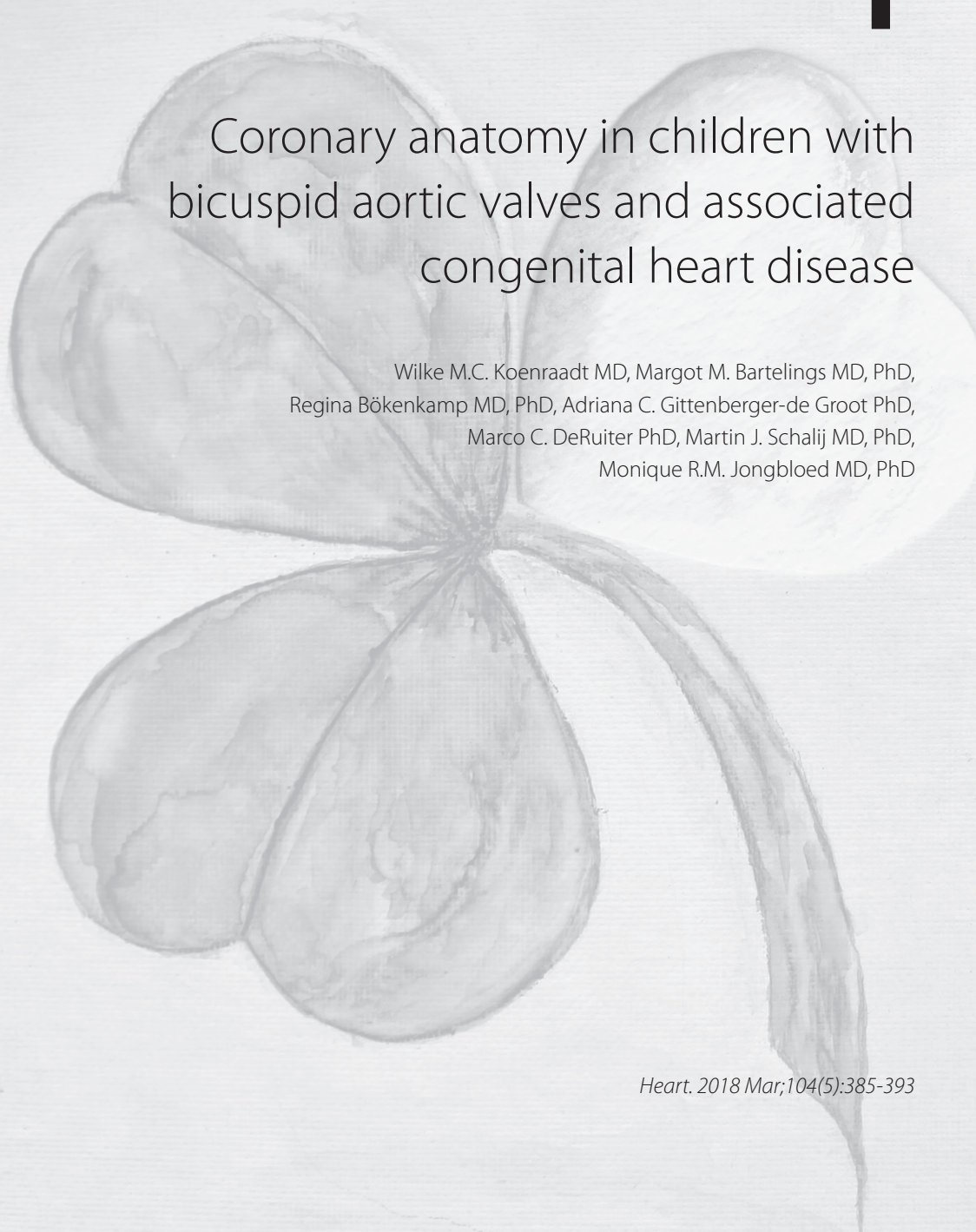
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Coronary anatomy in children with bicuspid aortic valves and associated congenital heart disease

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ABSTRACT

Background

In bicuspid aortic valve (BAV) patients, coronary anatomy is variable. High take-off coronary arteries have been described, but data are scarce, especially when associated with complex congenital heart disease (CHD). The purpose of this study was to describe coronary patterns in these patients.

Methods

In 84 post-mortem heart specimens with BAV and associated CHD, position and height of the coronary ostia were studied and related to BAV morphology.

Results

High take-off right (RCA) and left coronary arteries (LCA) were observed in 23% and 37% of hearts, respectively, most frequently in hearts with hypoplastic left ventricle (HLV) and outflow tract anomalies. In HLV high take-off was observed in 18/40 (45%), more frequently of LCA (n=14) than RCA (n=6). In hearts with aortic hypoplasia 8/13 (62%) had high take-off LCA and 6/13 (46%) high take-off RCA. High take-off was seen 19 times in 22 specimens with perimembranous VSD (RCA 8, LCA 11). High take-off was associated with type 1A BAV (raphe between right and left coronary leaflets), more outspoken for the RCA. Separate ostia of LAD and LCX were seen in 4 hearts (5%), not related to specific BAV morphology.

Conclusion

High take-off coronary arteries, especially the LCA, occur more frequently in BAV with associated CHD than reported in normal hearts and isolated BAV. Outflow tract defects and HLV are associated with type 1A BAV and high take-off coronary arteries. Although it's unclear whether these findings in infants with detrimental outcome can be related to surviving adults, clinical awareness of variations in coronary anatomy is warranted.

INTRODUCTION

Bicuspid aortic valve (BAV) is the most common congenital heart defect (prevalence 0.5-2%). BAVs with different morphology subtypes exist and BAV morphology correlates to outcome with respect to valve function and aortic dilation^{1,2}. In the majority of BAVs the right and left coronary leaflets are conjoined, in a minority the right and non-coronary leaflets and in only a small group the left and non-coronary leaflets are conjoined^{1,3}.

Variations in coronary anatomy have been described in BAV patients, including increased incidence of separate ostia of the left anterior descending coronary artery (LAD) and left circumflex coronary artery (LCX), and of a left dominant coronary system^{4,5}. Also differences in coronary ostial positions as related to the aortic sinus have been noted^{6,7}. In normal hearts, the coronary arteries arise mostly from the aortic sinuses below the sinotubular (ST) junction. Coronary ostia can be located a few millimetres above the ST junction in 5-22% percent of normal hearts, although literature is scarce^{8,9}. Coronary ostial take-off well above the ST junction, referred to as high coronary take-off, is rare, occurring in less than 1% of the normal population^{10,11}. Data concerning coronary height in BAV patients are scarce and not consistent, mostly confined to case reports^{6,7}. Lerer et al. reported the left coronary artery (LCA) in patients with isolated BAV above the aortic ST junction in 29% of cases, whereas the right coronary artery (RCA) is located too high in 11%¹². This study did not include hearts with additional congenital anomalies. In patients with Turner syndrome, only one out of 50 (2%) patients had a high takeoff RCA¹³. This patient also had a BAV whereas all other patients had a tricuspid aortic valve. The significance of recognizing high take-off coronary arteries lies in potential challenges encountered during catheter cannulation or surgery. When unrecognized, there have been cases of inadvertent transection and coronary cross-clamping during open-heart procedures^{14,15}. High take-off coronaries have also been associated with intramural coronary course¹¹ and may impair coronary blood flow due to formation of an acute angle with the ostial ridge and angulation may increase with increasing aortic dimensions¹⁶.

Currently available studies have largely been performed in patients with BAV as isolated anomaly. The influence of associated complex congenital heart disease (CHD) on coronary anatomy is unknown. Developmental issues may be of relevance, as many processes during heart development are influenced by signalling events from cell types that also influence coronary development¹⁷. Coronary arteries derive from angiogenic sprouts in the sinus venosus region, initially not connected to the aorta^{18,19}. Ingrowth in the aorta will occur during later development, orchestrated by epicardial derived cells, whereas endothelial cells will contribute to the proximal coronary orifices¹⁹⁻²¹. Disturbance in this process may lead to a too high position of the coronary arteries as

related to the aortic sinus (i.e. high take-off) and perhaps also to erroneous ingrowth of a coronary artery, usually the left, in the pulmonary artery²², although the developmental mechanism of the latter is still enigmatic.

The aim of the current study is to describe coronary patterns in post mortem hearts with BAV associated with complex CHD, as related to BAV morphology.

METHODS

Study population

This study was undertaken in accordance with the local ethics committee and Dutch regulations for proper use of human tissue for medical research purposes.

Eighty-four postmortem heart-lung specimens with BAV associated with other CHD from the Leiden collection of malformed hearts (Department of Anatomy&Embryology, Leiden University Medical Center, Leiden, The Netherlands) were studied macroscopically. This collection includes hearts preserved in ethanol and glycerin, dating from the 1950s to the current era.

Anatomical studies

Two experienced observers investigated the hearts. Cardiac morphology was assessed using sequential segmental analysis²³, performed as completely as possible. All cardiovascular anomalies as well as detailed morphology of the aortic valve and position and height of coronary ostia were noted. In some specimens cardiovascular anomalies could not be assessed due to incompleteness of the specimens.

Hypoplastic LV (HLV) was considered present when the lumen of the LV was smaller than the right ventricle (RV), but the apex was formed by the LV, or when the apex was formed by the RV, including cases with hypoplastic left heart syndrome (HLHS). HLHS was considered present when there was severe underdevelopment of the left heart with significant hypoplasia of the left ventricle including atresia, stenosis, or hypoplasia of the aortic or mitral valve, or both valves, and hypoplasia of the ascending aorta and aortic arch²⁴. The ascending aorta was considered hypoplastic if the aortic orifice diameter was smaller than expected as compared to the pulmonary orifice diameter. Tubular hypoplasia of the aortic arch was defined as a narrowed (often elongated) aortic arch segment. Aortic coarctation was defined as a localized obstructive ridge within the arch consisting of a constriction of the aortic wall and/or a shelf protruding into the aortic lumen²⁵.

BAV classification and terminology

BAV morphology was defined by orientation of the leaflets with respect to each other, determined by which of the leaflets (right coronary, left coronary, non-coronary) were conjoined and presence and position of a raphe. BAV morphology was classified as *Type 1* if the right and left coronary leaflets were conjoined; *Type 2* if the right and non-coronary leaflets were conjoined and *Type 3* if the left and non-coronary leaflet were conjoined. 'A' was added for valves in which a raphe could be recognized or 'B' for strictly bicuspid valves (no raphe) (**Figure 1**). A raphe was defined as a ridge located in the conjoined area of two leaflets, presumably representing a malformed commissure.

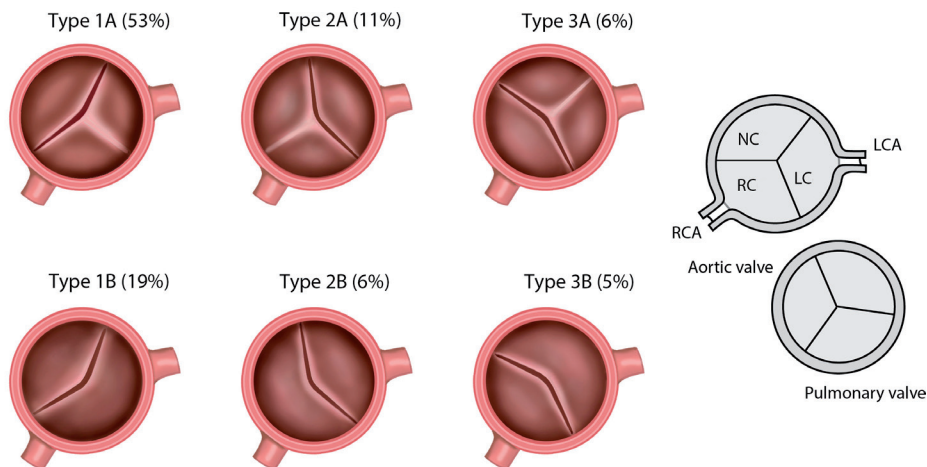


Figure 1. Schematic overview of different BAV morphologies. Drawings are oriented in anatomical view of the aortic valve, i.e. as seen from above. Upper panel: BAVs with a raphe; type 1 ("fusion" right and left coronary leaflets), type 2 ("fusion" right and non-coronary leaflets) and type 3 ("fusion" left and non-coronary leaflets). Lower panel: Strictly bicuspid valves (without a raphe), defined as a subgroup B. Leaflet size and symmetry, as well as the position of the commissures may vary from the schematic overview depicted here.

Abbreviations: LCA: left coronary artery; LC: left coronary leaflet; NC: non-coronary leaflet; RCA: right coronary artery; RC: right coronary leaflet. Modified after Schaefer et al[1].

Coronary ostia

In each specimen the number and position of the coronary arterial orifices were recorded. The position of each orifice was determined in relation to the ST junction and described as above, below, or at the junctional level (**Figure 2**). A coronary orifice was classified as being located above the ST junction when there was no communication between the coronary ostium and the ST junction.

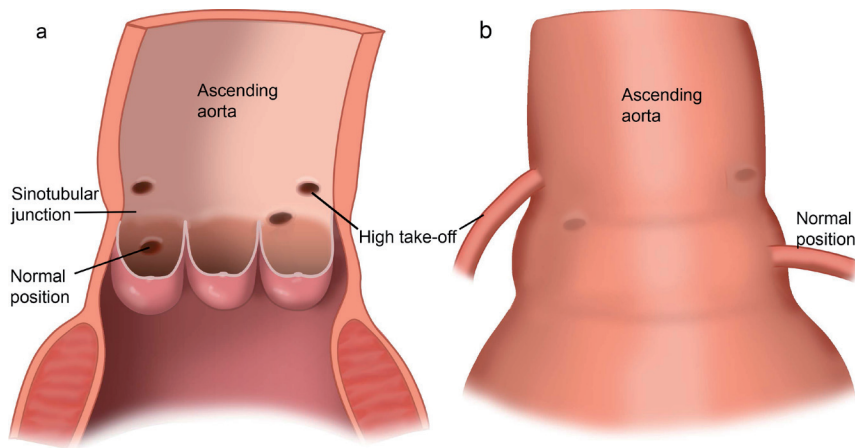


Figure 2. a: Schematic drawing of the aortic root with the aortic sinuses. The coronary ostia can be below, on or above the sinotubular junction. Drawing shows one normal coronary ostial position, one coronary ostium on the ST junction and two coronary ostia above the ST junction. Modified after Loukas et al[11].

b: Aortic root from another perspective, showing one high take-off coronary artery with sharper angulation and one normal position of the coronary artery.

Statistical analysis

Continuous variables are presented as mean $\pm$ standard deviation or as median and interquartile range. Categorical variables are presented as numbers and percentages. Cross-tabulations were made for binary categorical data, on which chi-square goodness-of-fit-tests were performed to test for independence. For comparing numerical data in more than 2 categories, logistic regression was used. Statistical analysis was performed using SPSS software (version 20.0, SPSS, Inc., Chicago, IL, USA). A P-value <0.05 , by a two-sided test, was considered statistically significant.

RESULTS

Specimen characteristics

A total of 84 hearts was studied, 48 male, 35 female. Age at demise ranged from fetal-24 years. **Table 1** provides an overview of patient characteristics including cardiovascular abnormalities.

The most frequent anomalies were HLIV (49%) and aortic coarctation (43%). Detailed morphological information of each of the 84 cases examined, is provided in **Supplementary table 1**.

Table 1. Patient characteristics and associated cardiovascular abnormalities

		n ^a	%
Age at demise	Fetal	4/82	5%
	< 1 year	66/82	80%
	1-2 years	1/82	1%
	2-4 years	3/82	4%
	4-5 years	2/82	2%
	> 5 years	6/82	7%
Cause of demise			
	Peroperative	11/84	13%
	Immediately postoperative	15/84	18%
	Heart failure	17/84	20%
	Arrhythmia (VF)	1/84	1%
	Trisomy 9	1/84	1%
	Trisomy 13	4/84	5%
	Trisomy 18	5/84	6%
Death attributed to severe form of CHD			
	Interrupted aortic arch/ extreme CoA	4/84	5%
	Uncorrected TGA	2/84	2%
Non cardiac causes			
	Intracerebral bleeding	3/84	4%
	Pulmonary problems	3/84	4%
	Sepsis	1/84	1%
	Other	7/84	8%
	Not documented	10/84	12%
Gender, M:F		48:35	
Aortic valve morphology	Type 1A	45/84	54%
	Type 2A	9/84	11%
	Type 3A	5/84	6%
	Type 1B	16/84	19%
	Type 2B	5/84	6%
	Type 3B	4/84	5%
Inflow and septal defects	PLSCV	15/83	18%
	Muscular VSD	20/84	24%
	ASD	11/83	13%
	AVSD	4/84	5%
	AV valve anomalies		
	TV Dysplasia	11/68	16%
	TV Hypoplasia	3/68	4%
	TV absent	1/68	1%
	MV hypoplasia	24/50	48%
	MV dysplasia	5/50	10%
	MV atresia	1/50	2%

Table 1. Continued

		n ^a	%
Outflow defects and LV hypoplasia	Perimembranous VSD	22/84	26%
	TGA	2/74	3%
	DORV	6/83	7%
	Hypoplastic LV	40/82	49%
	HLHS	6/40	
	aortic stenosis/mitral stenosis	4/40	
	aortic stenosis/mitral atresia	1/40	
	aortic atresia/mitral atresia	0/40	
	aortic atresia/mitral stenosis	0/40	
	Aortic arch hypoplasia	13/77	17%
	Aortic coarctation	33/77	43%
	LVOT obstruction	37/49	76%

VF = ventricular fibrillation; CHD = congenital heart disease; CoA = coarctation of the aorta; PLSCV=persistent left superior caval vein; VSD= ventricular septal defect; ASD=atrial septal defect; AVSD=atrioventricular septal defect; AV valve=atrioventricular valve; TV=tricuspid valve; MV=mitral valve; TGA=transposition of the great arteries; DORV=double outlet right ventricle; HLHS = hypoplastic left heart syndrome; LVOT=left ventricular outflow tract.

^aNumber of observed cases that could be assessed for this particular abnormality (2 unknown age, 1 unknown gender)

Aortic valve morphology

In 45 out of 84 hearts (54%) the raphe in the aortic valve was located between the left and right coronary leaflets (type 1A), whereas 9/84 (11%) had a type 2A morphology. In 5/84 (6%) hearts the raphe was located between the left and non-coronary leaflet (type 3A). In the BAVs without raphe, 16/84 (19%) had type 1B BAV, 5/84 (6%) type 2B and 4/84 (5%) type 3B, together comprising 25 cases (30%, (**Figure 1**)).

Coronary anatomy

In 84 specimens 54 (64%) coronary variations or anomalies were seen, of which 50 high take-off coronaries and separate ostia of the LAD and LCX in 4/84 (5%) specimens (**Figure 3**). This latter was not restricted to a specific valve morphology (2 had type 1A BAV, 1 had type 1B and 1 had type 3A BAV) and not associated with high take-off coronaries (high take-off LCA in 2/4 cases, no high-take-off RCA).

Overall, high coronary take-off was observed more often in the LCA than RCA (**Table 2**). The RCA arose from above the ST junction in 19/83 (23%) hearts (in 1 heart the RCA could not be evaluated). The LCA arose from above the ST junction in 31/84 (37%) hearts. Although the LCA more often showed high take-off than the RCA, this could not be correlated to a specific BAV morphology. Interestingly, in type A (i.e. with raphe) BAVs ostial height of the RCA was significantly more often above the ST junction than in type B (no raphe) BAVs (29% vs 8%, $p < 0.05$, **Table 3**).

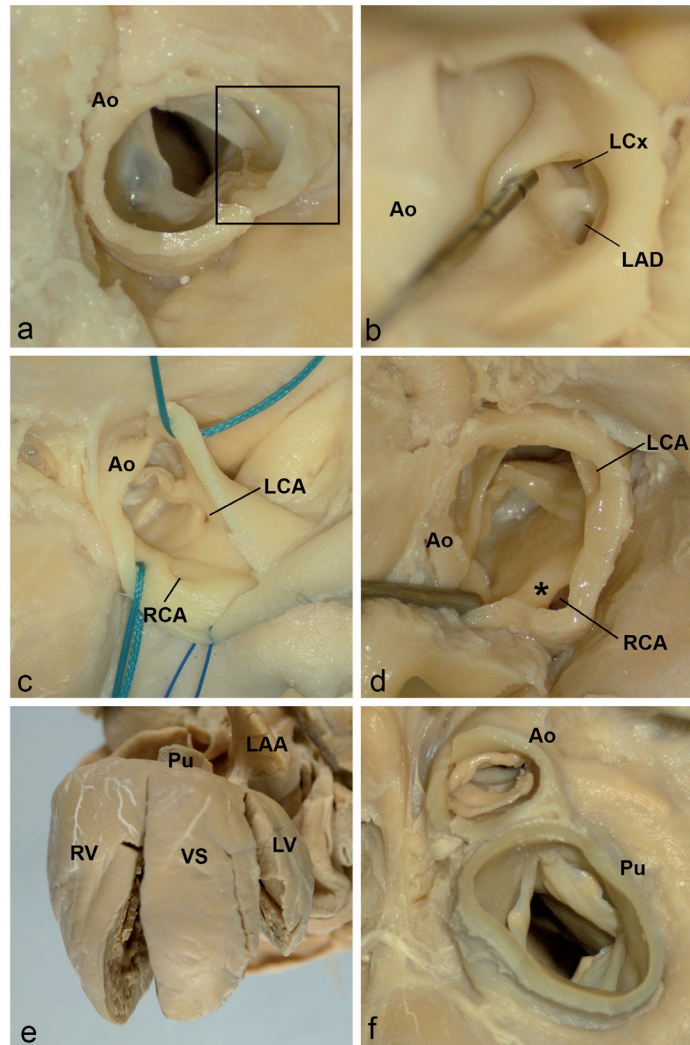


Figure 3. Representative examples of post-mortem heart specimens with variable valvular and coronary anatomy.

a,b: Type 3A BAV with separate ostia of the LAD and LCX (case 5577). The box (a) points out the origin of the LCA with a zoom in of the separate ostia (b).

c: Type 1B BAV (case 5932) with both coronary ostia located above the ST junction

d: Type 2A BAV (case 3642) with the LCA located above the ST junction and the RCA on the ST junction (*).

e,f: Specimens with a hypoplastic LV (e) and type 1A BAV (f) (case 6012). The raphe is not visible in this picture.

Abbreviations: Ao: aorta; LAA: left atrial appendage; LCA: left coronary artery; LCX: left circumflex artery; LV: left ventricle; Pu: pulmonary artery; RAA: right atrial appendage; RCA: right coronary artery; RV: right ventricle; VS: ventricular septum.

This was not observed for the LCA (**Table 3**). Examples of high take-off coronary arteries are shown in **Figure 3c,d**.

No significant difference was found in height of the coronary ostia when comparing type 1 vs type 2 or type 3 BAVs (**Table 3**).

Table 2. BAV morphology and height of coronaries in different anomalies

		BAV morphology	n (%)	RCA above ST	LCA above ST
Inflow and septal defects	PLSCV	Total	15	3	4
		Type 1A	6 (40%)	3 (100%)	1 (25%)
		Type 1B	2 (13%)	0 (0%)	0 (0%)
		Type 2A	3 (20%)	0 (0%)	1 (25%)
		Type 2B	1 (7%)	0 (0%)	0 (0%)
		Type 3A	1 (7%)	0 (0%)	1 (25%)
		Type 3B	2 (13%)	0 (0%)	1 (25%)
	Muscular VSD	Total	20	3	8
		Type 1A	12 (60%)	1 (33%)	5 (62%)
		Type 1B	5 (25%)	1 (33%)	2 (25%)
		Type 2A	0 (0%)	0 (0%)	0 (0%)
		Type 3A	2 (10%)	1 (33%)	1 (13%)
		Type 3B	1 (5%)	0 (0%)	0 (0%)
	ASD	Total	11	2	5
		Type 1A	5 (46%)	2 (100%)	2 (40%)
		Type 1B	3 (27%)	0 (0%)	2 (40%)
		Type 2A	2 (18%)	0 (0%)	0 (0%)
		Type 3A	1 (9%)	0 (0%)	1 (20%)
	AVSD	Total	4	1	2
		Type 1A	2 (50%)	1 (100%)	0 (0%)
		Type 2A	1 (25%)	0 (0%)	1 (50%)
		Type 2B	1 (25%)	0 (0%)	1 (50%)
	AV valve anomalies TV Dysplasia	Total	11	2	3
		Type 1A	5 (46%)	1 (50%)	1 (33%)
		Type 1B	3 (27%)	1 (50%)	1 (33%)
		Type 2A	3 (27%)	0 (0%)	1 (33%)
	TV Hypoplasia	Total	3	1	1
		Type 1A	1 (33%)	1 (100%)	1 (100%)
		Type 1B	1 (33%)	0 (0%)	0 (0%)
		Type 2A	1 (33%)	0 (0%)	0 (0%)
	TV absent	Total	1	0	0
		Type 1A	1 (100%)	0 (0%)	0 (0%)
	MV hypoplasia	Total	24	4	8
		Type 1A	12 (50%)	3 (75%)	4 (50%)
		Type 1B	6 (25%)	0 (0%)	2 (25%)
		Type 2A	2 (8%)	1 (25%)	0 (0%)
		Type 2B	1 (4%)	0 (0%)	1 (12.5%)
		Type 3A	3 (13%)	0 (0%)	1 (12.5%)
	MV dysplasia	Total	5	0	0
		Type 1A	4 (80%)	0 (0%)	0 (0%)
		Type 2B	1 (20%)	0 (0%)	0 (0%)
	MV atresia	Total	1	0	0
		Type 1A	1 (100%)	0 (0%)	0 (0%)

Table 2. Continued

		BAV morphology	n (%)	RCA above ST	LCA above ST
Outflow defects and LV hypoplasia	Perimembranous VSD	Total	22	8	11
		Type 1A	10 (45%)	6 (75%)	6 (55%)
		Type 1B	4 (18%)	2 (25%)	3 (27%)
		Type 2A	5 (22%)	0 (0%)	2 (18%)
		Type 2B	1 (5%)	0 (0%)	0 (0%)
		Type 3A	1 (5%)	0 (0%)	0 (0%)
		Type 3B	1 (5%)	0 (0%)	0 (0%)
	TGA	Total	2	0	0
		Type 1A	1 (50%)	0 (0%)	0 (0%)
		Type 3A	1 (50%)	0 (0%)	0 (0%)
	DORV	Total	6	1	2
		Type 1A	4 (66%)	1 (100%)	0 (0%)
		Type 1B	1 (17%)	0 (0%)	1 (50%)
		Type 2A	1 (17%)	0 (0%)	1 (50%)
	Hypoplastic LV	Total	40	6	14
		Type 1A	24 (60%)	6 (100%)	8 (57%)
		Type 1B	7 (17%)	0 (0%)	3 (22%)
		Type 2A	3 (7.5%)	0 (0%)	1 (7%)
		Type 2B	2 (5%)	0 (0%)	1 (7%)
		Type 3A	3 (7.5%)	0 (0%)	1 (7%)
		Type 3B	1 (3%)	0 (0%)	0 (0%)
	Aortic arch hypoplasia	Total	13	6	8
		Type 1A	7 (54%)	5 (83%)	5 (63%)
		Type 1B	2 (15%)	0 (0%)	1 (12%)
		Type 2A	3 (23%)	0 (0%)	2 (25%)
		Type 3A	1 (8%)	1 (17%)	0 (0%)
	Aortic coarctation	Total	33	4	8
		Type 1A	21 (64%)	4 (100%)	2 (25%)
		Type 1B	8 (24%)	0 (0%)	4 (50%)
		Type 2A	1 (3%)	0 (0%)	1 (12.5%)
		Type 3A	3 (9%)	0 (0%)	1 (12.5%)

RCA = right coronary artery; LCA = left coronary artery; PLSCV = persistent left superior caval vein; VSD = ventricular septal defect; ASD = atrial septal defect; AVSD = atrioventricular septal defect; AV valve = atrioventricular valve; TV = tricuspid valve; MV = mitral valve; TGA = transposition of the great arteries; DORV = double outlet right ventricle. ^a The number of observed cases that could be assessed for this particular abnormality

Table 3. Coronary height in different BAV subtypes

	RCA Above ST junction	p	LCA Above ST junction	p
Type A	17/58 (29%)		19/59 (32%)	
Type B	2/25 (8%)	0.03	12/25 (48%)	NS
Type 1	17/60 (28%)		23/61 (38%)	
Type 2	1/14 (7%)		6/14 (43%)	
Type 3	1/9 (11%)	NS	2/9 (22%)	NS
Type 1A	15/44 (34%)		14/45 (31%)	
Type 1B	2/16 (13%)	0.09	9/16 (56%)	0.07

RCA = right coronary artery; LCA = left coronary artery; ST = sinotubular

Subgroup analyses

As the included specimens comprise a heterogeneous group of associated congenital anomalies, subgroup analyses of valve morphology and coronary anatomy was performed, separating specimens with LV hypoplasia and outflow tract malformations from specimens with inflow tract anomalies and septal defects. Given their developmental origin, hearts with perimembranous ventricular septal defect (VSDs) were clustered among the outflow tract defects²⁶ (Table 2).

Left ventricular hypoplasia

In 40/82 hearts (49%, Table 1) LV hypoplasia was present, in 2 hearts the LV could not be evaluated. BAV morphology in this group was predominantly type 1A (24/40, 60%). Figure 3e,f shows HLW and a type 1A BAV. In 19/40 (48%) hearts, HLW was associated with aortic coarctation and in 9/40 (23%) with aortic hypoplasia (Table 4).

In 12/40 (30%) hearts, HLW was not associated with aortic arch anomalies. High coronary take-off was observed in 18/40 (45%) specimens with HLW, more frequently of the LCA (n=14) than the RCA (n=6, Table 4). Almost all specimens with HLW and one or more high take-off coronaries (16/18, 89%) had an abnormal aortic arch. All specimens with high take-off RCA had type 1A BAV (Table 2 and 4). The majority of specimens with high take-off LCA also had type 1A BAV, but the percentage was lower (57%) (Table 2).

Table 4. Arch anomalies and height of coronary arteries in LV hypoplasia

	Aortic Arch	AV type	LV hypoplasia	MV	RCA, above ST junction	LCA, above ST junction
1	normal	2B	+	dysplasia	-	-
2	coarctation	1A	+	nd	-	+
3	normal	1A	+	nd	-	-
4	normal	1A	+	normal	-	-
5	normal	2A	+	normal	-	-
6	hypoplasia	1B	+	hypoplasia	-	-
7	coarctation	1B	+	hypoplasia	-	+
8	coarctation	1B	+	hypoplasia	-	-
9	hypoplasia	1A	+	nd	+	-
10	coarctation	1A	+	hypoplasia	-	+
11	hypoplasia	1A	+	hypoplasia	-	-
12	coarctation	1B	+	hypoplasia	-	-
13	coarctation	3A	+	hypoplasia	-	-
14	coarctation	3A	+	hypoplasia	-	+

Table 4. Continued

	Aortic Arch	AV type	LV hypoplasia	MV	RCA, above ST junction	LCA, above ST junction
15	nd	1A	+	hypoplasia	-	-
16	coarctation	1A	+	hypoplasia	+	-
17	hypoplasia	1A	+	hypoplasia	+	+
18	coarctation	1A	+	hypoplasia	-	-
19	hypoplasia	1A	+	hypoplasia	-	+
20	hypoplasia	1B	+	nd	-	+
21	coarctation	3A	+	nd	-	-
22	hypoplasia	1A	+	nd	+	+
23	coarctation	1A	+	nd	-	-
24	coarctation	1A	+	hypoplasia	+	-
25	normal	2B	+	hypoplasia	-	+
26	hypoplasia	2A	+	hypoplasia	-	-
27	coarctation	1B	+	hypoplasia	-	-
28	coarctation	1A	+	hypoplasia	-	+
29	coarctation	1A	+	atresia	-	-
30	coarctation	1A	+	normal	-	-
31	nd	1A	+	left AV	-	-
32	hypoplasia	2A	+	right AV	-	+
33	coarctation	1A	+	dysplasia	-	-
34	interrupted	1A	+	nd	-	+
35	nd	1A	+	nd	+	-
36	normal	3B	+	nd	-	-
37	coarctation	1A	+	nd	-	-
38	interrupted	1A	+	normal	-	+
39	coarctation	1B	+	nd	-	+
40	normal	1A	+	normal	na	-

Outflow tract defects

In a total of 77 specimens in which the aortic arch could be evaluated, 13 (17%) showed aortic arch hypoplasia (9 in association with HLV) (**Table 1 and 2**). BAV morphology in this group was also predominantly type 1A. Again, a high take-off LCA was observed most frequently: 6/13 (46%) had high take-off RCA and 8/13 (62%) high take-off LCA. Specimens with aortic coarctation (33/77 (43%), 19 of which associated with HLV) showed high take-off RCA in 4/33 (12%) and high take-off LCA in 8/33 (24%) cases

(Table 2). High take-off was observed in none of the 2 specimens with transposition of the great arteries and 3 times in 6 hearts with double outlet right ventricle. Most of the hearts with an outflow tract defect and high take-off coronary arteries also had type 1A BAV (Table 2). Although a high take-off RCA was more frequent, especially high take-off RCA was correlated with type 1A BAV (Table 2).

Also in the group with perimembranous VSD subtype 1A was dominant. In these specimens (n = 22) 19 times a high take-off coronary artery was observed (8 RCA, 11 LCA). Most of the hearts with high take-off coronaries in this group had type 1A BAV (Table 2). Again, there was a correlation of high take-off RCA with type 1A BAV: 6/8 (75%) of the RCA with high take-off had type 1A BAV (Table 2).

Inflow and septal defects

In the group with muscular VSD (n=20), 11 high take off coronaries were seen (3 RCA, 8 LCA). Twelve out of these 20 specimens had type 1A BAV, in these 6 high take off coronary arteries were seen (Table 2). In 11 hearts with ASD, 7 high take-off coronaries were seen, 2/2 (100%) high take-off RCA had type 1A BAV and 2/5 (40%) high take-off LCA had type 1A BAV (Table 2). In mitral hypoplasia and atresia (related to HLIV), the majority showed type 1A BAVs (13/25 (52%) (Table 2).

In general, in both subgroups with inflow and outflow tract defects, high take-off RCA was strongly related to type 1A BAV, whereas a high take-off LCA was more divided over different BAV subtypes (Table 2).

DISCUSSION

To our knowledge, this is the first study that describes morphology of BAV and coronary anatomy in a large post-mortem group of specimens of BAV associated with complex congenital heart disease.

Key findings are 1) High take-off coronary arteries occur frequently in BAV associated with complex CHD; 2) High take-off LCA occurs more frequently than high take-off RCA; this cannot be correlated to BAV morphology 3) High take-off RCA is strongly related to type 1A BAV; 4) The subgroups of LV hypoplasia and outflow tract defects are associated with type 1A BAV and high take-off of both coronary arteries.

Coronary ostia and position in complex BAV

Separate ostia of the LAD and LCX were observed in 5% of specimens, considerably more than in the normal population but slightly less than our previous study in isolated BAV patients (11%). In contrast to that study, where separate ostia were linked to type B

BAVs⁵, in the current study there was no clear correlation to BAV morphology.

In normal hearts, high take-off coronary arteries from well above the ST junction are rare (prevalence <1%^{10, 11}). In isolated BAV, this prevalence was described as high as 11% (RCA) to 29% (LCA)¹². In the current study, we observed an even higher prevalence (37% for LCA and 23% for RCA), possibly due to a more complex study population with associated CHD. Similar to studies in isolated BAV, a predisposition for high take-off LCA was noted as compared to the RCA, possibly because left and right coronary ostia are patterned by different mechanisms. Animal studies indicate a left-right difference in coronary arterial development, as the transcription factor Tbx1, expressed in the outflow tract, promotes development of the left, but not right proximal coronary artery and ostium²¹.

Correct positioning of coronary artery stems depends on proper outflow tract morphogenesis²⁷. During development, the coronary arterial network develops from angiogenic sprouts from the sinus venosus^{18, 19} and will during later development connect to the aorta, influenced by epicardial derived cells²⁸. Given the heterogenous composition of the aortic vascular wall²⁹, it is likely that signaling from other cell populations, including neural crest and second heart field, also play a role. Inhibition of cardiac neural crest and second heart field development results in outflow tract septation/rotation defects, associated with abnormally placed coronary stems on the aorta²⁷.

Although a high take-off LCA was observed more frequently than a high take-off RCA, this was not correlated to BAV morphology. In contrast, RCA ostial height was significantly more often above the ST junction in type A (with raphe) than type B (no raphe) BAVs. A striking finding was that high take-off RCA was in almost all cases associated with type 1A BAV morphology, again indicating a potential right-left difference in coronary development. Whether or not this relates to a differential left-right contribution of epicardial (derived) cells³⁰, necessary for coronary ingrowth, is as yet undetermined.

Coronary anatomy in hypoplastic left heart syndrome (HLHS) and outflow tract anomalies.

HLHS is caused by left sided obstruction and has also been linked to BAV^{31, 32}, although no correlation has been made to BAV subtype. Aortic arch anomalies are associated with HLHS. In the current study HLHS was observed in 49% of hearts, although most specimens did not meet the criteria of HLHS. Aortic arch anomalies, including aortic arch hypoplasia and coarctation, were observed in most of these cases. In the current study the majority of HLHS hearts were associated with high take-off of both coronary arteries and had subtype 1A BAV morphology. Clinical consequence of this is unclear. In HLHS patients the arterial duct is the main source of systemic blood flow and provides

retrograde filling to the coronary arteries³³. High take-off coronary arteries have been associated with acute angulation¹⁶, potentially forming an impediment to this retrograde coronary flow. Whether this is a mechanism contributing to fetal demise in cases with HLIV and the consequences after Norwood procedures, is as yet unknown.

BAV morphology–developmental considerations

Different aortic valve morphologies are considered to have different developmental backgrounds. BAVs with left-right coronary leaflet “fusion” patterns (type 1A BAV) most likely result from abnormal septation of the proximal outflow tract, due to distorted behaviour of neural crest cells³⁴, whereas the right-non-coronary leaflet fusion pattern is considered an abnormality in epithelial-to-mesenchymal transformation³⁴. Indeed, in the current study, the majority of hearts with outflow defects showed type 1A BAV.

Study limitations

This is a descriptive study of a heterogeneous group of post-mortem specimens, comprising different forms of CHD. Many variations of CHD are encountered in clinical practice. This inter-individual variation makes a one-to-one correlation of current findings with clinical practice challenging. As this was a post-mortem group with therefore per definition a detrimental outcome, prevalence rates of cardiovascular abnormalities cannot be applied to the general CHD or BAV population. On the other hand, this is the largest group of post-mortem specimens with BAV associated with complex CHD described to date and different forms of CHD could roughly be clustered in order to compare groups based on a potential common developmental background.

CONCLUSION AND CLINICAL IMPLICATIONS

In this study in post-mortem specimens with BAV and associated complex CHD, prevalence of high take-off coronary arteries was high, especially in the group with outflow tract malformations. A predisposition to type 1A morphology was found, underlining a potential developmental background related to disturbed contribution of cardiac neural crest, as previously suggested based on animal studies³⁴. The clinical significance of high take-off coronary arteries is still debated. Potential harmful effects cannot be excluded as they have been linked to sudden cardiac death and ischemia due to impaired coronary blood flow^{14, 15}. Diagnosing these coronary anomalies is clinically relevant as a high take-off coronary artery can be injured during aortic clamping in cardiac bypass surgery. They can also cause technical difficulties during coronary angiography¹¹. Identifying separate ostia of LAD and LCX is of clinical importance in

using coronary artery perfusion as a means of myocardial protection during surgical aortic valve replacement, and in performing coronary angiography.

In conclusion, in patients with BAV with associated complex CHD -especially patients with HLIV and/or outflow tract defects and type 1A BAV- clinicians should be aware of variations in coronary anatomy like high take-off coronary arteries and separate ostia of LCX and LAD, potentially increasing risk of complications during procedures and affecting outcome. It is unclear whether the current findings in infants with detrimental outcome are representative of the population of surviving adults.

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Supplementary table 1.

Case	Age, weeks	Gender	AV type	PV	PLSCV	VSD		ASD	AVSD	TV	MV	HLV	TGA	DORV	Aortic arch hypoplasia	Aortic coarctation	Height RCA/LCA*	Separate ostia
10114	0	V	2A	T	-	-	-	+	-	D	n	+	-	-	-	-	on/on	-
10282	0	V	1A	T	-	-	-	-	-	n	n	-	-	-	-	-	above/above	-
1039	2	M	1A	T	-	-	+	-	-	Ab	nd	nd	+	-	-	-	on/on	-
10428	1	V	1A	T	-	-	-	-	-	D	At	+	-	+	-	+	on/on	-
11126	2	M	1A	T	+	-	-	-	-	n	H	+	-	-	-	+	on/on	-
11815	1	M	1A	T	-	-	+	-	-	n	H	+	-	-	-	+	on/above	-
1248	40	V	1B	U	-	-	+	-	-	n	nd	-	-	-	-	-	on/above	-
1784	520	M	1A	T	-	-	+	-	-	n	D	-	-	-	-	+	on/on	-
1899	0	V	2A	B	-	+	-	-	-	n	H	+	-	-	+	-	on/on	-
1900	832	V	2A	T	+	+	-	-	-	H	n	-	-	-	-	-	on/on	-
2025	19	V	2A	T	-	+	-	-	-	D	nd	-	-	-	-	-	on/above	-
2061	3	M	1B	T	+	-	-	+	-	n	H	+	-	-	-	+	on/on	-
2599	1	V	1A	T	-	-	+	-	-	n	H	-	-	-	-	+	on/on	-
2624	22	V	1A	T	-	-	+	-	-	n	D	-	-	-	-	-	on/on	-
2802	12	V	1A	T	-	-	+	-	-	n	D	+	-	-	-	+	on/on	-
29	1248	M	1A	T	-	-	-	-	-	n	n	-	-	-	-	+	above/on	-
2936	0	M	1B	T	-	+	-	-	-	n	nd	-	-	-	-	-	above/above	-
3046	2	V	1A	T	-	-	+	-	-	n	nd	+	-	-	-	-	on/above	-
3077	806	V	1B	T	-	-	-	-	-	n	nd	-	-	-	-	+	on/above	+
3090	6	V	1A	T	-	-	+	+	-	nd	H	+	nd	-	+	-	above/above	-
3103	3	M	1A	T	-	-	-	-	-	n	H	+	-	-	-	+	above/on	-
3136	260	M	1B	T	-	-	-	+	-	nd	nd	-	-	-	-	+	on/above	-

Supplementary table 1. Continued

Case	Age, weeks	Gender	AV type	PV	PLSCV	VSD		ASD	AVSD	TV	MV	HLV	TGA	DORV	Aortic arch hypoplasia	Aortic coarctation	Height RCA/LCA*	Separate ostia
						PM	M											
3224	260	V	1A	T	-	+	+	-	-	n	nd	-	-	-	-	-	on/on	-
3250	3	M	1A	T	-	-	-	-	-	n	nd	+	-	-	-	+	on/on	-
3369	4	M	1B	T	-	-	-	-	-	n	H	+	-	-	-	+	on/on	-
3608	104	M	1A	T	-	-	-	-	-	n	nd	-	-	-	-	+	on/on	-
3642	8	M	2A	T	-	-	-	-	-	nd	nd	-	-	-	-	+	on/above	-
3740	12	V	2A	B	+	+	-	+	-	D	nd	-	nd	-	-	-	on/on	-
3829	2	M	1B	T	+	-	+	-	-	n	nd	-	-	-	-	+	on/on	-
3860	676	V	1A	T	-	-	-	-	-	n	nd	-	nd	-	-	+	above/on	-
4064	208	V	1A	T	-	-	+	-	-	n	nd	-	-	-	-	-	on/above	-
4082			1A	T	+	-	-	-	-	nd	nd	-	-	-	-	-	above/on	-
4192	1	M	1A	T	-	-	-	-	-	nd	H	+	-	-	-	+	on/above	-
4325	3	M	1A	T	-	+	-	-	-	n	n	nd	-	-	-	-	above/above	-
4336	0	V	1A	T	-	-	-	-	-	n	n	+	-	-	-	+	on/on	-
449	108	M	1A	T	-	-	-	-	-	n	n	-	-	-	-	+	on/on	-
4771	9	V	2B	B	+	+	-	-	-	n	nd	-	-	-	-	-	on/on	-
4823	34	V	1A atretic		-	AVSD		AVSD	+	Right AV	Left AV	+	-	+	-	-	on/on	+
5010	2	M	1A	T	-	-	-	-	-	nd	H	+	-	-	+	-	on/on	-
5091	15	M	1B	T	-	-	+	-	-	nd	nd	+	-	-	+	-	on/above	-
5097	1	M	1A	T	-	+	-	-	-	n	n	+	-	-	-	-	on/above	-
5100	1	V	1B	T	-	-	-	-	-	nd	H	+	-	-	-	+	on/above	-
5189	2	M	1A	T	-	AVSD		AVSD	+	Right AV	Left AV	+	-	-	+	-	above/on	-

Supplementary table 1. Continued

Case	Age, weeks	Gender	AV type	PV	PLSCV	VSD PM	M	ASD	AVSD	TV	MV	HLV	TGA	DORV	Aortic arch hypoplasia	Aortic coarctation	Height RCA/LCA*	Separate ostia
5251	0	M	1A	U	-	-	-	+	-	D	nd	-	-	-	-	-	above/above	-
5371	2	M	1B	T	-	-	-	-	-	n	nd	+	-	-	-	+	on/above	-
5404	0	M	3A	T	-	-	-	-	-	n	H	+	-	-	-	+	on/on	-
5577	2	M	3A	T	+	-	+	+	-	n	H	+	-	-	-	+	on/above	+
5798	1	M	1A	B	+	+	-	-	-	n	nd	+	-	+	nd	nd	above/on	-
5844	1	V	1B	T	-	+	-	+	-	n	n	-	-	-	-	-	on/above	-
5932		V	1A	B	-	+	-	-	-	H	nd	-	-	-	+	-	above/above	-
5958	7	M	2A	T	-	-	-	-	-	n	H	-	-	-	-	-	above/on	-
5976	3	M	2A	T	-	+	-	-	-	nd	nd	-	-	-	+	-	on/above	-
6012	3	M	1A	T	-	+	-	-	-	n	H	+	-	-	+	-	on/above	-
6149	2	M	1A	T	-	-	+	-	-	n	nd	+	-	-	-	+	on/above	-
6199	208	V	1A	T	-	-	+	-	-	n	H	-	-	-	-	+	on/on	+
6294	2	V	1A	?	-	-	-	-	-	n	H	+	-	-	-	+	above/on	-
6300	8	V	1B	U	-	+	+	-	-	D	nd	-	-	-	-	-	above/on	-
6304	3	M	1A	T	-	-	-	-	-	D	nd	+	-	-	-	+	on/on	-
6314	3	M	1A	T	-	-	-	-	-	nd	nd	-	-	-	-	+	on/on	-
6575	1	M	3A	T	-	+	-	-	-	nd	nd	+	-	-	-	+	on/on	-
6602	3	M	1A	T	+	+	-	+	-	n	H	+	-	+	nd	nd	on/on	-
6813	0	V	1A	T	-	+	-	-	-	n	nd	-	-	-	-	-	above/on	-
6849	10	M	1A	T	+	-	-	+	-	D	H	-	-	-	-	+	on/on	-
6876	0	M	1A	T	-	+	-	-	-	nd	nd	+	-	-	+	-	above/above	-
6905	1	M	1B	T	-	-	-	-	-	D	H	+	-	-	-	+	on/on	-
692	17	M	1A	T	-	-	-	-	-	n	D	-	-	-	-	+	on/on	-

Supplementary table 1. Continued

Case	Age, weeks	Gender	AV type	PV	PLSCV	VSD PM	M	ASD	AVSD	TV	MV	HLV	TGA	DORV	Aortic arch hypoplasia	Aortic coarctation	Height RCA/LCA*	Separate ostia
6946	7	M	2B	T	-	-	-	-	-	nd	D	+	-	-	-	-	on/on	-
6977	8	M	3A	T	-	-	-	-	-	H	H	-	+	-	-	-	on/on	-
7213		M	3B	B	-	-	-	-	-	nd	nd	+	-	-	-	-	on/on	-
7409	2	V	1B	T	-	-	+	-	-	nd	nd	-	-	-	-	-	on/on	-
7418	11	V	1A	T	+	+	-	-	-	n	nd	-	nd	-	+	-	above/above	-
7457	884	M	3B	atretic	-	-	+	-	-	n	n	-	nd	-	-	-	on/on	-
747	47	V	3A	T	-	-	+	-	-	n	n	-	-	-	+	-	above/on	-
7506		M	2B	T	-	-	-	-	-	nd	H	+	nd	-	-	-	on/above	-
7576	2	V	1A	T	-	-	-	+	-	n	nd	+	-	-	-	-	on/on	-
7617	0	V	1A	T	-	-	-	-	-	n	n	+	-	-	-	-	na/on	-
8046	1	V	1B	T	-	-	-	-	-	n	H	+	-	-	+	-	on/on	-
8102	17	M	1B	atretic	-	+	-	-	-	D	H	-	-	+	nd	nd	on/above	-
8112		?	3B	B	+	+	-	-	-	nd	n	-	-	-	-	-	on/on	-
9222		M	2A	T	+	AVSD	AVSD	+	AVSD	Right AV	Left AV	+	-	+	+	-	on/above	-
9430	0	M	2B	T	-	-	-	-	-	n	n	-	-	-	-	-	on/on	-
9470	0	M	3B	B	+	-	-	-	-	n	n	-	-	-	-	-	on/above	-
9489	6	V	2B	T	-	AVSD	AVSD	+	AVSD	Right AV	Left AV	-	-	-	-	-	on/above	-
9913	0	M	1A	T	-	-	-	-	-	D	n	+	-	-	-	-	on/on	-

AV = aortic valve; PV = pulmonary valve; PLSVC = persistent left superior caval vein; VSD PM = perimembranous ventricular septal defect; VSD M = muscular ventricular septal defect; ASD = atrial septal defect; AVSD = atrioventricular septal defect; TV = tricuspid valve; MV = mitral valve; HL = hypoplastic left ventricle; TGA = transposition of the great arteries; DORV = double outlet right ventricle; RCA = right coronary artery; LCA = left coronary artery; T = tricuspid; B = bicuspid; U = unicommissural; D = dysplastic; n = normal; Ab = absent; H = hypoplastic; right AV = right atrioventricular valve; nd = not determined; na = not applicable

* Position of ostium of RCA and LCA in relation to the sinotubular junction

5

Coronary anatomy in Turner syndrome versus patients with isolated bicuspid aortic valves

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ABSTRACT

Objective

Variations in coronary anatomy, like absent left main stem and left dominant coronary system, have been described in Turner Syndrome (TS) patients and in patients with bicuspid aortic valves (BAV). It is unknown whether coronary variations in TS are related to BAV and to specific BAV subtypes.

Aim

To compare coronary anatomy in TS patients with/without BAV versus isolated BAV and to study BAV morphology subtypes in these groups.

Methods

Coronary anatomy and BAV morphology was studied in 86 TS patients (20 TS-BAV, 66 TS-TAV) and 86 isolated BAV patients (37 ± 13 years vs. 42 ± 15 years respectively) by computed tomography.

Results

There was no significant difference in coronary dominance between TS patients with and without BAV (25% vs 21%, $p=0.933$). BAVs with fusion of right and left coronary leaflets (RL BAV) without raphe showed a high prevalence of left coronary dominance in both TS-BAV and isolated BAV (both 38%). Absent left main stem was more often seen in TS-BAV as compared to isolated BAV (10% vs 0%). All TS-BAV patients with absent left main stem had RL BAV without raphe.

Conclusion

The equal distribution of left dominance in RL BAV without raphe in TS-BAV and isolated BAV suggests that presence of left dominance is a feature of BAVs without raphe, independent of TS. Both TS and RL BAV without raphe seem independently associated with absent left main stems. Awareness of the higher incidence of particularly absent left main stems is important to avoid complications during hypothermic perfusion.

INTRODUCTION

Turner Syndrome (TS) is caused by complete or partial monosomy of the X chromosome in all or part of the cells and affects one in 2500 females¹. The mortality rate in patients with TS is around three-times higher than in the general female population, mainly as a result of cardiovascular disorders². This includes coronary artery disease³, the presence of a bicuspid aortic valve (BAV, 12-30%), coarctation of the aorta (CoA, 7-18%), partial anomalous pulmonary venous return (1-25%)^{4,5,6} and hypoplastic left heart syndrome. Also coronary anomalies are reported frequently in patients with TS (20% versus 5% in the general population), although data are scarce⁷. These coronary anomalies mainly seem to involve the left coronary artery system, with immediate bifurcation of the two major branches of the left coronary artery (LCA) (i.e. absent main left coronary stem) being the most common anomaly reported with an incidence of 14%⁷. A higher prevalence of left coronary dominance has also been noted in patients with TS (28% vs. 20% in healthy controls)⁷. Furthermore, the prevalence of BAV is common in TS, described in 12-30% of TS patients^{5,8,9}, versus 1-2% in the general population^{10,11}.

BAV is the most common congenital cardiac abnormality and variations in coronary anatomy, such as an absent main left coronary stem¹² have been described. The incidence of a left dominant coronary artery system in patients with BAV is reported to be 25-29%^{13,14}, much higher than the reported incidence of 10-12% in the general population with tricuspid aortic valves^{15,16}. Interestingly, a left dominant coronary artery system as well as immediate bifurcation of the LCA appears to be associated to the left-right fusion BAV without a raphe (i.e strictly bicuspid)¹⁷.

Thus, patients with TS are reported to have an increased prevalence of coronary anomalies, increased left coronary dominance and increased prevalence of BAV, but data on these issues are limited. Furthermore, little is known about the relation between coronary anomalies, left coronary dominance and BAV and the various morphologies of BAV in TS patients. In addition, the relation with the BAV associated aortic disease (mainly aortic coarctation) is unclear. The current study will focus on these issues in TS patients in comparison to patients with isolated BAV. The aims of the current study are: 1) To investigate coronary anatomy in TS patients; 2) To study coronary anatomy in TS patients in relation to the presence of BAV and BAV morphology subtypes; 3) To compare coronary anatomy in Turner patients with BAV versus isolated BAV; 4) To investigate the relation with aortic pathology, mainly CoA, in Turner patients, versus patients with isolated BAV and 5) To evaluate the potential role of gender on coronary anatomy in the different groups.

METHODS

Study population

This is a multicentre study performed in three different academic medical centers in The Netherlands. The study population consisted of 89 patients with Turner Syndrome and 89 non-Turner syndrome patients with isolated BAV, who were regularly visiting the outpatient clinics of the Erasmus Medical Center, the Radboud University Nijmegen Medical Centre or Leiden University Medical Center, The Netherlands.

The inclusion criteria were: Age ≥ 18 years and no prior aortic valve surgery for all patients. In addition, genetic confirmation of chromosome XO was an inclusion criterion for Turner syndrome patients. Patients with non-determinable aortic valve morphology were excluded from analysis, leaving a total number of 172 patients included in the study. In included patients, multi-phase CT scanning was performed to assess aortic valve morphology and coronary artery anatomy. The study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO). The medical ethical committee of the participating centers approved the study (P15.167). All patients provided informed consent in writing. The current study is conducted by the research consortium “Unravelling etiology and risk factors in patients with Bicuspid Aortic valves”, conducted by Erasmus Medical Center; Rotterdam, the Radboud University Nijmegen Medical Center; Nijmegen and Leiden University Medical Center; Leiden, the Netherlands.

Computed tomography coronary angiography data acquisition and image analysis

ECG-triggered multi-phase computed tomography scanning was performed in the three centers according to a standardized imaging protocol with local adjustments (Erasmus Medical Center: Siemens Medical Systems, Germany; Radboud University Nijmegen Medical Center: Toshiba Medical Systems; Leiden University Medical Center: Toshiba Medical Systems, Otawara, Japan). Dose modulated ECG-pulsing was employed. Pitch was adjusted to the heart rate and if necessary beta blockers were administered prior to the scan. Iodinated contrast material was administered through an antecubital vein followed by a mixture of contrast and saline. Imaging started using bolus tracking in the aorta. Images were reconstructed at 1.0 mm with a 0.4 mm interval. Aortic valve morphology was evaluated and identified as **RL** with “fusion” of right and left coronary leaflets; **RN** with right and non-coronary fusion and as **LN** with left and non-coronary fusion. In addition to this, presence or absence of a raphe was described (**Figure 1**).

Coronary anatomy and coronary vessel dominance were assessed in a standardized manner by dividing the coronary artery into 17 segments according to the guidelines of the American Heart Association¹⁸. The coronary artery system was classified as right

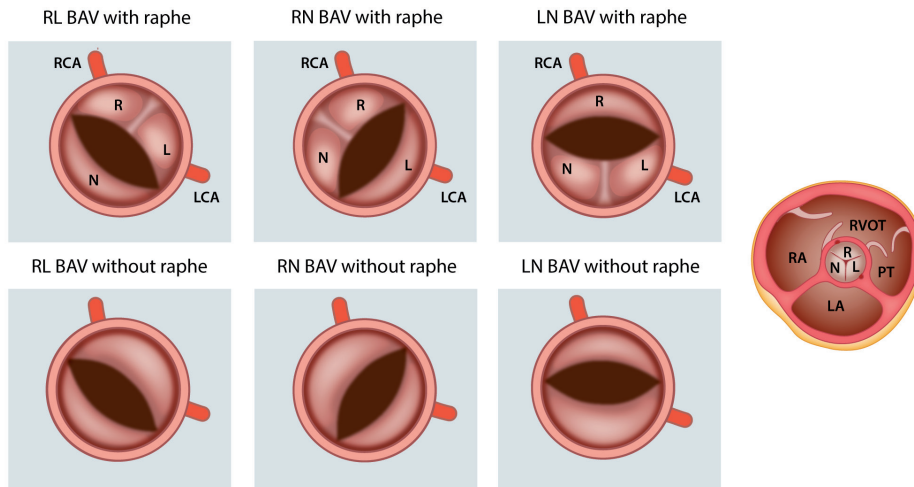


Figure 1. Schematic overview of different BAV morphologies.

Upper panel: BAVs with a raphe; top left: BAV with fusion of right and left (RL) coronary leaflets; middle: BAV with fusion of right and non-coronary (RN) leaflets, right; BAV with fusion of left and non-coronary (LN) leaflets. Lower panel: Strictly bicuspid valves (without a raphe). Leaflet size and symmetry, as well as the position of the commissures may vary from the schematic overview depicted here.

Abbreviations: L: left coronary leaflet; R: right coronary leaflet; N: non-coronary leaflet; Modified after Schaefer et al²⁰.

dominant when the posterior descending artery (PDA) originated from the right coronary artery (RCA) and considered left dominant if the PDA originated from the left circumflex artery (LCX). The coronary artery system was classified as balanced, when the PDA originated from the RCA in combination with a large posterolateral branch originating from the LCX, reaching to or close to the posterior interventricular groove. Subsequently, the presence of a left main stem and localization of the coronary ostia were assessed. If there was no left main stem, the LCA was designated as having separate ostia of the LAD and LCX. A selection of representative CT scans was 3D reconstructed using the AMIRA v4.0 Software Package (Template Graphics Software, San Diego, CA, USA).

Statistical analysis

Continuous variables are presented as mean $\pm$ standard deviation or as median and interquartile range. Categorical variables are presented as numbers and percentages. Cross-tabulations were made for binary categorical data, on which chi-square goodness-of-fit-tests were performed to test for independence. Statistical analysis was performed using SPSS software (version 23.0, SPSS, Inc., Chicago, IL, USA). A P-value <0.05 , by a two-sided test, was considered statistically significant.

RESULTS

Patient characteristics

The study population comprised 172 patients with a mean age of 39 ± 14 years, 65 patients (38%) were male, 107 female (62%). Eighty-six patients (50%) had Turner syndrome, inherently all female. Out of 172 patients, 106 (62%) had a bicuspid aortic valve, of which 20 patients with TS (**Figure 2**). Baseline patient characteristics are depicted in **Table 1**.

Table 1. Patient characteristics

	Total (n = 172)	Isolated BAV (n = 86)	TS-BAV (n = 20)	TS-TAV (n = 66)
Age (years)	39 ± 14	42 ± 15	37 ± 12	37 ± 13
Gender (male)	65 (38%)	65 (76%)	0 (0%)	0 (0%)
CoA	20 (12%)	11 (13%)	5 (25%)	4 (6%)
Length (cm)	168 ± 15	180 ± 11	154 ± 7	156 ± 9
Weight (kg)	71 ± 16	79 ± 14	57 ± 10	65 ± 16

Abbreviations: BAV = bicuspid aortic valve; TS-BAV = Turner Syndrome and bicuspid aortic valve; TS-TAV = Turner Syndrome and tricuspid aortic valve; CoA = coarctation of the aorta

BAV morphology

BAV morphology was studied in patients with TS and BAV (TS-BAV) and in patients with isolated BAV.

Turner syndrome and BAV

In the group of patients with TS-BAV (n=20), 11 (55%) had a BAV with raphe versus 70% in the isolated BAV group. In 9/20 patients (45%), the raphe was located between the left and right coronary cusp (RL BAV). In 2 patients (10%) the raphe was located between the right and non-coronary cusp (RN BAV). BAVs without raphe were observed in 9/20 patients (45%, as compared to 30% in the isolated BAV group), 8 patients had a RL BAV, 1 patient a RN BAV (**Figure 2**).

Isolated BAV

In the group of patients with isolated BAV, the majority of patients (60/86 (70%)) had a BAV with a raphe. In 43/86 patients (50%), a RL BAV with raphe was present, in accordance with the prevalence in previous studies in isolated BAV patients[17,19,20]. In 17/86 patients (20%), a RN BAV with a raphe was present. In 26/86 patients (30%), no raphe was identified. In this group of strictly bicuspid aortic valves, 13 patients had RL BAV, 10 a RN BAV and 3 a LN BAV.

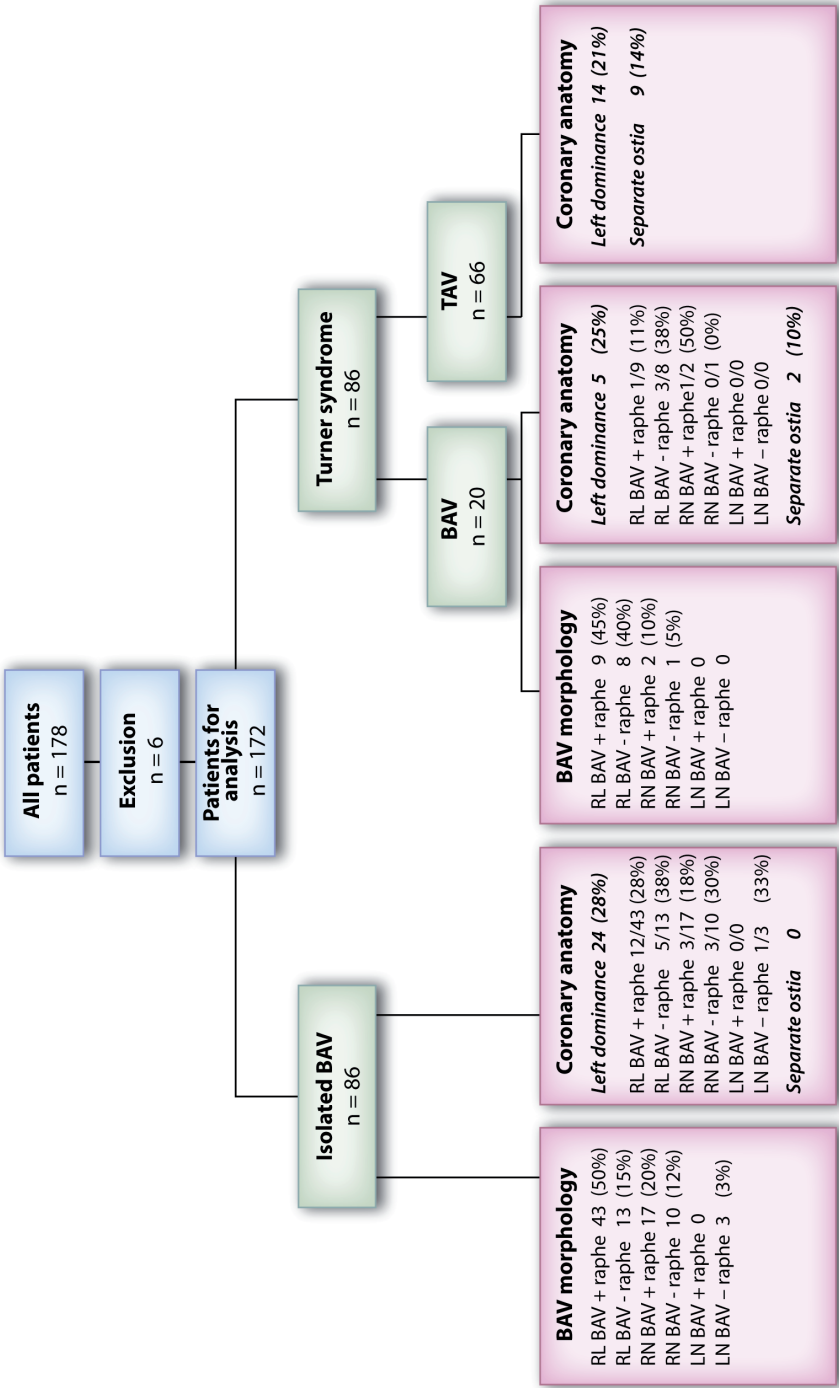


Figure 2. Flowchart indicating the study groups and morphological outcomes

Coronary anatomy

Of all 172 patients analysed, 43 (25%) had a left dominant coronary artery system and 122 patients (71%) had a right dominant coronary artery system, in 1 patient there was a balanced coronary artery system and in 6 patients dominance was not clear. Separate ostia of the left coronary artery (i.e. no left main stem) were seen in 11 patients (6%).

Coronary anatomy in Turner patients with and without BAV

There was no significant difference in presence of left dominant coronary artery systems between TS-BAV patients and TS patients with a tricuspid aortic valve (25% vs. 21%, $p = 0.933$, **Table 2a**). In addition, no significant differences were noted in the occurrence of separate ostia between patients TS-BAV and TS-TAV patients (10% vs 14%, $p = 0.655$, **Table 2a**).

Table 2a. BAV morphology subtypes in relation to coronary anatomy; TS-BAV vs. TS-TA

	TS-BAV	TS-TAV	P
Left dominance	5/20 (25%)	14/66 (21%)	0.933
Type 1A	1/9 (11%)		
Type 1B	3/8 (38%)		
Type 2A	1/2 (50%)		
Type 2B	0		
Type 3B	0		
Separate ostia	2/20 (10%)	9/66 (14%)	0.655
Type 1B	2/2 (100%)		

Table 2b. BAV morphology subtypes in relation to coronary anatomy; Isolated BAV vs. TS-BAV

	TS-BAV	Isolated BAV	P
Left dominance	5/20 (25%)	24/86 (28%)	0.517
Type 1A	1/9 (11%)	12/43 (28%)	
Type 1B	3/8 (38%)	5/13 (38%)	
Type 2A	1/2 (50%)	3/17 (18%)	
Type 2B	0	3/10 (30%)	
Type 3B	0	1/3 (33%)	
Separate ostia	2/20 (10%)	0	< 0.05
Type 1B	2/2 (100%)		

Abbreviations: BAV = bicuspid aortic valve; TS-BAV = Turner Syndrome and bicuspid aortic valve; TS-TAV = Turner Syndrome and tricuspid aortic valve

Coronary anatomy in Turner patients with BAV versus isolated BAV patients

There was no significant difference in prevalence of left dominant coronary artery systems between patients with isolated BAV and patients with TS and BAV (28% vs. 25%, $p = 0.517$, **Table 2b**). Separate ostia were more often observed in patients with TS and BAV as compared to patients with isolated BAV, although numbers were low (2 vs. 0 patients resp., **Table 2b**).

BAV morphology subtypes in relation to coronary anatomy

BAV morphology in patients with TS-BAV and patients with isolated BAV is summarized in **Table 2b**. In both groups, RL BAVs without raphe showed a relatively high percentage of left dominant coronary artery systems (38% in both groups, **Table 2b**), as compared to the general population. In the TS-BAV group, both patients with separate ostia had a RL BAV without raphe (**Table 2b**). Separate ostia were not observed in the group with isolated BAV in this cohort.

Aortic pathology in Turner patients versus patients with isolated BAV

Nineteen patients out of 172 (11%) had CoA. Of these 19 patients, 6 (32%) showed a left dominant coronary artery system. No significant difference in occurrence of left coronary dominance was found between patients with CoA and patients without CoA (32% vs. 25%). In addition, there was no difference in the presence of left dominance between patients with CoA and TS and those without TS (33% vs 30%). A representative example of a TS patient with CoA and RL BAV without a raphe is shown in **Figure 3**.

Role of gender

Finally, the role of gender was evaluated. Naturally, all patients in the Turner group were female. In the group with isolated BAV 65 patients (76%) were male, 21 patients (24%) were female. 20/65 (31%) males in this isolated BAV group had a left dominant coronary artery system. Of the 21 females with isolated BAV, 4 (19%) showed left dominance as compared to 25% in the TS-BAV group and 21% in the TS-TAV group. In summary, there was no significant difference in presence of left dominance between females in the different groups.

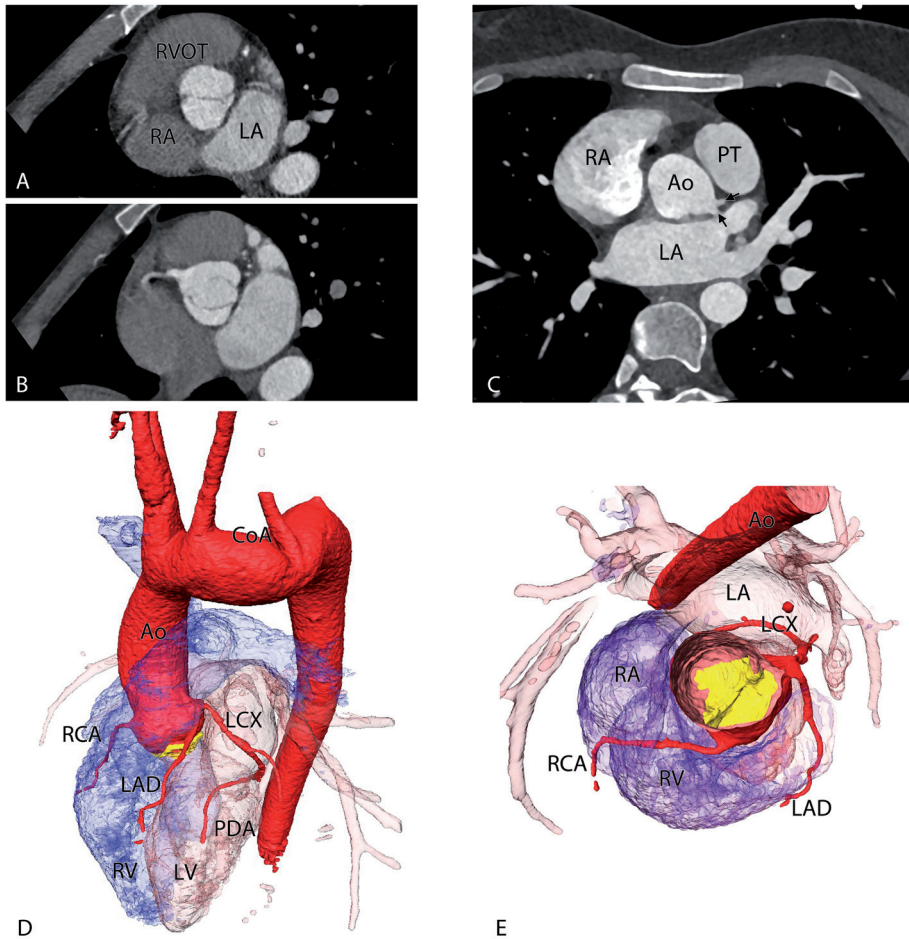


Figure 3. Example of type 1B BAV, closed (A) and open (B), with separate ostia (arrows) of the LAD and LCX (C).

D, E: 3D reconstruction of a heart with type 1B BAV, aortic coarctation (CoA), a left dominant coronary artery system and separate ostia of the LAD and LCX. The aortic valve is indicated in yellow.

Ao: aorta LA: left atrium, LV: left ventricle, PT: pulmonary trunk, RA: right atrium, RV: right ventricle

DISCUSSION

The key findings of this study were

1. No significant differences in coronary anatomy with regard to coronary dominance or absence of left main stem, were found between TS patients with and without BAV. In addition, no significant difference in prevalence of left dominant systems between BAV patients with versus without Turner syndrome were observed.
2. Patients with RL BAVs without raphe showed a high prevalence of left coronary dominance in the group of TS-BAV as well as in isolated BAV.
3. Separate ostia were more often seen in patients with TS and BAV as compared to patients with isolated BAV. No significant differences in the occurrence of separate ostia between TS-BAV and TS-TAV patients were observed.
4. There was no significant difference in occurrence of left coronary dominance between patients with CoA and without CoA and between patients with CoA and TS and those without TS.

Coronary anatomy - Dominance

As previously described, there is an association between BAV and the presence of a left dominant coronary artery system¹⁷. Whether this also applies to BAV in TS, was to date unknown. One previous study described a higher prevalence of left dominant coronary artery systems in TS, however this was not specified to aortic valve morphology (BAV versus TAV)⁷. The current study shows that patients with TS and BAV do not show significantly more left dominant coronary artery systems as compared to patients with TS with TAV (25% vs. 21%). The higher prevalence of left dominance in TS therefore does not seem to be attributed to the presence of BAV, suggesting that the presence of TS may be an independent factor associated with left coronary artery dominance, regardless of valve morphology. If there was a cumulative effect of TS and BAV, we would have expected the group of TS-BAV to have a much higher prevalence of left dominance, but this was not the case (25% vs. 21%).

In general, isolated BAV patients showed more left dominant coronary artery systems (28%) than reported in the general population. This is in accordance with our previous study, in which we found a higher prevalence of left dominance in RL BAV without raphe in isolated BAV patients¹⁷, and it is in line with the literature^{16,17}. Identical results were obtained in the current study, for both TS-BAV and isolated BAV patients. The equal percentages of this distribution in both TS and isolated BAV groups, suggests that the presence of left dominance is a feature of BAVs without raphe and not affected by the presence of TS. In summary, both TS and RL BAV without raphe seem to be independently associated with left coronary dominance.

Coronary anatomy – Separate ostia

Separate ostia or absent left main trunk has been described in 14% of patients with TS, compared to less than one percent in the general population, which was not specified to aortic valve morphology^{7,21}. The current study shows comparable results, showing separate left sided coronary ostia in 13% of patients with TS. No significant differences in absence of main stem with regard to valve morphology (BAV or TAV) were found. Remarkably, in the current study separate ostia were not observed in patients with isolated BAV, whereas separate ostia were previously observed in isolated BAV patients in 11-15%^{12,17}. Within the TS-BAV group, both of the separate coronary ostia occurred in RL BAV without raphe, which corresponds to previous data in isolated BAV¹⁷. Therefore, we postulate that, as observed in the analysis of coronary dominance, both TS and RL BAV without raphe are independently associated with absent left main stems. The fact that RL BAVs without raphe often showed a left dominant coronary artery system and separate ostia of the LAD and LCX, may be explained by molecular mechanisms guiding the ingrowth of coronary arteries^{22,23}. It is tempting to speculate that in BAV patients, where strict bordering of the cusps is hampered, variations in coronary anatomy occur more often. This might be more outspoken in patients with strictly bicuspid valves, where a raphe is missing and no valvular commissures can be determined.

Coarctation of the aorta

TS is associated with aortic pathology, especially CoA and (tubular) hypoplasia^{24,25}. In contrast to previous results in isolated BAV¹⁷, TS patients with CoA did not show more left dominant coronary artery systems as compared to patients without CoA. In addition, there was no difference in the presence of left dominance between patients with CoA and TS and those without TS.

Role of gender

The role of gender in distribution and outcome of heart disease has received more attention in recent years. Gender differences play a role in coronary artery disease (CAD). Cardiovascular risk factors are similar among women and men, but it is only the male sex that comprises a risk factor for CAD. In young women, the first symptoms of CAD develop years later than in man, related to the benefit of the protective effect of estrogens³. Previous studies have reported a higher incidence of CAD (obstructive and non-obstructive) in patients with left dominant coronary anatomy^{26,27}. In addition, patients with RL BAVs without raphe have been reported to be more at risk of developing significant coronary artery disease¹⁷. BAV is almost 3 times more common in males than females¹⁰, which might be related to the increased risk of males for coronary artery disease, although the mechanism of such a relation is to date unresolved¹⁷. Patients with

Turner syndrome are more at risk of CAD as well, probably due to the lack of estrogens, but possibly also due to the fact that there's more left dominance in patients with TS. Whether or not this is related to strictly bicuspid (no raphe), is yet to be explored.

CONCLUSIONS AND CLINICAL RELEVANCE

This study shows that there are no differences in coronary artery dominance between TS patients with and without BAV and between patients with TS-BAV and isolated BAV. Patients with RL BAVs without raphe show a high prevalence of left coronary dominance in the group of TS-BAV as well as in isolated BAV. The equal distribution in both TS and isolated BAV groups, suggests that both TS and RL BAV without raphe are independently associated with left coronary dominance.

Patients with TS-BAV show more separate ostia of the LAD and LCX as compared to patients with isolated BAV. In addition, separate ostia were more often observed in TS patients with RL BAV without raphe, suggesting that both TS and RL BAV without raphe are independently associated with absent left main stems.

Patients with TS are more prone to develop CAD at a younger age, due to lack of estrogens. The presence of a left dominant coronary artery system and CoA, exposing to early hypertension, carry an additional risk of premature coronary artery disease. In case of the need for diagnostic coronary angiography or surgery, it is important to be aware of the higher incidence of particularly separate left coronary ostia, to avoid complications during hypothermic perfusion.

Study limitations

This study was conducted in a relatively small group of patients, bearing the risk of overestimation of results. Although we found an association of BAV subtypes with some variations in coronary anatomy in this descriptive study, this does not mean that there is a causal relationship. However, as limited data are currently present on coronary anatomy in Turner syndrome patients in different BAV morphologies, the current study adds insight into this issue with respect to the general, non-syndromal, population and could raise awareness of potential variations in coronary anatomy in different subgroups of BAV patients.

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6

Pulmonary valve morphology in patients with bicuspid aortic valves

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ABSTRACT

Background

The aortic and pulmonary valve share a common developmental origin from the embryonic arterial trunk. Bicuspid aortic valve is the most common congenital anomaly and can occur isolated as well as in association with other congenital heart disease (CHD). Data on pulmonary valve morphology in these cases is scarce. In this study we aimed to determine pulmonary valve morphology in hearts with BAV associated with CHD.

Methods

In 83 post-mortem heart specimens with BAV and associated CHD, pulmonary valve morphology was studied and related to BAV morphology.

Results

In 14/83 (17%) hearts the pulmonary valve was affected, bicuspid in 8/83 (10%), dome-shaped in 3/83 (4%) and atretic in 3/83 (4%). In specimens with a bicuspid pulmonary valve, 5/8 (63%) had a strictly bicuspid aortic valve (without raphe), 2/3 hearts (67%) with dome-shaped pulmonary valves and 2/3 hearts (67%) with atretic pulmonary valves had BAV without raphe. Six out of 8 (75%) specimens with a bicuspid pulmonary valve had a perimembranous ventricular septal defect (VSD). 4/8 (50%) specimens with a bicuspid pulmonary valve were associated with chromosomal abnormalities: 3 (38%) had trisomy 18, 1 (13%) had trisomy 13.

Conclusion

In BAV with associated CHD, abnormal pulmonary valve morphology was observed in 17% of the hearts. The majority of hearts with abnormal pulmonary valve morphology had a type B bicuspid aortic valve (without raphe). Bilateral semilunar valvular disease is associated with type B BAVs and in many cases related to chromosomal abnormalities. As this study was performed in post-mortem specimens with high frequency of associated CHD, caution is warranted with application of these results to the general BAV population.

INTRODUCTION

Bicuspid aortic valve (BAV) is the most common congenital anomaly, occurring in 1–2% of the general population¹, whereas bicuspid pulmonary valves are rare (incidence of 0.1%)². The aortic valve and the pulmonary valve arise from a common arterial trunk, which is initially unseparated. During development, separation at the level of the outflow tract, valves and great vessels needs to occur. Neural crest cells initiate the aorto-pulmonary septum formation, after which a separate aorta and pulmonary valve can be distinguished³.

Bicuspid pulmonary valves are usually associated with other congenital heart disease such as tetralogy of Fallot⁴ or transposition of the great arteries⁵. Bilateral bicuspid semilunar valves are rare and diagnosed mainly during surgery⁶ or post-mortem². One study reports a case of bicuspid pulmonary and aortic valves diagnosed by three-dimensional transoesophageal echocardiography⁷. Animal studies report a higher incidence of bilateral bicuspid semilunar valves, up to 4% in the Syrian hamster⁸. One study in human post-mortem hearts reports an incidence of 12%, mostly in hearts with associated congenital heart disease (CHD)⁹. Given the common developmental origin of aorta and pulmonary valves¹⁰, the question arises whether pulmonary valve morphology is affected in specific subgroups of BAV patients. In general, this does not necessarily seem the case. Animal studies have pointed out that bicuspid aortic and pulmonary valves may have a different morphogenetic origin, at least in valves not associated with major malformations of the heart⁸. Studies in humans, especially in hearts with associated CHD, are however scarce^{2, 6, 9} and the relation with BAV morphology (i.e. location and presence of raphe) has not been studied to date.

The aim of the current study is to describe the incidence of abnormal pulmonary valve morphology in post mortem hearts with BAV and associated congenital heart disease (CHD), and to correlate findings with BAV morphology.

METHODS

Study population

This study was performed in accordance with the local ethics committee and Dutch regulations for proper use of human tissue for medical research purposes. Eighty-four postmortem heart-lung specimens with BAV from the Leiden collection of malformed hearts (Department of Anatomy & Embryology, Leiden University Medical Center, Leiden, The Netherlands) were studied macroscopically. These hearts were obtained from autopsies and preserved in an ethanol/glycerin solution, collected from the early 1950s

until now. Two experienced observers investigated the hearts. Cardiac morphology was assessed using sequential segmental analysis¹¹. All cardiovascular anomalies as well as detailed morphology of the aortic valve and pulmonary valve were noted. In some cases cardiovascular anomalies could not be assessed due to incompleteness of the specimens. Coronary anatomy in these hearts was described in a separate paper¹².

Valve classification and terminology

BAV morphology was defined by orientation of leaflets with respect to each other, based on attachment of leaflets (right coronary, left coronary, non-coronary) and presence and position of a raphe. BAV morphology was classified as *Type 1* if right and left coronary leaflets were conjoined; *Type 2* if right and non-coronary leaflets were conjoined and *Type 3* if left and non-coronary leaflet were conjoined. 'A' was added for valves in which a raphe could be recognized or 'B' for strictly bicuspid valves (no raphe) (**Figure 1a**). A raphe was defined as a ridge located in the conjoined area of two leaflets, presumably representing a malformed commissure. Subsequently, pulmonary valve morphology (tricuspid, bicuspid, unicommissural/dome shaped or atretic) was assessed as well as presence or absence of a raphe.

RESULTS

The study group consisted of 84 specimens, 1 specimen was excluded due to non-assessable pulmonary valve morphology. Age at demise ranged from fetal-24 years. Of the 83 patients, 24 died of cardiac causes, for example heart failure or arrhythmia. Twenty-five patients died perioperative or immediately postoperative. Twenty-four patients died of non-cardiac causes, such as chromosomal abnormalities, intracerebral bleeding or pulmonary problems. Of 10 patients cause of death was unknown.

Characteristics of the specimens are listed in table 1.

In 69/83 (83%) specimens (42 male, 26 female, 1 unknown) the pulmonary valve was normal (**Figure 1b**). The majority of the 83 included specimens had associated congenital anomalies (**Table 1**). In 14/83 (17%) hearts the pulmonary valve was affected; the pulmonary valve was bicuspid in 8/83 hearts (10%), dome-shaped in 3/83 hearts (4%) and 3 pulmonary valves were atretic (4%). Examples are shown in **Figure 1d**.

Of the 14 specimens with an affected pulmonary valve, 9 (64%) had a type B (strictly bicuspid) aortic valve (**Figure 1b and Table 2**). For comparison, in the complete study group, 26/84 hearts (31%) had type B BAV.

In the subgroup of specimens with a bicuspid pulmonary valve, 5/8 (63%) also had a strictly bicuspid aortic valve (1 type 1B, 1 type 2B and 3 type 3B, **Figure 1b and Table**

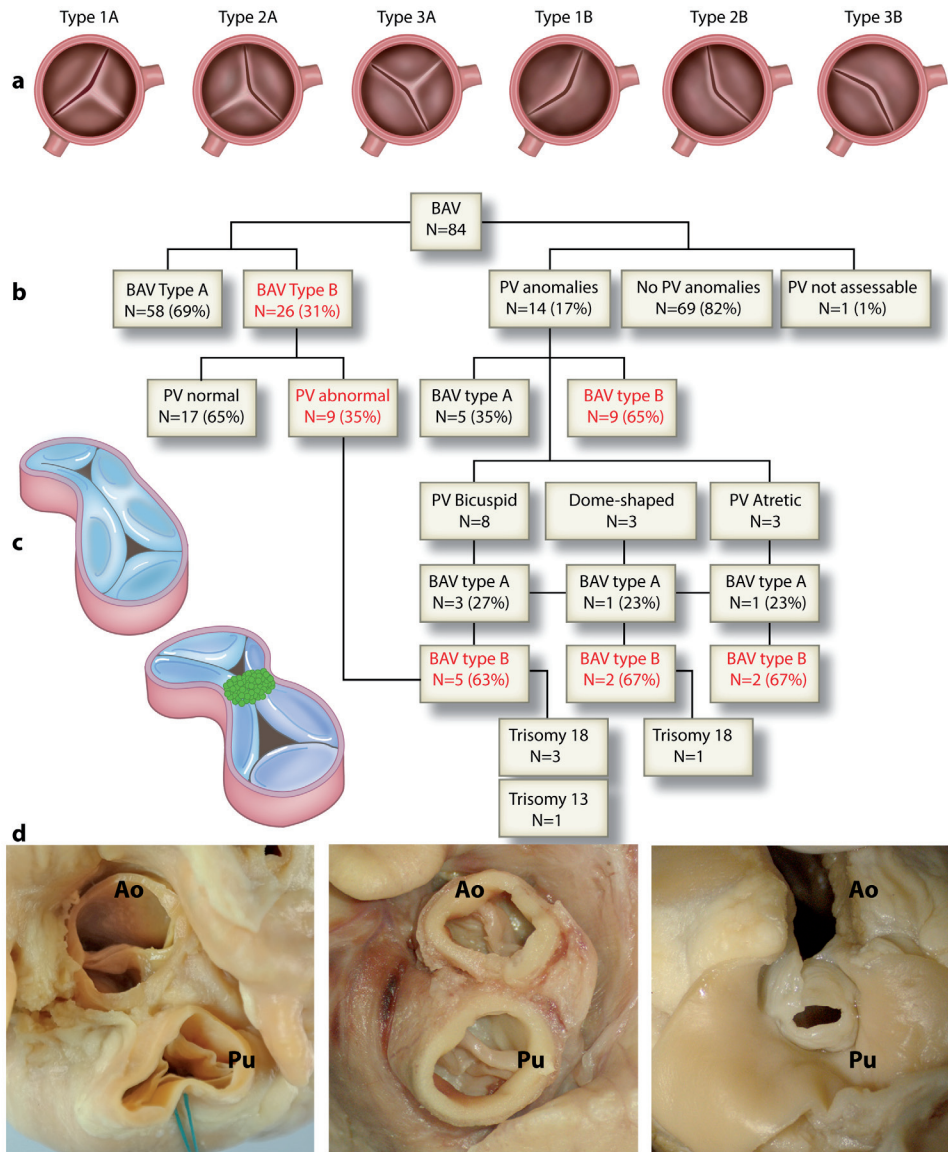


Figure 1. a: Schematic overview of different BAV morphologies (Modified after [17]). **b:** Schematic overview of results in the study population. **c:** Development of semilunar valves. The initially unseparated common arterial trunk contains endocardial cushions (blue), that will be separated, orchestrated by neural crest cells (green dots). **d:** Examples of post-mortem heart specimens with variable pulmonary valve morphology. *Left:* Type 1A BAV and tricuspid pulmonary valve. *Middle:* Type 3B BAV and bicuspid pulmonary valve. No raphe can be determined in the pulmonary valve. *Right:* Type 1B bicuspid aortic valve and dome-shaped pulmonary valve.

2). Of these, 1 pulmonary valve was also strictly bicuspid, the other 4 did have a raphe. In 3/8 (38%) hearts with a bicuspid pulmonary valve, a raphe was present (type A): 1 of these was type 1A BAV and 2 were type 2A (Table 2).

Table 1. Patient characteristics and associated cardiovascular abnormalities

Complete study group		
	n ^a	%
Gender (male:female)	48:35	
Aortic valve morphology		
Type 1A	45/84	54%
Type 2A	9/84	11%
Type 3A	5/84	6%
Type 1B	16/84	19%
Type 2B	5/84	6%
Type 3B	4/84	5%
Pulmonary valve morphology		
Tricuspid	69/83	83%
Dome-shaped	3/83	4%
Bicuspid	8/83	10%
Atresia	3/83	4%
Associated anomalies		
Aortic arch anomalies	46/77	60%
<i>Hypoplasia</i>	13/77	17%
<i>Coarctation</i>	33/77	43%
ASD	11/83	13%
AVSD	4/84	5%
AV valve anomalies		
<i>TV anomalies</i>	15/68	22%
<i>MV anomalies</i>	30/50	60%
DORV	6/83	7%
PLSCV	15/83	18%
TGA	2/74	3%
VSD	42/84	50%
<i>Muscular</i>	20/84	24%
<i>Perimembranous</i>	22/84	26%
Chromosomal abnormalities		
Trisomy 9	1	
Trisomy 13	2	
Trisomy 18	5	

ASD = atrial septal defect; AVSD = atrioventricular septal defect; AV valve = atrioventricular valve; TV = tricuspid valve; MV = mitral valve; DORV = double outlet right ventricle; PLSCV = persistent left superior caval vein; TGA = transposition of the great arteries; VSD = ventricular septal defect; PM VSD = perimembranous VSD.

^aThe number of observed cases that could be assessed for this particular abnormality

Of the 3 hearts with dome-shaped pulmonary valves, 2 (67%) had a type B BAV (1 type 1B and 1 type 3B BAV, **Figure 1b and table 2**), whereas 2/3 hearts (67%) with atretic pulmonary valves had a type B BAV (1 type 1B and 1 type 3B).

In 5/14 specimens (36%), not only the semilunar valves but also the atrioventricular (AV) valves were affected (**Table 2**).

Of the 8 specimens with a bicuspid pulmonary valve, 6 (75%) had a perimembranous ventricular septal defect (VSD).

4/8 (50%) specimens with a bicuspid pulmonary valve were associated with chromosomal abnormalities: 3 (38%) had trisomy 18, 1 (13%) had trisomy 13, these all had a type B BAV.

Table 2. Associated cardiovascular abnormalities in patients with pulmonary valve anomalies

Subgroup with pulmonary valve anomalies (n=14)	AoV morphology	Associated anomalies
Dome-shaped (n=3)	1A	• ASD, TV dysplasia
	1B	• Muscular VSD
	3B	• PLSCV, PM VSD, trisomy 18
Bicuspid (n=8)	1A	• DORV, PM VSD
	1B	• TV hypoplasia, arch hypoplasia, PM VSD, trisomy 18
	2A (n=2)	• PLSCV, ASD, TV dysplasia, PM VSD; MV hypoplasia, arch hypoplasia, PM VSD
	2B	• PLSVC, PM VSD, trisomy 18
	3B (n=3)	• Trisomy 13; PLSVC, TV dysplasia; PLSCV, PM VSD, trisomy 18
Atretic (n=3)	1A	• AVSD, DORV
	1B	• DORV, TV dysplasia, MV hypoplasia, PM VSD
	3B	• Muscular VSD

ASD = atrial septal defect; AVSD = atrioventricular septal defect; AV valve = atrioventricular valve; TV = tricuspid valve; MV = mitral valve; DORV = double outlet right ventricle; PLSCV = persistent left superior caval vein; TGA = transposition of the great arteries; VSD = ventricular septal defect; PM VSD = perimembranous VSD.

^a The number of observed cases that could be assessed for this particular abnormality

DISCUSSION

Key findings of the current study are: 1) In the BAV population with associated CHD, abnormal PV morphology was encountered in 17% of hearts; 2) The majority of hearts with abnormal pulmonary valve morphology had a type B BAV; 3) Bilateral bicuspid semilunar valves were associated with chromosomal abnormalities in 50% of specimen, predominantly trisomy 18.

Given the common developmental background of the aortic and pulmonary valves (both derived from outflow tract cushions with contributions of endocardium derived

cells, neural crest cells¹⁰ and, possibly, epicardial derived cells¹³, one could expect that in case of BAV also the pulmonary valve is affected. The observed 17% of the cases (bicuspid, dome-shaped and atretic pulmonary valves) in the current study, is a minority but still a higher percentage than reported in previous studies^{2, 6}, indicating at least a partly common development of aorta and pulmonary valves. Almost two-third of the hearts with an abnormal pulmonary valve had a strictly bicuspid aortic valve, indicating a potential predisposition of maldevelopment of the pulmonary valve in a subgroup of patients with BAV type B. Although the mechanism is unclear at this point, it seems that in cases where tripartition of the valve is severely disturbed to the extent that an anatomical third leaflet and a raphe cannot be recognised, the development of the pulmonary valve (that evolves from the same endocardial cushions that contribute to the aorta during development, **Figure 1c**) is also prone to be affected. In this respect, it is interesting that in 75% of the hearts with bilateral semilunar valves, there was a perimembranous VSD, suggesting an early defect in endocardial cushion remodelling, as these cushions are also involved in closure of the interventricular foramen.

In 10% of the specimens, there was concurrence of a bicuspid aortic and pulmonary valve, which is a similar percentage as has been found in a previous study⁹. The association with chromosomal abnormalities, especially a higher incidence of Trisomy 18, is in accordance with previous studies^{9, 14, 15}.

The fact that not only the semilunar valves but also the AV valves were affected in 36% of the cases, as well as the high percentage of perimembranous VSDs that were observed, could indicate a developmental cause affecting primarily endocardial cushion derived tissues¹⁶.

CONCLUSION

Pulmonary valve pathology was encountered in 17% of the cases with BAV. Two third of these cases had a BAV type B. In addition, over one third of specimens with abnormal pulmonary valve morphology had a chromosomal abnormality, and all of these had a BAV type B. We conclude that bilateral semilunar valve disease is associated with type B BAVs, in many cases related to chromosomal abnormalities. As this study was performed in post-mortem specimens with high frequency of associated CHD, caution is warranted with application of these results to the general BAV population.

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Coding of coronary arterial origin and branching in congenital heart disease: the modified Leiden Convention

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ABSTRACT

Variations in coronary anatomy are commonly encountered and may relate to the position of the coronary ostium with regard to the aortic sinus, the angle of the coronary take off as well as the course of the coronary arterial branches. Several classification systems have been proposed in the past decades. However, they all lack a simple non-confusing rationale that is applicable irrespective of the relative position of the great arteries, as well as in situations where the valve has 2 leaflets (bicuspid aortic valve). In this paper, we present a modification of a relatively simple system introduced in the early eighties, designated the "The Leiden Convention". The first step of the Leiden Convention is that the clinician takes a position in the non-facing sinus of the aorta looking towards the pulmonary orifice. The right hand facing sinus is sinus 1 and the left hand facing sinus is sinus 2 (Central Picture). The coronary branch(es) arising from sinus 1 are annotated proceeding in a counter-clockwise fashion towards sinus 2. "Usual" (normal) coronary anatomy would be 1R-2LCx.

This coding system was originally designed and highly applicable in hearts with an altered great artery relationship such as in the variable and complicated patterns seen in transposition of the great arteries (discordant ventriculo-arterial connections) and double outlet right ventricle. We show that this coding system can also be used in cases with normally related great arteries (concordant ventriculo-arterial connections), including cases with a bicuspid aortic valve. Given their clinical relevance, single sinus coronary arteries will be discussed as a separate group.

INTRODUCTION

Coronary arterial origin and course of the proximal main stems can be an important indication for analysis of cardiac patho-morphology. In the morphologically normal heart there is a fairly consistent coronary pattern consisting of three main branches connecting by way of two orifices to the semilunar sinuses of the aorta. The left coronary artery (LCA) arises from the left sinus which, taking a curving course posterior to the pulmonary orifice, divides into a left anterior (LAD) and a left circumflex (LCx) branch. These branches run subepicardially in the anterior interventricular sulcus and the left atrioventricular sulcus, respectively. The right coronary artery (RCA) connects to the right sinus and runs in the right atrioventricular sulcus¹. From the LAD, LCx and RCA several marginal, septal and diagonal branches arise, that enter the myocardium and perfuse the septum and free wall of the ventricles. Smaller separate branches are important for perfusion of the sino-atrial and atrioventricular nodes. They usually originate from a main branch that is closest to the respective node, although diversity in branching patterns is found^{2,3}. As a general rule, coronary arteries will never cross over other coronary artery branches in their course.

Variations in sinus terminology as related to position of the great arteries

Variations in coronary anatomy are commonly encountered and may relate to the position of the coronary ostium in the aortic sinus, the angle of the coronary take off as well as the course of the coronary arterial branches. Specifically in case of congenital heart disease (CHD) involving the cardiac outflow tract, variations are seen in origin and branching patterns¹. This is even more so when there are variations in the *relative position of the orifices* of the great arteries. In the normal situation, the aortic orifice is located right posterior with regard to the pulmonary orifice. In cases of CHD affecting the outflow tract, the position of the aorta may vary from right posterior, to right side-by-side, to right anterior, to frontal, or to left anterior as related to the pulmonary orifice (**Figure 1a**). When evaluating the position of the aortic valve leaflets with regard to the pulmonary valve, regardless of the position, two sinuses can be recognised facing the pulmonary valve (referred to as “facing” sinuses) as well as one “non-facing” sinus (**Figure 1a:l, b:l**). In the aorta the non-facing sinus is often referred to as the non-coronary sinus. To enable the proposed simple unambiguous coding, first a terminology for the aortic sinuses was adopted that is independent of the relative anatomical position of the great arteries and the position of the aortic orifice with regard to the anterior surface of the heart. In this way it has been possible to avoid the change in positional nomenclature for the semilunar sinuses when the aorta is not in its normal position with regard to the pulmonary orifice, as is the case in transposition of the great arteries (TGA) (reviewed

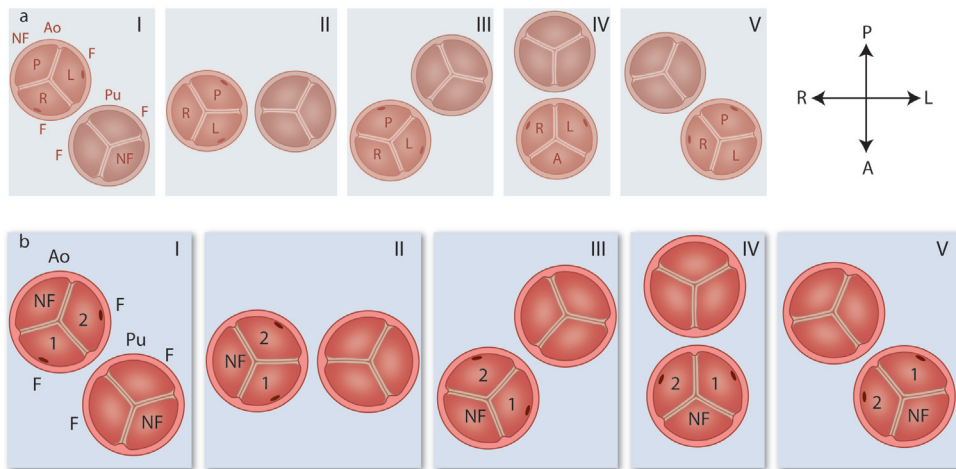


Figure 1. View from above.

a. Influence of positional changes of the great arteries on the description of the semilunar sinuses. Regardless of the position of the aortic valve (Ao), two sinuses can be recognised facing the pulmonary valve (Pu). These are referred to as the facing sinuses (F), leaving one non-facing sinus (NF).

b. Unambiguous numerical terminology that is not influenced by relative position of the great arteries.

L: left, R: right, P: posterior, A: anterior. Relative position of the aortic orifice: I right posterior, II right side-by-side, III right anterior, IV anterior, V left anterior.

in⁴, congenitally corrected transposition and double outlet right ventricle (DORV). With a positional terminology for the semilunar sinuses this may lead to a myriad of variations (**Figure 1a: II-V**). The terminology for the semilunar sinuses in the Leiden Convention is independent of the position of the great arteries (**Figure 1b: II-V**). This is supported by cardiac development, when the endocardial outflow tract cushions, present in the initially unseparated outflow tract, become involved in septation. Neural crest derived cells form the so-called condensed mesenchyme separating the future aortic and pulmonary orifices (**Figure 2a-d**). At the level of the orifices, two facing commissures are formed, one in the aortic and one in the pulmonary orifice (**Figure 2b,c**). At both opposite sides two more commissures are formed between the intercalated (future non-facing leaflets) and the main endocardial outflow tract cushions (facing leaflets) (**Figure 2b,c**). This eventually results in the definitive aortic and pulmonary valves (**Figure 2c,d**) with their typical position of the commissures, including two facing commissures.

The general rule holds that the mentioned main coronary branches take the shortest course towards an aortic semilunar sinus. This finding is supported by the developmental background showing that the coronary arteries grow into the aortic sinuses instead of

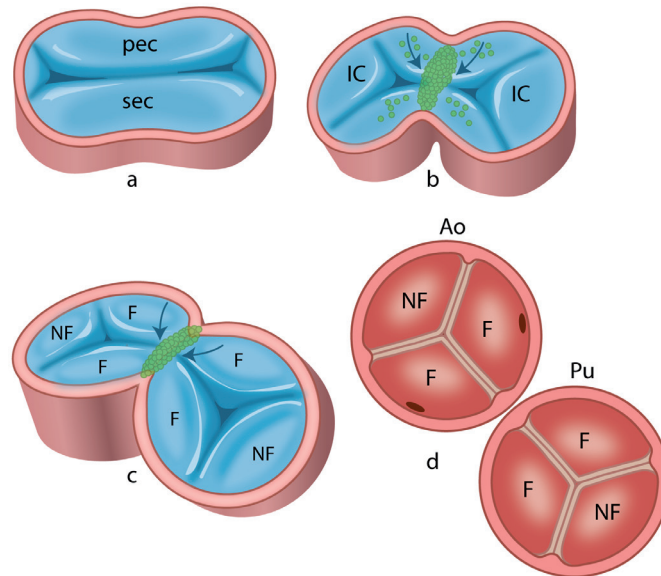


Figure 2. View from above.

a. Unseptated cardiac outflow tract at future orifice level, showing a large septal (sec) and parietal (pec) outflow tract endocardial cushion and two small intercalated cushions (ic).

b. Septation of the orifice level with ingrowth of neural crest cells (green), in which two facing commissures (arrows) are formed between the aortic (Ao) and the pulmonary (Pu) orifice (**b, c**).

d. The definitive situation in which each orifice contains two facing (F) and one non-facing (NF) sinus.

growing out^{5,6}. There is still an unexplained preference for the facing sinuses (99% of the cases), leaving the non-facing sinus mostly without an orifice of a connecting branch (non-coronary sinus). Over time molecular research in mainly mouse models attempted to elucidate this phenomenon. A recent publication is enlightening in this respect, as it shows a separate developmental program guiding the LCA and the RCA, however, without explaining the preference for the facing sinuses of the aorta and the avoidance of the non-facing/non-coronary sinus⁷.

With the advance of corrective surgery for CHD and the introduction of sophisticated diagnostic imaging techniques over the past decades, it has become possible to document the coronary artery anatomy before corrective or palliative surgery is performed. Specifically the arterial switch procedure for transposition of the great arteries (TGA) and double outlet right ventricle (DORV) with sub-pulmonary ventricular septal defect (Taussig-Bing malformation), requiring an additional transfer of the coronary arterial ostia into the neo-aorta, triggered an increased interest in variations in coronary artery anatomy⁸.

A special group of coronary malformations concern the coronary arteries arising from one coronary sinus. These are referred to as single sinus coronary arteries, irrespective of whether there is only one shared coronary ostium or more ostia lying close together in the same coronary sinus. Single sinus coronary arteries can exist isolated or in the setting of other congenital malformations. In contrast to the common variations encountered in coronary anatomy, single sinus coronary arteries seem to be more clinically relevant, especially when there is an interarterial/intramural course of a coronary branch⁹⁻¹¹. Pre-operative information is also of relevance in patients with bicuspid aortic valves (BAV).

Principles of the coronary artery coding system: The Leiden Convention (Figure 3a-c).

Several classification systems have been proposed in the last thirty years (reviewed in¹²), all lacking, however, a simple non-confusing rationale that is applicable irrespective of the position of the great arteries, as well as in situations where only two valve leaflets are present as in bicuspid aortic valve (BAV). In the early eighties, we have provided a relatively simple coding system⁴ which was later on dubbed "The Leiden Convention"¹⁸. However, this system has a few flaws, mainly because it does not adequately show the course of the coronary branches (curving) around the great arteries. A modification to this system was proposed¹³, but never made it to referenced literature. The *modified* Leiden Convention, as is presented here, is applicable in hearts with an altered great artery relationship such as encountered in TGA and DORV, presenting with variable and complicated patterns, as well as in cases with normally related great arteries, including BAV. Given their clinical relevance, single sinus coronary arteries will be discussed as a separate group.

For application of the Leiden Convention, as a general rule, an aortic valve with 3 leaflets *always carries a non-facing/non-coronary sinus*. The first step of the Leiden Convention is that the clinician or researcher takes position in the non-facing sinus of the aorta looking towards the pulmonary orifice and then referring to the right hand facing sinus as *sinus 1* and to the left hand facing sinus as *sinus 2* (**Figure 3a**). Independent of the position of the great arteries, this relationship is always maintained, thus acquiring the desired simplification of semilunar sinus terminology (**Figure 1b**).

The second step is to simplify the description of the coronary ostium (defined by the position of the visible orifice) and branching patterns of the main coronary arteries. When describing the coronary anatomy according to the Leiden Convention, the sinus number is indicated first (as explained in step 1), and thereafter the coronary branch that arises from sinus 1 is annotated proceeding in a *counter clockwise fashion* (**step 2; Figure 3b**). For a normal heart this would be: 1R-2LCx, indicating the 3 main branches being the RCA (R), the LAD (L) and the LCx (Cx) (**Figure 3b**). The LCA, comprised of the

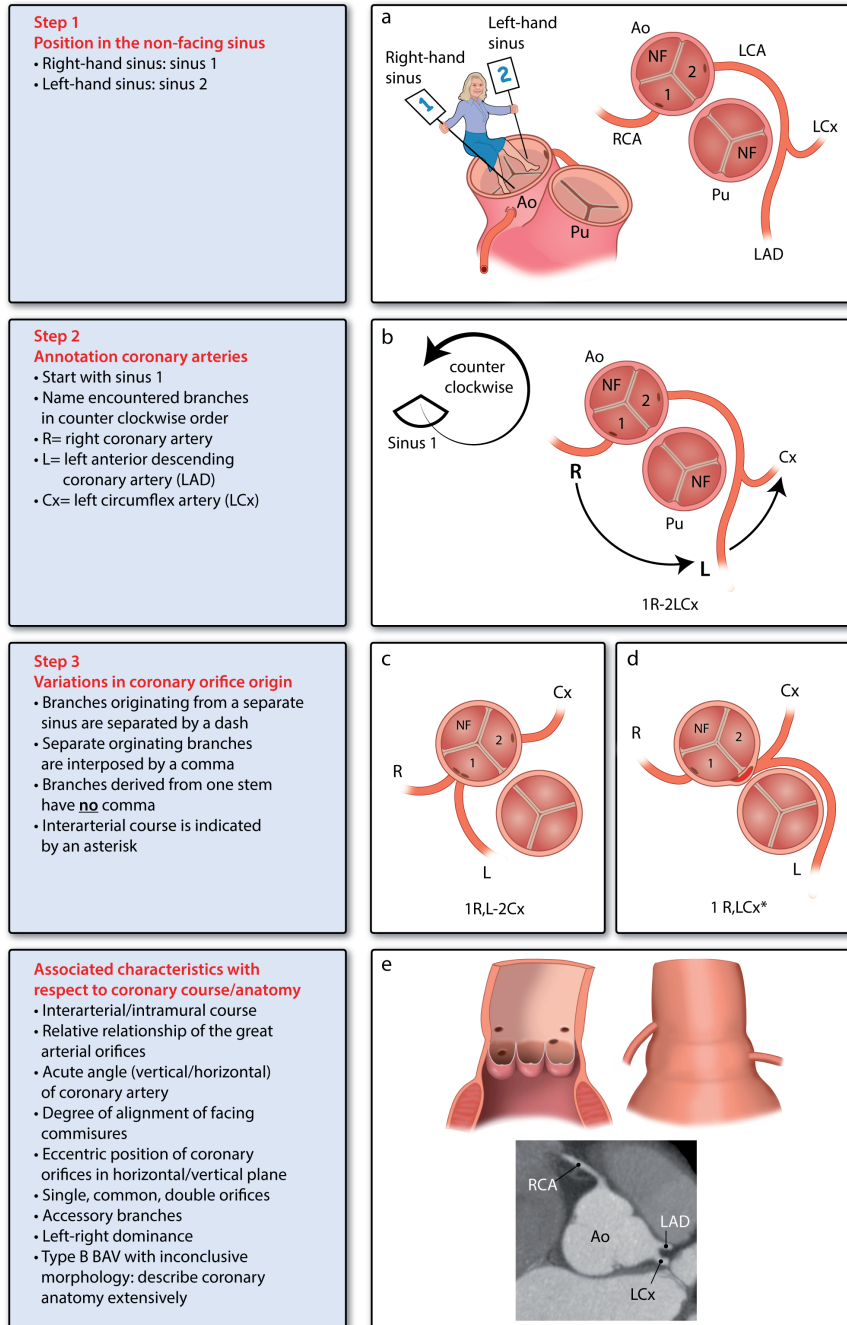


Figure 3. Application of the Leiden Convention applying 3 subsequent steps (a,b,c) and additional information (d). For explanation see text.

L and Cx (with or without a main stem), does not have a separate annotation. To cover variations in coronary orifice origin step 3 is necessary (**Figure 3c,d**). The use of a dash (-) between artery branches arising from a different sinus is applicable in all cases with double sinus origin (**Figure 3b,c**). When coronary artery branches share a common ostium or stem, there is no comma (**Figure 3b**) between the annotations, whereas a comma is interposed when two (or more) arteries arise separately from the same sinus (**Figure 3c,d**). For instance, in case of normal anatomy with an LCA as a main stem, the annotation is: 1R-2LCx, as described above. In case of absent main stem (separate origin of L and Cx from sinus 2), the annotation would be 1R-2L,Cx. An asterisk (*) is provided for a coronary artery branch that has an interarterial, often partly intramural, course (**Figure 3d**). These cases may clinically be most at risk of complications, such as sudden cardiac death (SCD). Additional features such as a prepulmonary or a retro-aortic course become evident when the system is applied consequently, and indicating the course of the coronary artery separately is not necessary.

Finally, additional information (**Figure 3e**) regarding associated anomalies and clinically relevant issues such as an interarterial/intramural coronary artery course, acute angulation and position/height of coronary orifices within the sinus should be described. This is particularly important to underline potentially harmful clinical findings, e.g. during the conveyance of medical information to the thoracic surgeon and other professionals involved in patient management.

Coronary artery coding in TGA and DORV

The proposed simple coding system was originally devised for TGA and the Taussig Bing malformation when the arterial switch procedure, initiated by Jatene¹⁴ became the preferred surgical technique, replacing the atrial switch procedures. In the arterial switch procedure, it is necessary not only to switch the main great arteries but additionally, the coronary arteries have to be transferred to the neo-aortic root (former pulmonary root). Coding systems in which positional semilunar sinus terminology have been used, combined with other morphological characteristics of the coronary orifice origin and branching patterns, could lead to as many as 28 variations¹⁵. Realizing that this was too cumbersome for practical clinical use, several more simple classifications were introduced, mostly making use of an alphabetical identification such as type A to D¹⁶ or of a numerical grouping with subgroups (reviewed¹²). The original 'Leiden Convention', using facing sinus 1 and 2 with an additional numerical identification, left 6 basic types, nevertheless requiring memorizing the numerical indications⁴.

The *modified* Leiden Convention includes the course of the main branches around the great arteries, resulting in a further redundancy of the number of variations in the coding system. It is fascinating that a rather stable pattern of coronary origin (1LCx-2R)

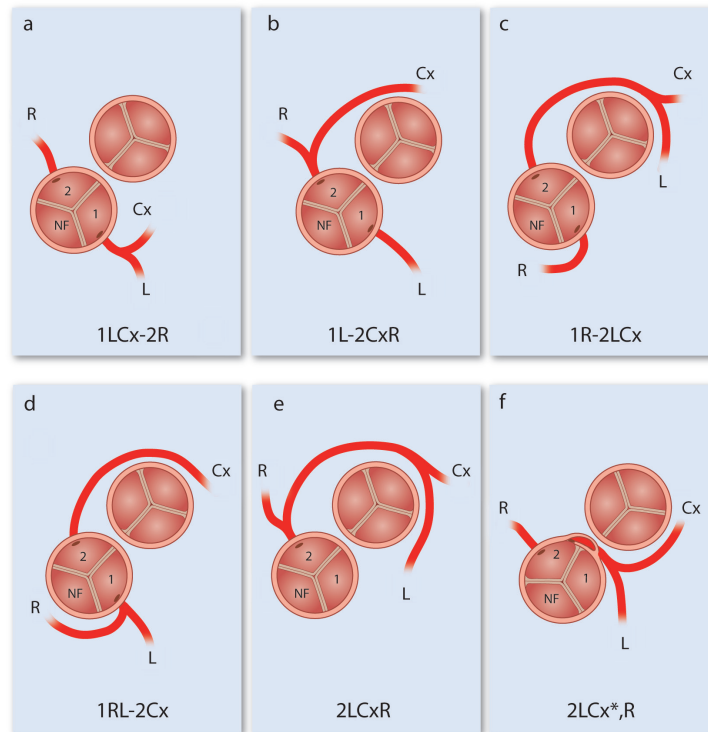


Figure 4. Variations in coronary anatomy in transposition of the great arteries, view from above. **a.** the most common form. **b-d** show variations with a double sinus origin. **e.** example of a single sinus 2 origin. In **f.** the clinically most hazardous form is shown with the LCx taking off directly behind the facing commissure from sinus 2 and thereafter taking an interarterial/intramural course to the anterior aspect of the heart. The combination of L and Cx thereafter courses over the right ventricular outflow tract crossing anteriorly to the pulmonary orifice.

exists in the most common form of TGA with the aortic orifice in a right anterior position (**Figure 4a**) as observed in a series of 103 post-mortem specimens with TGA (**Figure 5a**)⁴ In case of side by side great arteries, as often seen in Taussig Bing type DORV, the greatest variation in coronary origin like 1L-2CxR, 1R-2LCx or 1RL-2Cx (**Figure 4b-d, 5b**) is to be expected⁴ as if the coronary artery has difficulty in finding its shortest course towards a facing semilunar sinus. In case of a frontal position of the aorta this variation is diminished as a shortest course is again clear (**Figure 5c**).

It is important that during surgical transfer of the coronary arteries no kinking occurs, as it may lead to hypoxia of the coronary arterial myocardial perfusion area. Surgical risks are encountered in cases with a single sinus coronary artery pattern¹². A surgical challenge is also found in cases with an interarterial/intramural course (**Figure 4f, 5a,b**) of the coronary artery between the aortic and pulmonary orifice, requiring an adaptation

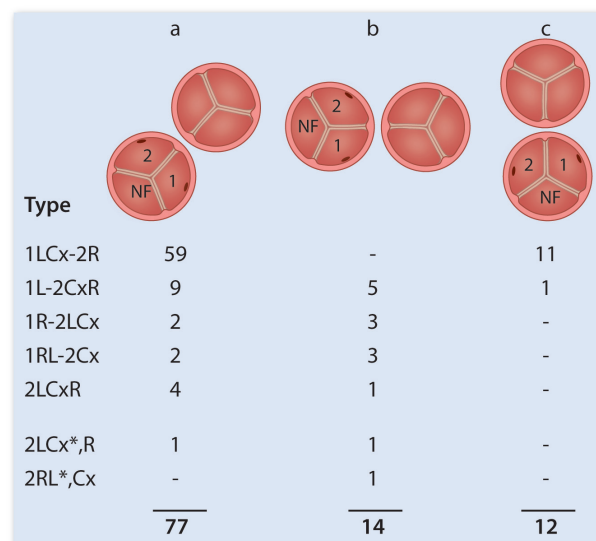


Figure 5. Frequency of variations in 103 cases transposition of the great arteries with right anterior position of the aorta (a), side by side position of the great arteries in in DORV with subpulmonary ventricular septal defect (b) and anterior position of the aorta (c)^{(modified after (4))}. The most common form (1LCx-2R) is found in cases with a right anterior position of the aorta. Of the complicated cases with an interarterial/intramural artery (1LCx*,R), 1 is found in the most common position seen in TGA, being right anterior, while 2 cases (1LCx*,R and 2RL*,Cx) were seen in side-by-side great artery positions, mostly encountered in DORV.

of the transfer procedure^{12,17,18}. These cases often present with a double orifice from one sinus, with the interarterial coronary artery originating from the opposite sinus (e.g. an interarterial LCA originating from the right sinus) and coursing directly behind or most often just above the facing commissure of the aortic and pulmonary orifices. In these cases there is an acute take off with a slit-like coronary orifice while the first part of the artery takes an intramural course in the wall of the aorta, where both the aorta and the coronary artery have a joined media without a separating adventitia^{10,19}.

Coronary artery coding variations in normally related great arteries

The estimated incidence of coronary anomalies in structurally normal heart ranges between 0.1 and 1.0%, although this is difficult to estimate as many variations are likely asymptomatic¹⁰. Although the incidence is low, unfavourable anatomy can have high clinical impact, and coronary anomalies are held responsible for almost 20% of cases of SCD in competitive athletes²⁰. A large magnetic resonance imaging screening program found 0.7% of the population had an interarterial course. The origin of the

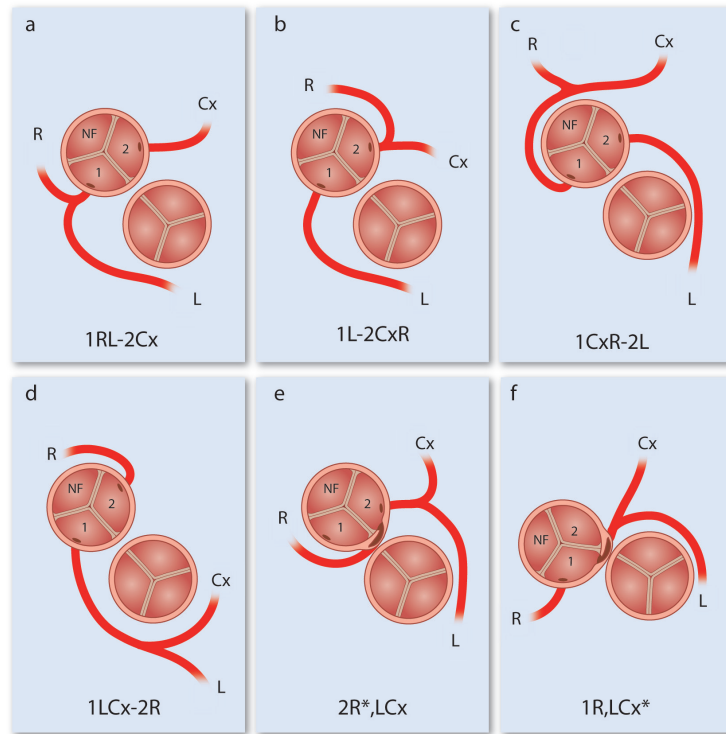


Figure 6. This schematic figure presents the most common variations in coronary arterial patterning, viewed from above. **a.** presents the normally encountered situation whereas **b-f** are less common variations. A dash (-) is used between branches that derive from a separate sinus (**a-d**). A comma is interposed when the branches arise from a separate orifice in the sinus (**e,f**). An asterisk is placed after an intra-arterial/intramural branch (**e,f**). Variation **f** is clinically the most hazardous with an interarterial/intramural course of a branch. In these cases the artery commonly shares part of the aortic wall media with no adventitia interposed. The orifice of the artery is commonly present in the direct neighbourhood of the facing commissure.

right coronary artery from the opposite sinus was 3 to 6 times more common than an aberrant left coronary artery²¹. The coronary anatomy mostly related to SCD is single sinus coronary anatomy, especially a left coronary artery originating from the right sinus, that is associated with an interarterial/intramural course and acute take-off¹⁰. When applying the modified Leiden Convention on cases with normally related great arteries, this results in a set of variations which is exemplified in (Figure 6a-f). This Figure covers multiple possibilities of the system but is not meant to be complete. As elaborated above, the abnormally located ostium of the left coronary artery branch in combination with an intramural course which specifically tends to cross above or behind the facing aortic commissure, carries the highest risk of sudden death (Figure 6f)¹⁰.

Coronary artery coding in bicuspid aortic valves

Classification of coronary artery origin tends to be challenging when not all three valvular commissures can be distinguished, as is the case in BAV. BAV has recently gained increased interest because of the often concomitant dilation of the ascending aorta in early adult life²². Surgical treatment strategies focus on the possibility to predict development of aortic dilation based on the types of aortic valve morphology. We have adopted and modified the Sievers and Schaeffer numerical classification^{23,24}, to 3 types based on which valve leaflets are conjoined and whether or not a raphe can be distinguished. In contrast to the Sievers classification²⁴, this adjusted classification does not specifically mention the number of raphe. We propose that a numerical valve annotation can also be used for BAV variations (**Figure 7**). It is generally assumed that in BAV, where only two functional leaflets develop, there is a fusion (or non-separation) of two of the three leaflets. In the classification used in the current paper, Type 1 has

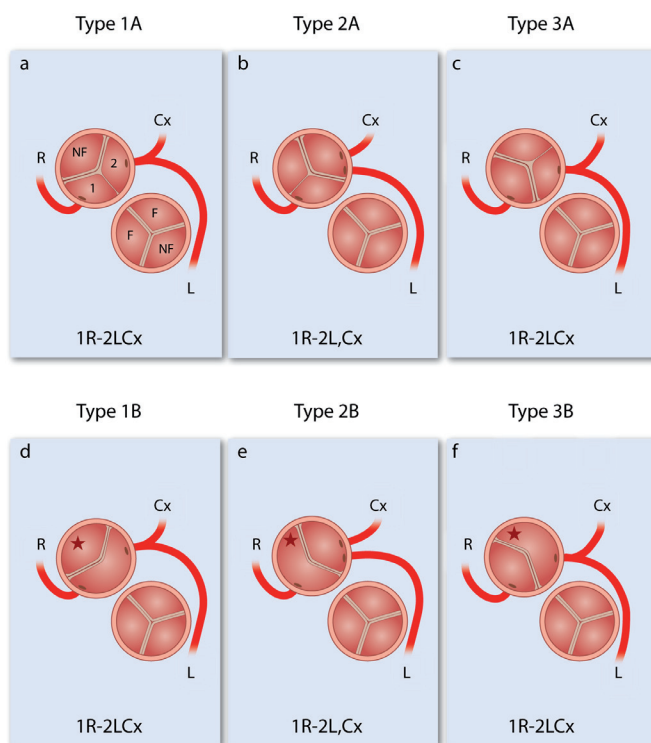


Figure 7. Schematic representation of classification in bicuspid aortic valve disease with associated variations in coronary anatomy viewed from above, for example separate ostia of the left main stem, (**b, e**). The supposed “non-facing/non-coronary” part of the aortic leaflet in case of a strictly bicuspid valve is indicated by a star in **d-f**.

a joined right (facing sinus 1) and left (facing sinus 2) semilunar sinus, while in Type 2 the right (sinus 1) and the non-coronary (non-facing sinus) are joined. Type 3 is very rare with a fusion of the left (sinus 2) and the non-facing sinus (**Figure 7**). Based on the presence of a commissural ridge or raphe or the complete absence of a raphe, the letter A for presence (**Figure 7a-c**) and B for absence (**Figure 7d-f**) was assigned.

The modified Leiden Convention coronary coding system can be used in the majority of BAVs with a raphe (Type A) based on the general rule that in 99% of the hearts the coronary artery connects to a facing sinus 1 and 2 and the non-facing sinus remains non-coronary. A single sinus coronary origin has been reported to be very rare in BAV patients²⁵. The most common varieties observed in BAV are a left dominant coronary anatomy²⁶ and absence of or a short left main stem (**Figure 7b,e**) which may complicate cardioplegia when this is not recognised. Coronary coding is challenging in type B BAV (without raphe). Here, reasoning could be reversed and the modified Leiden Convention could be used to predict which leaflets have been fused or not separated during development and thus could be decided whether it is a Type 1, 2 or 3 related morphology. The normal (tricuspid) aortic valve is composed of two “facing” leaflets and a facing commissure (facing the corresponding pulmonary valve leaflets and commissure) and one “non-facing” leaflet (see **Figure 2c,d**). In case of type B BAV, we know that the most common form is Type 1B²⁶. If this is the case, the “potential raphe” between the coronary branches should point towards the facing commissure of the pulmonary semilunar valve (**Figure 7a and d**). In case of type 2B and 3B, one of the commissures of the BAV aortic valve points towards the facing commissure of the pulmonary valve, thus allowing distinction between type 1B and types 2 and 3 B. It is more difficult to distinguish between a Type 2B and 3B. Here the clinically observed opening pattern of the aortic valve should be assessed. After identification of the (potential) non-facing/non-coronary leaflet (red stars in **Figure 7d-f**), the Leiden Convention nomenclature can be applied. If determination of valve morphology is inconclusive, a descriptive approach is recommended. This should be added to the report of associated characteristics (**Figure 3e**). Data regarding coronary arterial patterning and origin in BAV as related to BAV subtypes are scarce. Recently, our group published data showing variations in left-right dominance²⁶ and an increased incidence of high-take off coronary arteries in BAV, as compared to CHD in general²⁷. This also belongs to the additional information that needs to be provided to the modified Leiden Convention coding system (**Figure 3e**).

Associated characteristics with respect to coronary orifices, course of coronary arterial branches and anatomical variations

In some cases it is necessary to describe a number of essential aspects of coronary variations that may have an impact on the surgical treatment of choice.

These include (**Figure 3e**):

1. The presence of an intramural course in cases with an interarterial branch, which is especially hazardous if it concerns the left coronary artery branches.
2. The relative relationship of the great arterial orifices (also see **Figure 1**).
3. The eccentric positioning of an orifice in the horizontal plane is of importance for the possibility to excise a proper button. Positioning close to a valvular commissure and acute angle take-off are features that hamper the procedure.
4. The degree of alignment of the facing commissures to determine the site of repositioning the buttons of the new coronary orifice in an arterial switch procedure.
5. The positioning of an orifice in a vertical plane above the sino-tubular junction, referred to as high take-off, often seen in concordance with an acute angle take-off and a variable intramural course. This angle is also influenced by aortic diameter; an acute angle can become more outspoken with aortic dilation²⁸.
6. The presence of a single, double or common orifice for branches is an essential part of preoperative knowledge.
7. Accessory branches that bud-off the coronary sinuses should be indicated as well as the origin of the sino-atrial node branch and a possible conal artery.
8. Information on right, left or co-dominance of the coronary artery system.

CONCLUSIONS

Coronary arterial patterning is of relevance, especially in the setting of CHD where pre-operative information before the often complex surgery is mandatory. TGA and DORV with subpulmonary ventricular septal defect require special adjustments for the transfer of the coronary arteries to the neo-aorta specifically in case of an interarterial/intramural course. In normally related great arteries, the interarterial/intramural course of specifically the left coronary artery branches poses a life threatening hazard which can be fatal in young adults during athletic performances(9;10). Here we propose an accessible unambiguous system based on the modification of a previously developed and widely implemented coding system referred to as The Leiden Convention, extending its use for several forms of CHD and including information on origin and branching patterns.

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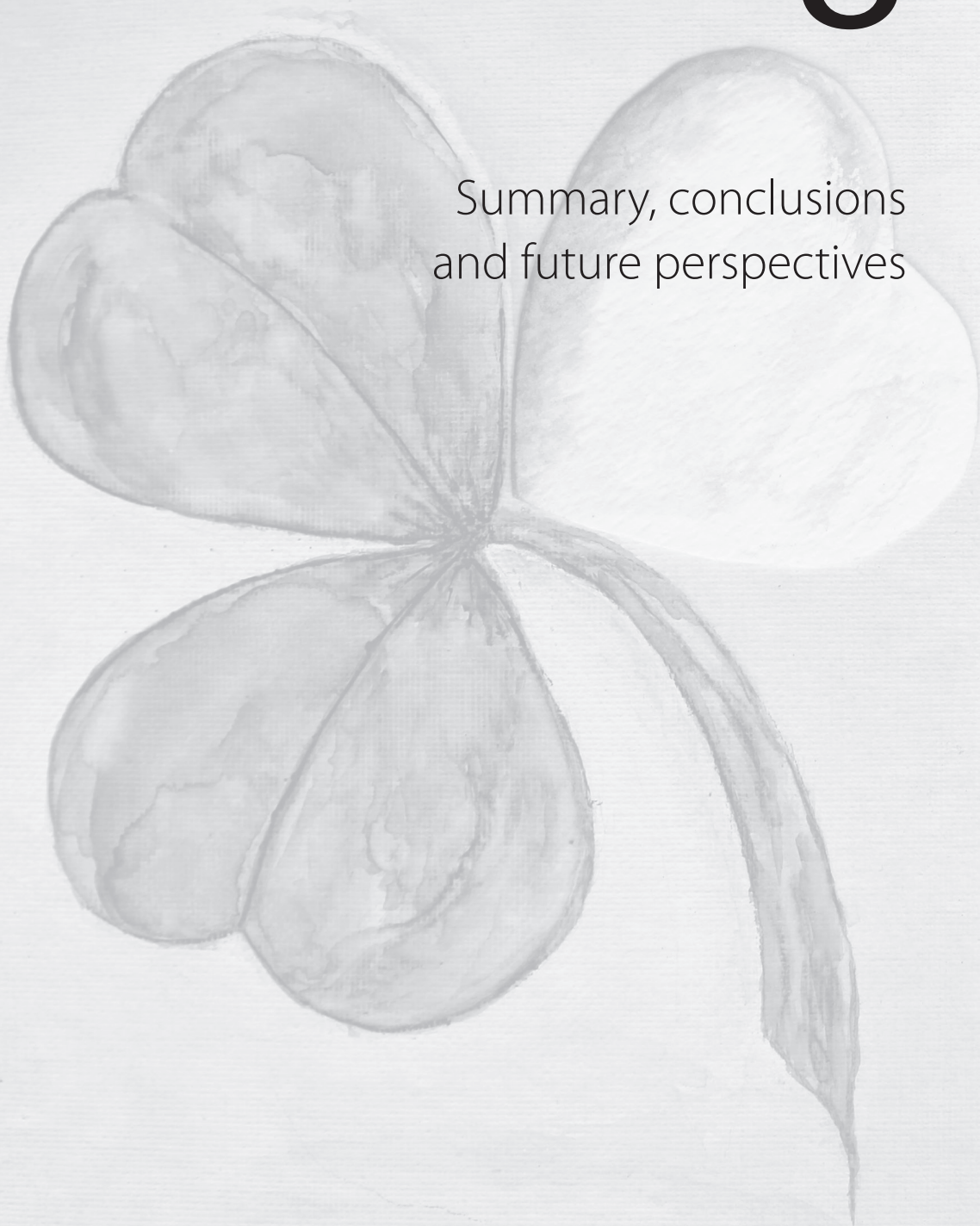
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8

Summary, conclusions
and future perspectives



SUMMARY

This thesis focuses on the morphology of the bicuspid aortic valve (BAV) in relation to coronary anatomy, pulmonary valve anatomy and outcome. The prevalence of bicuspid aortic valves is 1-2%. It is almost 3 times more common in males than in females. In this regard it is striking that BAV is also a dominant feature of heart disease in female patients with Turner syndrome (Monosomy X or X chromosome mosaicism, incidence 12-30%).

In the introduction of this thesis (**Chapter 1**) a brief overview of cardiac morphogenesis and development of the aortic valve is given. During development the aortic and pulmonary semilunar valves develop from two endocardial cushions in the undivided embryonic outflow tract of the heart. During development, septation at the levels of the cardiac outflow tract, arterial valves and great arteries has to occur. After normal septation, a 3-leaflet aortic and pulmonary semilunar valve can be recognised at the valvular level.

A bicuspid or bi-leaflet aortic valve consists of 2 (functional) leaflets instead of 3 leaflets. In many cases, an incomplete tri-leaflet partition can still be recognised, due to the presence of a raphe, i.e. fusion of 2 commissures. Different morphological subtypes of bicuspid aortic valves can be distinguished. Three main types exist, that we have designated in this thesis as type 1, 2 and 3. Type 1 is the most common, with fusion of the right and left coronary leaflets; in type 2 the right and non-coronary leaflets are fused and in the rare type 3 there is fusion of the left and non-coronary leaflets. In addition, these types were subdivided further with respect to the presence or absence of a raphe, i.e. whether a site of supposed commissural fusion can be recognised (type A), or whether there is a strictly bicuspid valve in which no raphe can be recognised (type B). Bicuspid aortic valves are associated with several complications including valvular dysfunction and aortic dilation. They have also been associated with coronary abnormalities, the relation with BAV morphological subtype, however, was largely unexplored. In addition, given the partly common development, also pulmonary valve abnormalities could be expected in cases with BAV, although information regarding pulmonary valve morphology in BAV patients is limited in literature. These issues are the subject of the current thesis. Below the main findings of the different studies included in this thesis are summarised.

Chapter 2: in a group of 255 patients with bicuspid aortic valves, valve morphology and aortic dimensions were evaluated by echocardiography. This study showed that not only valve morphology, but also the presence or absence of a raphe, and even extent of the

raphe influenced outcome. Patients with BAV with a complete raphe had a significantly higher prevalence of valve dysfunction - especially aortic regurgitation - as compared to BAVs with incomplete raphes. Patients with type 1A BAV (BAV with fusion of the left and right coronary leaflet) and a complete raphe had larger aortic sinus diameters compared to the rest of the population. This could indicate that patients with BAV with a complete raphe, as well as patients with a type 1A BAV need closer monitoring and more regular follow-up. In the subgroup with aortic coarctation, incomplete raphes were observed more frequently, associated with smaller aortic root diameters and less valve regurgitation, although the smaller aortic root diameters might also be attributed to the fact that these patients were younger.

Chapter 3: coronary anatomy was studied in 186 patients with bicuspid aortic valves. Coronary anatomy was evaluated by computed tomography coronary angiography and/or invasive coronary angiography. The results were correlated with coronary events, like severe coronary artery disease (> 70% luminal narrowing), having undergone revascularisation by percutaneous coronary intervention or coronary artery bypass grafting or any admission for unstable angina pectoris. We found that variations in coronary anatomy, like a left dominant coronary artery system and separate ostia of the LAD and LCX, correlate to BAV morphology, and that type 1B BAV (without a raphe) show more variations. Patients with this anatomy also seem to be at risk of developing coronary artery disease. Patients with type 1 BAV without a raphe might therefore need closer monitoring and careful regulation of risk factors. In patients with BAV and CoA, more left dominance was seen compared with the group with isolated BAV, but there was no difference in significant coronary artery disease or events. This might be attributed to the fact that these CoA patients were younger.

Chapter 4: in this chapter coronary anatomy in 84 post-mortem heart specimens with BAV and associated complex congenital heart disease is described. Position and height of the coronary ostia were studied and related to BAV morphology. High take-off coronary arteries, especially the left coronary artery (LCA), occurred more frequently in BAV associated with complex CHD than reported in structurally normal hearts and in hearts with isolated BAV. It was also shown that outflow tract defects and a hypoplastic left ventricle were associated with type 1A BAV and high take-off coronary arteries. Clinical awareness of variations in coronary anatomy, causing possible risk of complications during procedures, is warranted, especially in BAV associated with complex CHD.

Chapter 5: variations in coronary anatomy and aortic valve morphology were studied by computed tomography in 3 groups of patients: Patients with Turner Syndrome (TS) and BAV, patients with Turner syndrome and a tricuspid aortic valve (TAV) and patients

with isolated BAV. The study population comprised 86 TS patients and 86 patients with isolated BAV. There were no significant differences in coronary anatomy with regard to coronary dominance or absent left main stem between TS patients with BAV and with TAV. In addition, no significant difference in prevalence of left dominant systems between BAV patients with versus without Turner syndrome were observed. Although there were no significant differences in the occurrence of separate ostia of the LAD and LCX between TS-BAV and TS patients with a TAV, separate ostia were more often seen in patients with TS and BAV as compared to patients with isolated BAV. All TS-BAV patients with separate left coronary ostia (absent left main stem) had type 1B BAV. Patients with type 1B BAVs showed a high prevalence of left coronary dominance in the group of TS-BAV as well as in isolated BAV. There was an equal distribution of left dominance in type 1B BAV in the group TS-BAV and isolated BAV, suggesting that presence of left dominance is a feature of BAVs without raphe independent of the presence of TS. Additionally, both TS and type 1B BAV seemed independently associated with absent left main stems. Awareness of the higher incidence of particularly absent left main stems is important to avoid complications during hypothermic perfusion.

Chapter 6: in this chapter pulmonary valve morphology was studied in 83 post-mortem heart specimens with BAV and associated complex congenital heart disease. As the aortic valve and pulmonary valve share part of their morphogenetic process, pulmonary valve abnormalities might be expected in (subgroups of) patients with BAV. In this study, pulmonary valve morphology was evaluated and related to BAV morphology. In BAV with associated CHD, abnormal pulmonary valve morphology was observed in 17% of the hearts. The pulmonary valve was bicuspid in 10%, dome-shaped in 4% and atretic in 4%. The majority of the hearts with abnormal pulmonary valve morphology had a bicuspid aortic valve without a raphe (type B). Bilateral semilunar valvular disease was associated with type B BAVs and in many cases related to chromosomal abnormalities (trisomy 13 and 18).

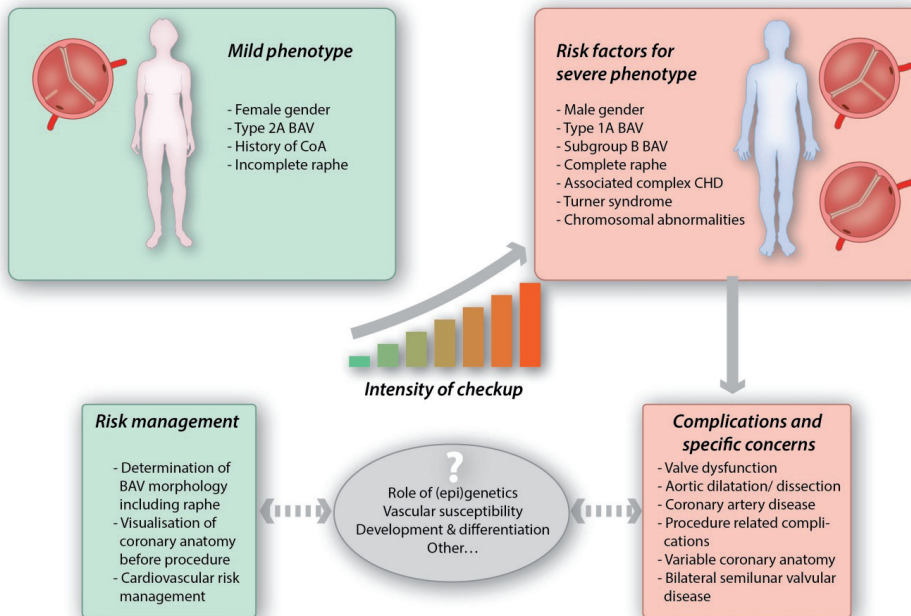
The **final Chapter** describes a classification system for coronary anatomy. The system is applicable in hearts with normally related great arteries, as well as in hearts with an altered great artery relationship. This classification system is based on the modification of a previously developed and widely implemented classification system referred to as the Leiden Convention. A section describing coronary classification in the setting of BAV, is included in this chapter. Coronary arterial patterning is of relevance, especially in the setting of congenital heart disease (CHD) where pre-operative information before the often complex surgery is mandatory to avoid problems with perfusion during hypothermic cardioplegia in case of variant anatomy.

CONCLUSIONS AND CLINICAL IMPLICATIONS

BAV morphology and especially the presence and extent of a raphe is of clinical importance, as a complete raphe predisposes to more valvular dysfunction and aortopathy. Moreover, type 1A BAVs with a complete raphe show more aortic regurgitation and root dilatation. On the other hand, patients with BAV and CoA have smaller aortic root diameters and less valve regurgitation, possibly due to the fact that in this group of patients the majority have an incomplete raphe. Regarding variations in coronary anatomy, it turns out that patients with type 1B BAVs and patients with BAV and CoA more often have a left dominant coronary artery system. Patients with type 1B BAVs also seem more at risk of developing significant coronary artery disease. In addition, separate ostia of the LAD and LCX are more common in patients with type 1B BAV, which could be of clinical importance in using coronary artery perfusion as a means of myocardial protection during surgical aortic valve replacement. Patients with Turner syndrome and BAV do not show significant differences in coronary dominance as compared to patients with Turner syndrome with TAV and to patients with isolated BAV. However, patients with TS and BAV show more separate ostia of the LAD and LCX as compared to patients with isolated BAV. Since patients with TS are more prone to develop coronary artery disease at a younger age, in case of the need for diagnostic coronary angiography or surgery, it is important to be aware of the higher incidence of particularly separate left coronary ostia, to avoid complications during hypothermic perfusion. In BAV with associated complex congenital heart disease, a high take-off of the coronary arteries, occurred more frequently than reported in structurally normal hearts and in hearts with isolated BAV, which is also of clinical importance during procedures. Finally, there should be awareness of possible bilateral semilunar valvular disease, which is associated with type B BAVs and in many cases related to chromosomal abnormalities.

Below a Working Model based on results described in this thesis is depicted.

Working model for risk stratification in BAV patients



FUTURE PERSPECTIVES

In this thesis, pathomorphology of BAV with regard to different BAV subtypes, coronary arterial distribution and pulmonary valve morphology has been explored. Future research will focus on embryonic development of the aortic valve, aortic wall and coronary arteries in animal models with BAV. These three morphological entities all receive contributions from different cellular sources, such as neural crest, endocardium, epicardium and second heart field and its derived cells. These cellular contributions to the aortic valve, aortic wall and coronary arteries, and their developmental overlap, will be the subject of future studies. In addition, signalling pathways involved in formation of bicuspid aortic valves, such as the nitric oxide synthase (NOS) pathway will be further explored in animal models in embryonic, fetal and postnatal stages. These studies will likely shed light on the, to date, insufficiently understood association between bicuspid aortic valves, aortic complications and coronary abnormalities.

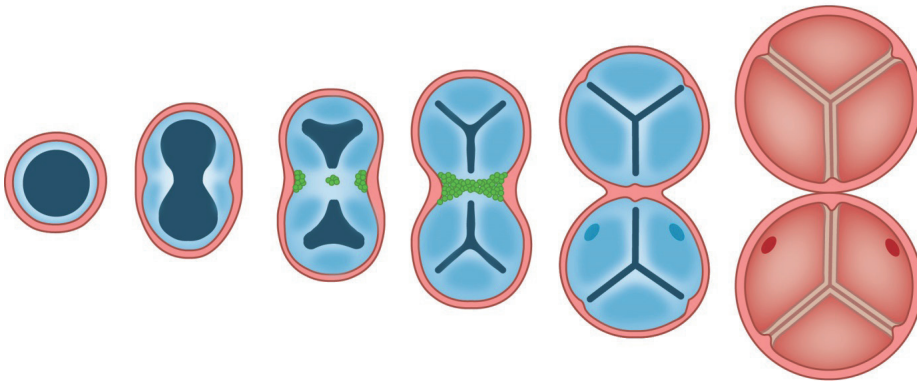
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Nederlandse samenvatting



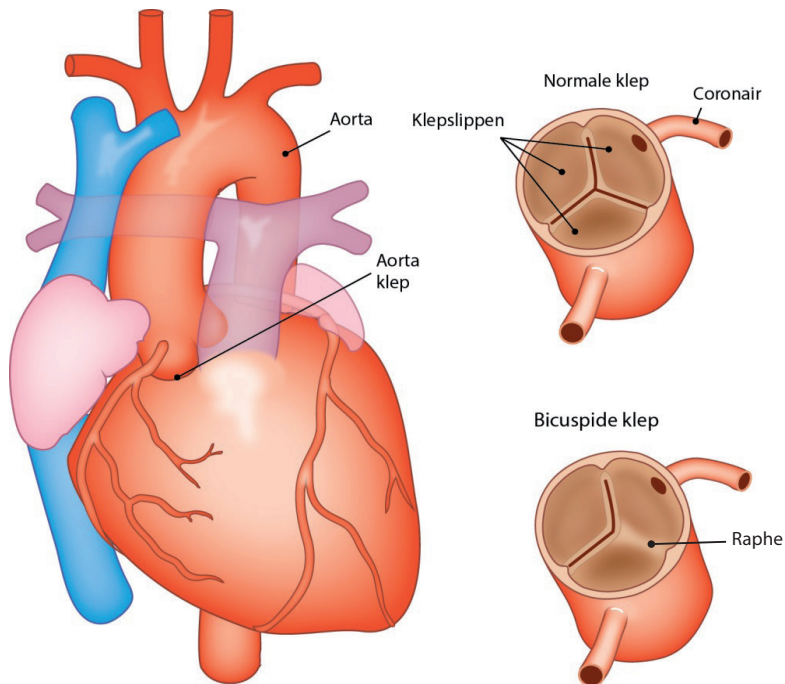
NEDERLANDSE SAMENVATTING

In dit proefschrift ligt de focus op de vorm van de bicuspide (tweeslippige) aortaklep in relatie tot de anatomie van de kransslagaders (coronairen), longslagader (pulmonalis) klep anatomie en complicaties. De introductie van het proefschrift geeft een beknopt overzicht van de ontwikkeling van het hart, de aortaklep en de kransslagaders. Tijdens de ontwikkeling ontstaan de aorta- en pulmonalisklep uit de zogenaamde endocard kussens in het uitstroom traject van het hart. Aanvankelijk is de uitstroom ongescheiden, d.w.z. dat er nog geen aparte aorta-en pulmonalisklep is. Gedurende de verdere ontwikkeling vindt splitsing plaats, waarna in de normale situatie tricuspide (3 slippige) aorta- en pulmonaliskleppen kunnen worden herkend (**Figuur 1**).



Figuur 1.

Een bicuspide aortaklep (bicuspid aortic valve (BAV)) komt voor in 1-2% van de bevolking, bijna 3 keer zo vaak bij mannen als bij vrouwen. In dit opzicht is het verrassend dat BAV ook een veel voorkomend probleem is in vrouwelijke patienten met Turner syndroom (incidentie 12-30%). Een bicuspide aortaklep bestaat uit slechts 2 (functionele) klepslippen. In veel gevallen kan een incomplete driedeling nog steeds worden herkend, door aanwezigheid van een raphe (richeltje ter plaatse van de fusie van 2 klepbladen, **figuur 2**).



Figuur 2.

Er bestaan verschillende subtypes van bicuspide aortakleppen, gebaseerd op de fusie van de klepslippen. Er zijn in de regel 2 klepholtes die een kransslagader (linker en rechter coronaire klepslip) bevatten en 1 zonder (non coronaire klepslip) (figuur 2). De belangrijkste kleptypes hebben we in dit proefschrift type 1, 2 en 3 genoemd. Type 1, met "fusie", dan wel incomplete splitsing, van de linker en rechter coronaire klepslip is het meest voorkomend; in type 2 zijn de rechter en non coronaire klepslippen gefuseerd en in het zeldzame type 3 is er fusie van de linker en non coronaire klepslip. Een verdere onderverdeling werd gemaakt op basis van aan- of afwezigheid van een raphe, d.w.z. of er een fusieline zichtbaar is (type A, figuur 2) of er sprake is van een "strikt bicuspide" klep zonder herkenbare fusieline (type B). In figuur 2 wordt een voorbeeld getoond van een type 1A klep.

Bicuspide aortakleppen zijn geassocieerd met complicaties, zoals klepdysfunctie en aorta verwijding. Ook zijn ze geassocieerd met afwijkingen van de kransslagaderen. In dit proefschrift was de hypothese dat het subtype BAV (dus type 1, 2 of 3, en type A of B) van belang was voor eventuele complicaties en afwijkingen van de kransslagaders, met in sommige gevallen een slechtere prognose. Ook zouden er, gezien de gedeeltelijk gezamenlijke ontwikkeling van aorta- en pulmonalisklep, in het geval van BAV ook

afwijkingen aan de pulmonalisklep verwacht kunnen worden, maar hier zijn nog weinig gegevens over bekend. Deze vraagstukken zijn onderwerp van dit proefschrift. Hieronder worden de belangrijkste bevindingen uiteengezet.

In **hoofdstuk 2** werden in 255 patiënten het klep subtype en de aorta afmetingen geëvalueerd middels echocardiografie. Hieruit bleek dat niet alleen het kleptype, maar ook de aan- of afwezigheid en zelfs de lengte van een raphe een rol speelde. Patiënten met BAV met een complete raphe hadden meer kans op klepdysfunctie (voornamelijk lekkage), vergeleken met BAVs met incomplete raphe. Patiënten met type 1A BAV en een complete raphe hadden een wijdere aortawortel dan de rest van de patiëntengroep. Dit zou kunnen impliceren dat patiënten met type 1A BAV en patiënten met BAV met een complete raphe vaker gecontroleerd moeten worden. Er werd ook een subgroep patiënten met een lokale vernauwing van de aorta (coarctatie van de aorta) geanalyseerd, waarbij bleek dat er in deze groep vaker incomplete raphes voorkwamen, geassocieerd met smallere diameters van de aortawortel en minder kleplekkage. De smallere diameters van de aortawortel zouden echter ook toegeschreven kan worden aan het feit dat de patiënten in deze subgroep jonger waren.

Hoofdstuk 3: beschrijft de kransslagader anatomie in 186 patiënten met BAV. In deze studie werd de aortaklep-anatomie gecorreleerd aan uitkomsten, zoals ernstig coronairlijden of het hebben ondergaan van dotter behandeling of bypass operatie. Patiënten met type 1B BAV bleken meer variatie in coronair anatomie te hebben, zoals een links dominant coronair systeem (d.w.z. dat de linker kransslagader het belangrijkste deel van het hart van zuurstof voorzien) of een linker kransslagader, die meteen bij de oorsprong splitst (gescheiden oorsprong). Patiënten met deze anatomie bleken ook een groter risico op het ontwikkelen van coronairlijden (bijv. vaatverkalking) te hebben. Patiënten met type 1B BAV moeten daarom mogelijk vaker gecontroleerd worden.

In **hoofdstuk 4** wordt de coronair anatomie beschreven in 84 harten met BAV en geassocieerde complexe aangeboren hartafwijkingen. De positie en hoogte van coronair openingen vanuit de aorta werd bekeken en gerelateerd aan BAV morfologie. Een hoge afgang van met name de linker kransslagader, bleek meer voor te komen in BAV met geassocieerde aangeboren hartafwijkingen dan in structureel normale harten en harten met geïsoleerde BAV (d.w.z. zonder bijkomende complexe aangeboren hartafwijkingen). Ook bleek dat afwijkingen aan het uitstroomtraject en een te kleine (hypoplastische) linker kamer geassocieerd waren met type 1A BAV en een hoge afgang van de coronairen. Kennis van de coronair anatomie is belangrijk om mogelijke complicaties tijdens chirurgische ingrepen te voorkomen.

Hoofdstuk 5 beschrijft de coronair anatomie in patiënten met Turner syndroom en BAV, patiënten met Turner syndroom en een tricuspidale aortaklep (TAV) en patiënten met geïsoleerde BAV. Er werden geen belangrijke verschillen in coronair anatomie gezien (ten aanzien van dominantie of directe splitsing van de linker kransslagader) tussen Turner patiënten met BAV en Turner patiënten met TAV. Er werd wel vaker een gescheiden oorsprong van de linker kransslagader gezien in patiënten met Turner syndroom en BAV vergeleken met patiënten met geïsoleerde BAV. Alle patiënten met Turner syndroom en BAV en gescheiden oorsprong van de linker kransslagader hadden type 1B BAV, dus zonder raphe. Er was een gelijke verdeling van links dominantie in type 1B BAV in de groep met Turner syndroom en BAV en de groep met geïsoleerde BAV, hetgeen suggereert dat een links dominante coronair anatomie een kenmerk is van type B BAV, onafhankelijk van de aanwezigheid van Turner syndroom. Tevens lijken zowel Turner syndroom als type 1B BAV onafhankelijk geassocieerd met gescheiden oorsprong van beide takken van de linker coronair arterie.

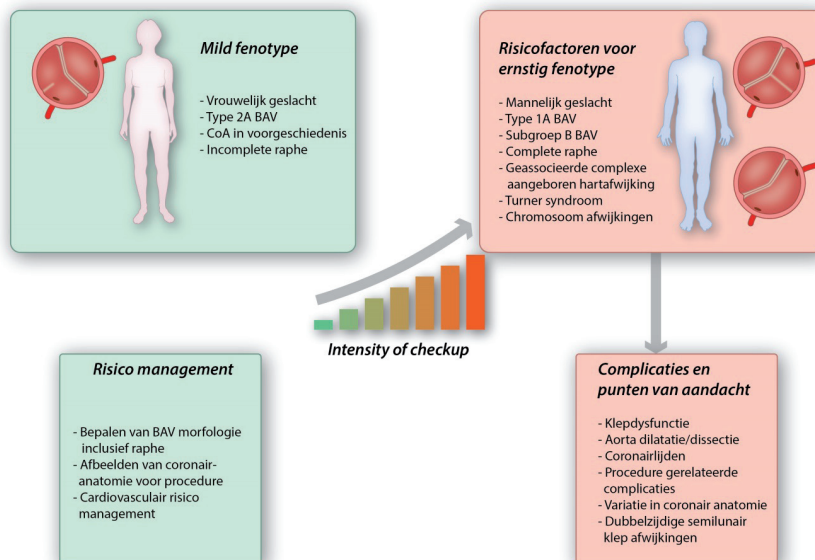
Hoofdstuk 6: in dit hoofdstuk wordt de morfologie van de pulmonalisklep beschreven in 83 harten met BAV en geassocieerde aangeboren hartafwijkingen. Door de deels gezamenlijke ontwikkeling van aorta- en pulmonalisklep, zouden afwijkingen aan de pulmonalisklep verwacht kunnen worden in patiënten met BAV. In dit hoofdstuk werd in 17% van de harten met BAV en geassocieerde aangeboren hartafwijking ook een abnormale morfologie van de pulmonalisklep gezien. De meerderheid van de harten met abnormale pulmonalisklep had een BAV zonder raphe (type B). Gecombineerde afwijkingen aan de aorta- en pulmonalisklep blijken ook geassocieerd met chromosomale afwijkingen (trisomie 13 en 18).

In het **laatste hoofdstuk** wordt een classificatie systeem voor anatomie van de kransslagaders beschreven. Bij een normale anatomie zijn er 2 kransslagaders, die in principe uit de linker en rechter klepholte van de aorta komen. De linker kransslagader splitst zich na een kort gezamenlijk beloop in 2 vaten, de rechter kransslagader bestaat uit 1 vat en is meestal het grootst (dominant). Het classificatie systeem is een modificatie van een eerder ontwikkeld systeem, genaamd de 'Leiden Convention'. Het classificatie systeem is toepasbaar op harten met een normale en met een afwijkende stand van de grote vaten. Ook de coronaire classificatie in harten met BAV wordt in dit hoofdstuk beschreven. Het beschrijven van coronair anatomie is belangrijk, vooral in geval van bijkomende aangeboren hartafwijkingen, om complicaties door afwijkende anatomie van de kransslagaders tijdens een operatie te voorkomen.

CONCLUSIE EN TOEKOMSTPERSPECTIEF

In dit proefschrift werd het subtype van bicuspide aortakleppen in relatie tot anatomie van de kransslagaders en anatomie van de pulmonalisklep bestudeerd. Het subtype BAV en in het bijzonder de aan- of afwezigheid van een raphe zijn van belang om klepdysfunctie en aorta problemen te kunnen voorspellen. Ook is het subtype BAV van belang als voorspeller van een mogelijk afwijkende anatomie van de kransslagaders, hetgeen weer van belang is bij eventuele chirurgische ingrepen. Tenslotte moet er bij BAV rekening worden gehouden met het bestaan van een eveneens afwijkende pulmonalisklep, hetgeen geassocieerd is met chromosomale afwijkingen. Hieronder wordt een werkmodel weergegeven, gebaseerd op de resultaten beschreven in dit proefschrift.

Werkmodel voor risico inschatting in BAV patiënten



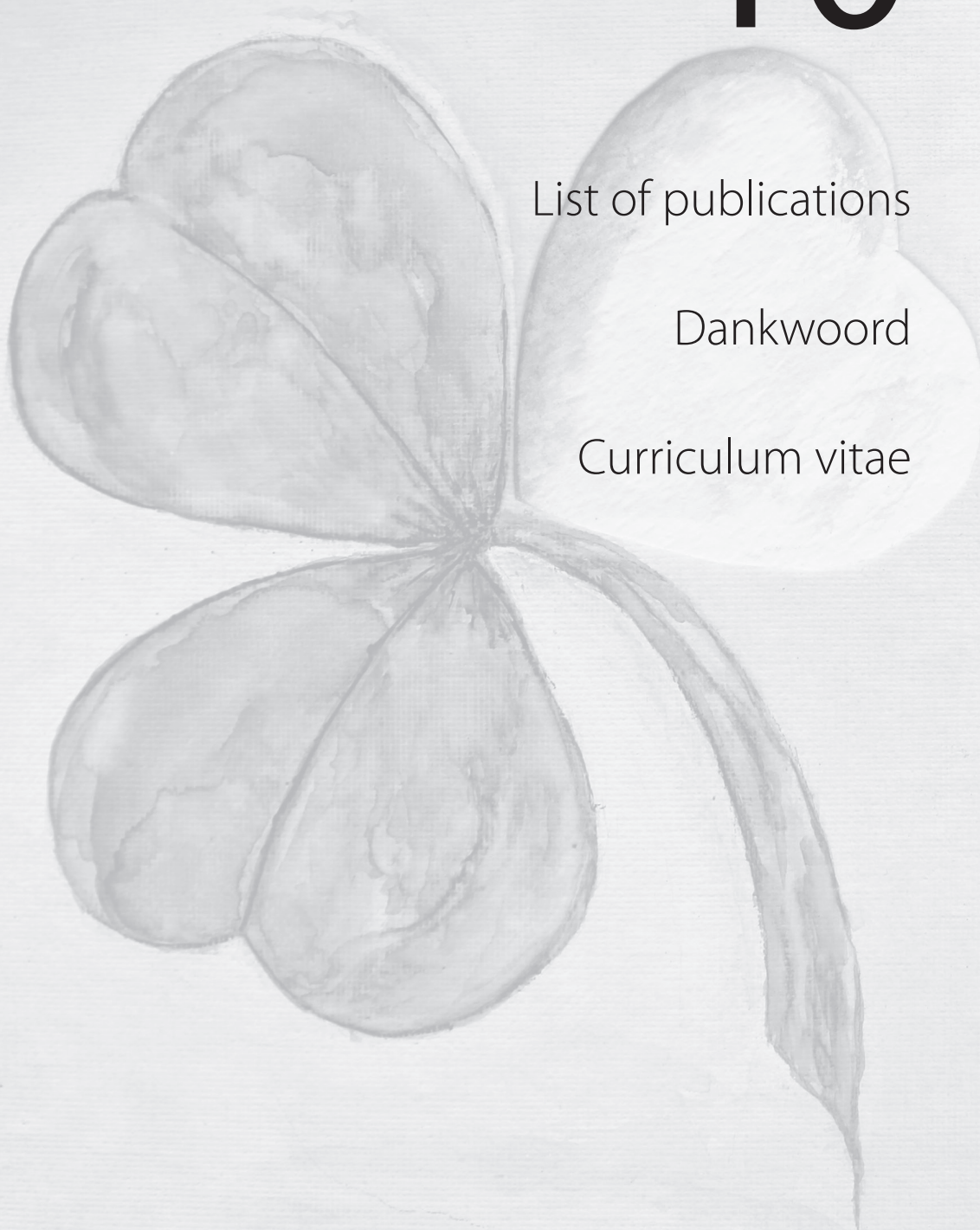
Toekomstig onderzoek zal focussen op de embryonale ontwikkeling van de aortaklep, aortawand en kransslagaders in diermodellen met BAV. Tevens zullen moleculaire signaleringspaden die betrokken zijn bij de vorming van bicuspide aortakleppen verder onderzocht worden in diermodellen. Deze studies zullen waarschijnlijk meer licht werpen op de tot nu toe onvoldoende begrepen associatie tussen bicuspide aortakleppen, aorta complicaties en coronair afwijkingen.

10

List of publications

Dankwoord

Curriculum vitae



LIST OF PUBLICATIONS

Koenraadt WMC, Siebelink HJ, Schalij MJ, van der Vlugt MJ, van den Bosch AE, Budde RPJ, Roos-Hesselink JW, Duijnhouwer AL, van den Hoven AT, DeRuiter MC, Jongbloed MRM. Coronary anatomy in Turner patients versus patients with isolated bicuspid aortic valves, *Heart* 2018, *In press*

Gittenberger-de Groot AC, **Koenraadt WMC**, Bartelings MM, Bökenkamp R, DeRuiter MC, Hazekamp M, Bogers AJJC, Quaegebeur JM, Schalij MJ, Vliegen HW, Poelmann RE, Jongbloed M. Coding of coronary arterial origin and branching in congenital heart disease: the modified Leiden Convention. *J Thorac Cardiovasc Surg.* 2018 Aug 18. pii: S0022-5223(18)32134-2

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CURRICULUM VITAE

Wilke M.C. Koenraadt werd geboren op 26 juli 1978 in Deurne, waar zij in 1997 haar eindexamen haalde aan het St. Willibrord Gymnasium. Aansluitend startte zij met de studie Geneeskunde in Maastricht. Na behalen van het artsexamen in 2003, heeft zij een jaar als spoedeisende hulp arts gewerkt in Australië. Nadien startte zij als arts assistent Interne Geneeskunde in het Groene Hart Ziekenhuis in Gouda. Daar werd haar enthousiasme voor het vak cardiologie aangewakkerd. Op 1 januari 2008 begon zij met haar opleiding cardiologie, die zij in 2013 afrondde. Daarna was zij als cardioloog werkzaam in het HMC en het Groene Hart Ziekenhuis. Eind 2016 heeft zij zich volledig toegelegd op het afronden van dit proefschrift (promotoren: prof. dr. M.J. Schalij en prof. dr. M.C. de Ruiter, co-promotor: dr. M.R.M. Jongbloed). Vanaf 2017 was zij hiernaast werkzaam als cardioloog in Cardiologie Centrum Voorschoten. Per 1 oktober 2018 werkt zij als cardioloog in ziekenhuis Gelderse Vallei in Ede.

