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# **VASCULAR FUNCTION IN BICUSPID AORTIC VALVE DISEASE**

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**Karolinska  
Institutet**

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“You must understand that there is more than one path to the top of the mountain” — Miyamoto Musashi, A Book of Five Rings.

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# **Vascular function in bicuspid aortic valve disease**

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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# ABSTRACT

The aortic valve normally consists of three cusps (tricuspid aortic valve, TAV), but in 0.5-2% of the population it consists of only two cusps (bicuspid aortic valve, BAV), which is the most common congenital cardiac malformation. BAV is prone to aortic stenosis (AS) and regurgitation (AR), and >25% of BAV patients will require surgery of the aortic valve and/or aorta within 20 years of diagnosis. BAV is associated with impaired function, dilation and dissection of the ascending aorta, while little is known about the abnormalities of the descending aorta in BAV.

The aims of this thesis were to investigate morphological and functional alterations in the aorta of BAV and TAV patients, with a focus on the descending aorta, and to assess the feasibility of a new ultrasound-based method for studies of the elastic properties of the aorta. This was performed using transesophageal echocardiography (TEE) in consecutive patients without significant coronary artery disease having aortic valve disease and/or ascending aortic aneurysms requiring surgery (>50 % with BAV).

In Study I ( $n = 300$ ), we examined if different phenotypes of BAV (according to surgical inspection) were associated with different types of ascending aortic dilation. We did not find any such association. Ascending aortic dilation was common in patients with AS and BAV but not with TAV.

In Study II ( $n = 85$ ), a new modality, Velocity Vector Imaging (VVI), which is based on speckle tracking, was evaluated for automated deformation analysis of the descending aorta using TEE images. The method was found to be feasible for aortic studies. We could compute elasticity indices of the aorta with low variability and a strong correlation to indices calculated with a standard method (M-mode).

In Study III ( $n = 192$ ), we used VVI to compare aortic elasticity between BAV and TAV patients. After correction for age, dimension of the ascending aorta, cholesterol, and stroke volume in a multivariable regression model, BAV was associated with lower strain and distensibility of the descending aorta in the AR group, and higher distensibility in the AS group.

In Study IV ( $n = 369$ ), we examined the intima-media thickness (IMT) in the descending aorta and found no difference between BAV and TAV. Thus, the functional alterations of the aorta found in Study III seem not to depend on structural wall changes. Furthermore, we could show that genetic markers (single nucleotide polymorphisms, SNPs), which influence IMT in the carotid artery seem to correlate to IMT in the descending aorta in patients with TAV.

In conclusion, we found no association between dilation of the ascending aorta and a specific BAV phenotype. We demonstrated that VVI technique is feasible for analysis of elastic properties of the aorta. In patients with AR, BAV was associated with lower strain and distensibility than TAV, suggesting impairment of the elastic aortic properties in the descending aorta. IMT was not influenced by presence of BAV.

# Populärvetenskaplig sammanfattning

Aortaklaffen består normalt av tre klaffblad (TAV), men hos 0,5-2 % av befolkningen är klaffen missbildad och har bara två klaffblad, bikuspid aortaklaff (BAV). Detta är den vanligaste medfödda hjärtnissbildningen och BAV är ofta associerad med klaffsjukdom i form av förträngning (aortastenosis, AS) eller läckage (aortaregurgitation, AR), och >25 % av patienter med BAV kommer att behöva genomgå kirurgi av aortaklaffen och/eller uppåtstående delen av aorta (stora kroppspulsådern) inom 20 år från att BAV upptäckts. BAV är kopplat till vidgning/aneurysm av uppåtstående delen av aorta, vilket ger en ökad risk för kärlväggsbristning, s.k. dissektion. Förändringar i den nedåtstående delen av aorta, dvs. mellan aortabågen och diafragma, är studerad i betydligt mindre utsträckning i BAV. Syftet med denna avhandling var att kartlägga morfologiska och funktionella förändringar i aorta hos BAV och TAV patienter med ny ekokardiografisk teknik för bestämning av elastiska egenskaper hos aorta.

I Studie I (300 patienter) undersökte vi med transesophagealt ultraljud om olika konfigurationer av BAV (enligt kirurgisk bedömning) är kopplade till grad och förekomst av olika former av aortavidgning. Vi fann ingen sådan koppling. Vidgning av uppåtstående delen av aorta var vanligare hos patienter med BAV och AS, än hos patienter med TAV och AS.

I Studie II (85 patienter) utvärderade vi ett nytt mätprogram (Velocity Vector Imaging, VVI) för automatisk beräkning av kärlstyvhet hos nedåtstående aorta. Vi kunde konstatera att VVI metoden är användbar, har liten spridning vid upprepade mätningar av kärlfunktion och ett starkt samband till kärlstyvhet beräknat med standard metod (M-mode).

I Studie III (192 patienter) använde vi VVI metoden för att beräkna kärlstyvheten hos patienter med BAV och TAV. Patienter med BAV och AR hade nedsatt elasticitet i nedåtstående aorta jämfört de med TAV. Detta talar för att elastiska kärlgenskaper vid BAV är påverkade även i den delen av aorta. Ålder var den viktigaste faktor som påverkade kärlstyvhet, följd av förekomst av BAV.

I Studie IV (369 patienter) undersökte vi tjockleken av intima-media komplexet (IMT) i kroppspulsådern mellan bågen och diafragma. Vi fann ingen signifikant skillnad i aortas IMT mellan BAV och TAV patienter, trots att vi i Studie III har visat skillnader i aortas funktion. Vi kunde även visa att genetiska markörer (SNPs) som påverkar vägg tjockleken i halspulsådern korrelerar till vägg tjockleken även i aorta hos patienter med TAV.

Sammanfattning: Vi fann inget samband mellan BAV konfiguration och dilatation av uppåtstående aorta. VVI tekniken kan användas för analyser av aortadeformation. Vid jämförelse med TAV hade BAV patienter med AR nedsatt funktion i den nedåtstående delen av aorta. IMT påverkades inte av förekomsten av BAV.

# LIST OF SCIENTIFIC PAPERS

- I. Jackson, V., Petrini, J., Caidahl, K., Eriksson, M. J., Liska, J., Eriksson, P., Franco-Cereceda, A. Bicuspid aortic valve leaflet morphology in relation to aortic root morphology: a study of 300 patients undergoing open-heart surgery. *Eur J Cardiothorac Surg*, 2011. 40(3): p. e118-24
- II. Petrini, J., Yousry, M., Rickenlund, A., Liska, J., Hamsten, A., Eriksson, P., Franco-Cereceda, A., Caidahl, K., Eriksson, M. J. The feasibility of velocity vector imaging by transesophageal echocardiography for assessment of elastic properties of the descending aorta in aortic valve disease. *J Am Soc Echocardiogr*, 2010. 23(9): p. 985-92.
- III. Petrini, J., Jenner, J., Rickenlund, A., Eriksson, P., Franco-Cereceda, A., Caidahl, K., Eriksson, M. J. Elastic properties of the descending aorta in patients with a bicuspid or tricuspid aortic valve and aortic valvular disease. *J Am Soc Echocardiogr*, 2014. 27(4): p. 393-404.
- IV. Petrini, J., Yousry, M., Björk, H. M., Eriksson, P., Rickenlund, A., Franco-Cereceda, A., Caidahl, K., Eriksson, M. J. Intima-media thickness of the descending aorta in patients with bicuspid or tricuspid aortic valves: *Manuscript*.

# CONTENTS

1	INTRODUCTION .....	1
1.1	Valves of the heart .....	1
1.2	Bicuspid aortic valve.....	1
1.3	Mechanical environment of the aortic valve .....	7
1.4	Clinical manifestations of bicuspid aortic valve disease .....	8
1.5	Aortopathy associated with BAV .....	9
1.6	Elastic properties of the aorta .....	11
1.7	Noninvasive imaging of aortic function.....	12
1.8	Two-dimensional strain imaging.....	13
1.9	Intima-media thickness .....	14
2	AIMS .....	17
3	PATIENTS AND METHODS .....	19
3.1	Patients .....	19
3.2	Study population of the thesis .....	21
3.3	Methods .....	22
3.4	Ethical considerations .....	31
4	RESULTS.....	33
4.1	Morphology of the aortic valve and aorta .....	33
4.2	Vascular function .....	38
4.3	Intima- media thickness of the descending aorta .....	44
4.4	Reproducibility .....	46



5	DISCUSSION .....	47
5.1	Morphology .....	47
5.2	Aortic function .....	51
5.3	Intima- media thickness of the descending aorta .....	52
5.4	Methodology .....	54
5.5	Limitations .....	57
6	FURTHER PERSPECTIVES .....	58
7	CONCLUSIONS.....	59
8	ACKNOWLEDGEMENTS.....	61
9	REFERENCES.....	63

# LIST OF ABBREVIATIONS

AR	Aortic regurgitation
AoIMT	Intima-media thickness of the descending aorta
AS	Aortic stenosis
ASAP	Advanced Study of Aortic Pathology
AVA	Aortic valve area
BAV	Bicuspid aortic valve
CIMT	Carotid intima-media thickness
CoA	Aortic coarctation
CT	Computed tomography
CV	Coefficient of variation
DBP	Diastolic blood pressure
ECM	Extracellular matrix
ICC	Intraclass correlation coefficient
IMT	Intima-media thickness
LN	Left- and noncoronary cusps fused in BAV
MRI	Magnetic resonance imaging
$P_{\max}$	Peak transvalvular pressure gradient
$P_{\text{mean}}$	Mean transvalvular pressure gradient
PWV	Pulse wave velocity
RL	Right-and left-coronary cusps fused in BAV
RN	Right-and non-coronary cusps fused in BAV
SAC	Systemic arterial compliance
SBP	Systolic blood pressure
SNP	Single nucleotide polymorphism

STJ	Sinotubular junction
SV	Sinus of Valsalva
TAA	Thoracic aortic aneurysm
TAV	Tricuspid aortic valve
TEE	Transesophageal echocardiography
TTE	Transthoracic echocardiography
VVI	Velocity Vector Imaging
UAV	Unicuspid aortic valve
Zva	Valvulo-arterial impedance



# 1 INTRODUCTION

## 1.1 Valves of the heart

The heart has four valves, ensuring that blood is flowing in the correct direction during the cardiac cycle. The aortic valve is located between the left ventricle and the ascending part of the aorta. This semilunar valve normally consists of three equally sized leaflets: a tricuspid aortic valve (TAV). In 0.5-2% of the population, the aortic valve consists of two leaflets, almost always unequally sized (92% of cases) [1]. This malformation is called a bicuspid aortic valve (BAV) and is the most common congenital cardiac anomaly, with a male predominance of 3:1 (Figure 1).

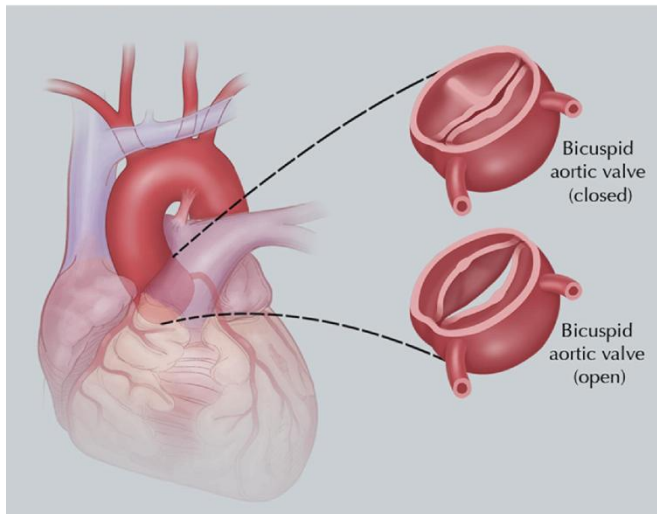


Figure 1. *Bicuspid aortic valve (BAV)*. Reprinted with permission from Elsevier [1].

## 1.2 Bicuspid aortic valve

There are a number of different classifications of BAV with regard to: the orientation of the leaflets [2], which particular cusps are fused/not separated [3] and the morphology of the ascending aorta, specifically the coronary sinuses [4]. In our studies, we have chosen to classify the BAV morphology according to which cusps are fused (Figure 2). Throughout the literature most bicuspid aortic

valves in humans demonstrate a continuity between the right- and the non-coronary cusps. The current understanding is that BAV is a congenital cardiac malformation and the misnomer ‘functionally bicuspid’ is no longer a valid description of an aortic valve.

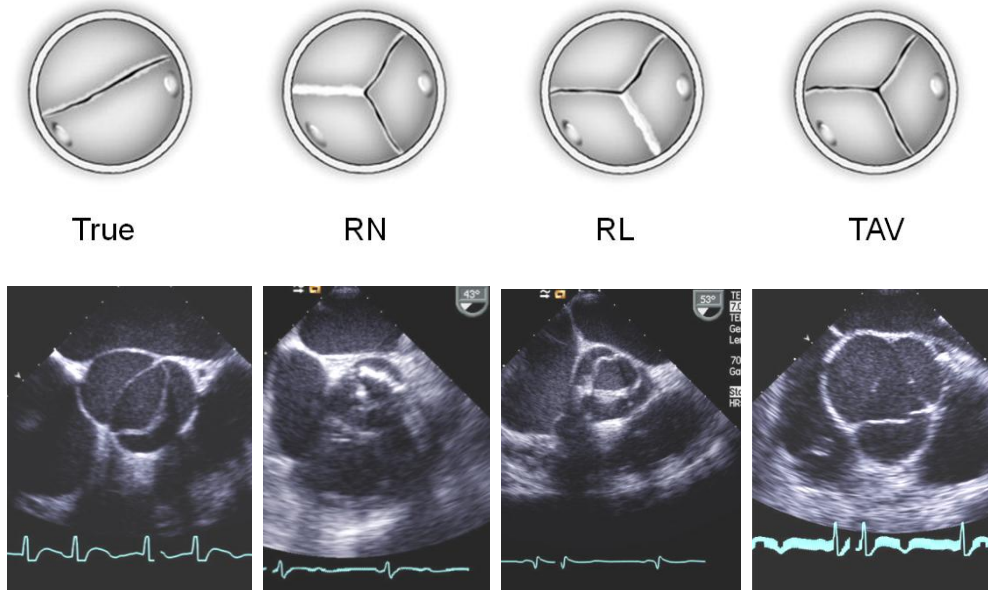


Figure 2. *Three phenotypes of BAV (True, RN - fusion of the right- and non-coronary cusps, RL – fusion of the right- and left-coronary cusps) and a TAV, are shown schematically\* and as images from transesophageal ultrasound. \*Adapted with permission from Elsevier [Study I].*

Malformation of the leaflets and excess tissue in a BAV may result in asymmetrical closure of the valve or valve prolapse, causing alterations to the aortic flow dynamics [5].

### 1.2.1 History

The first description of BAV can be found in the drawings of Leonardo da Vinci, who studied the human anatomy thoughtfully and thoroughly in the late 1490s and early 1500s. He made drawings of the aortic valve [6] including a bicuspid variant (Figure 3). In 1764, Hunter wrote “on examining the valves of the aorta, I found that there had been two only, instead of three; and that one of them had a kind of fraenum or cross-bar, attaching its middle to the sides of the artery” [7, 8]. However, the first formal and most recognized description of BAV is

attributed to Paget in 1844 [9], who noted “that in the majority of cases in which only two valves have been found in the aorta or pulmonary artery, those valves have been diseased, and often extremely diseased”.

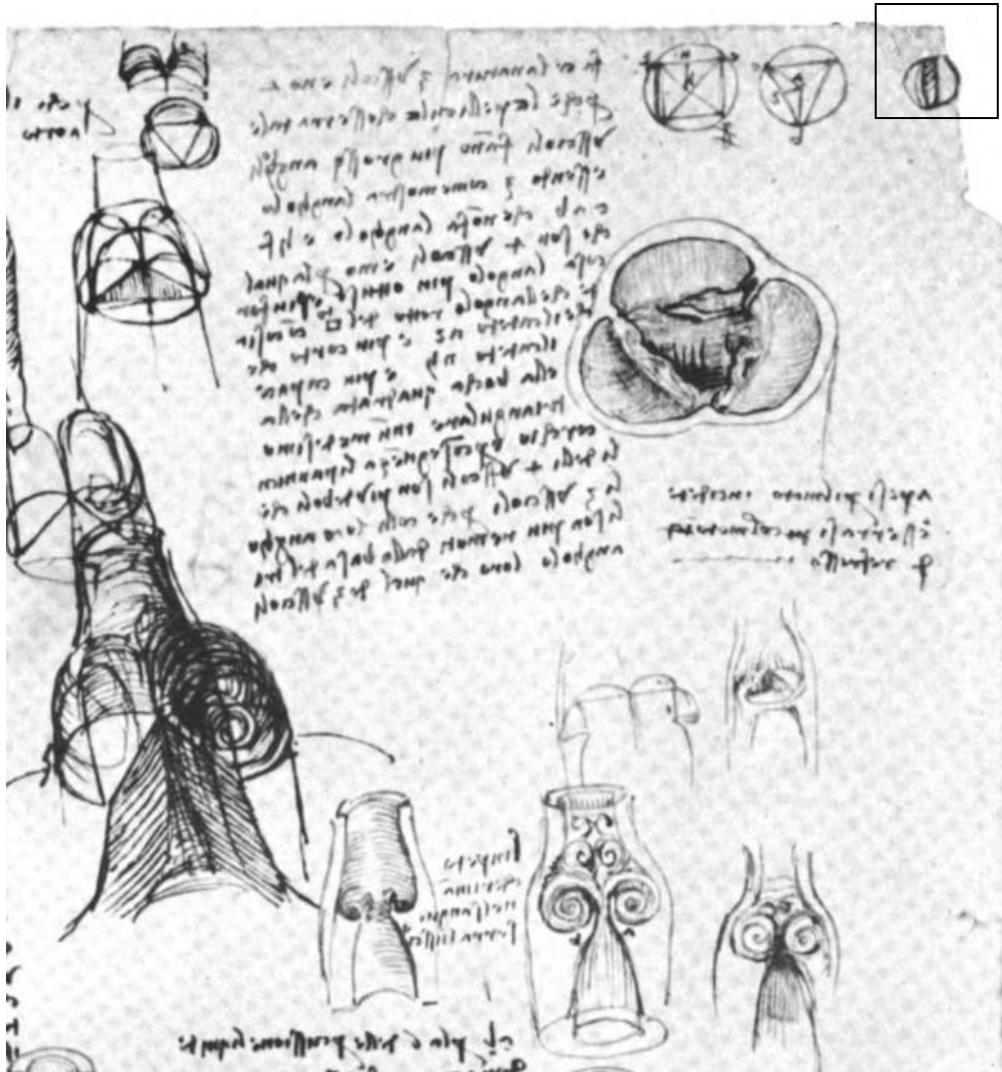


Figure 3. Leonardo da Vinci's drawings of the aortic valve with a BAV in the upper right corner. Available from Hathitrust.org [6].

In England in the mid-19th century Peacock also studied BAV [10] (Figure 4) and described its liability for valve regurgitation and stenosis. In Canada during the 1880s Osler [11] noted that BAV was prone to endocarditis [12, 13]. At the

same institution, Abbot [14] reported a BAV patient with dissection of the ascending aorta in a case with aortic coarctation, an association verified 50 years later in a larger necropsy study [15]. Today, we know that a majority of patients undergoing aortic valve replacement have BAV [16].

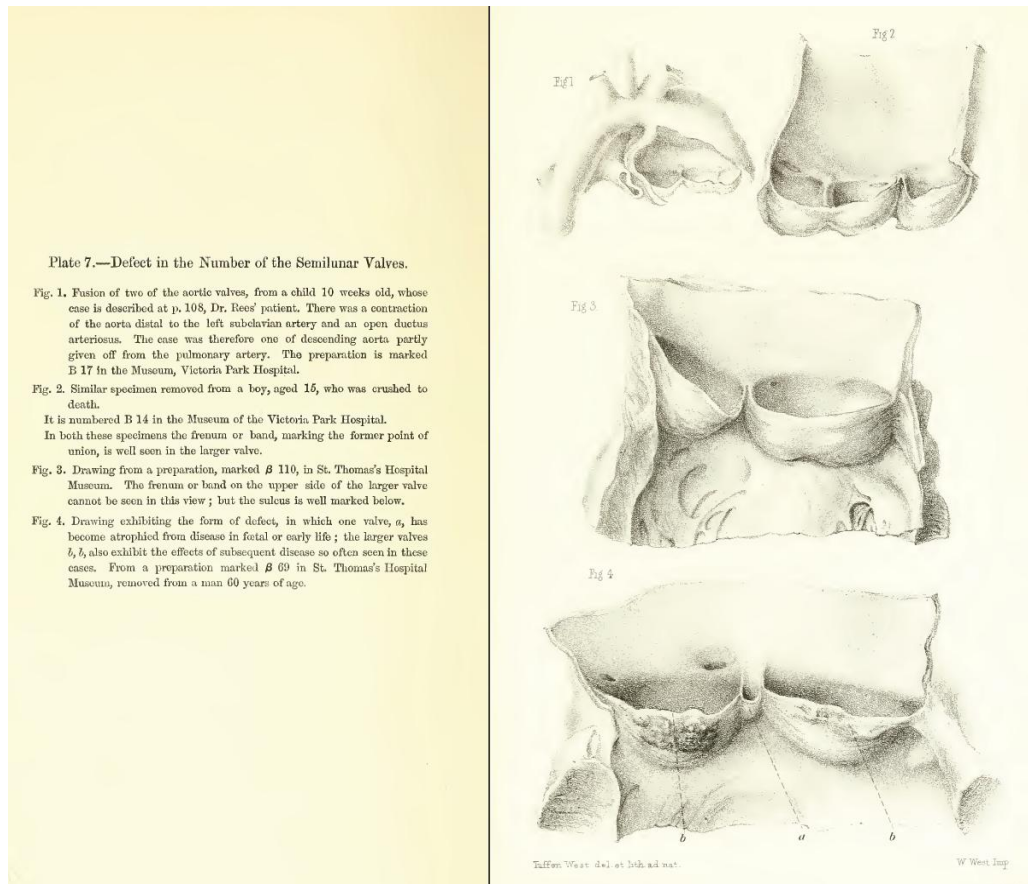


Figure 4. “Defect in the Number of Semilunar Valves” Peacock, 1866. Available from [Openlibrary.org](https://openlibrary.org) [10].

## 1.2.2 Genetics

The genetic susceptibility of BAV is far from being identified. Mutations present in specific families have been identified (Table 1) but the genetic variants involved in BAV formation are still unknown for the majority of population. No genome-wide association studies to identify genetic modifiers have been



published so far. Furthermore, whether the same genetic variants involved in the formation of BAV are also associated with increased risk of ascending aortic complications is still unknown. An inheritance pattern consistent with autosomal dominance with reduced penetrance and prevalence in the first degree relatives of BAV patients, has been demonstrated as high as 9% in families studied [17]. Different phenotypes of BAV can be seen in different animal models, indicating that they depend on different genotypes and processes [18], as described below. BAV aortopathy is not a monogenetic disease like Marfan syndrome, but is associated with many different mutations [19]. BAV is also associated with other genetic syndromes, such as Turner (X0), Shone's and Williams syndromes.

Table 1. *Genes associated with bicuspid aortic valve.*

Gene	Gene name	Chromosome	Human disease
45, X	Unidentified	X0	Turner syndrome
5q, 9q34, 13q, 15q25.1–26, 18q	Undefined	5, 9, 13, 15, 18	BAV
ACTA2	$\alpha$ -smooth muscle actin	10	Familial aortic aneurysm, Type 6
AXIN1	Axin-1	16	BAV
ELN	Elastin	7	Cutis laxa/BAV
ENG	Endoglin	9	Hereditary haemorrhagic telangiectasia
FBN1	Fibrillin-1	15	Marfan syndrome
FGF8	Fibroblast growth factor 8	10	BAV
FN1	Fibronectin-1	2	BAV
GATA5	GATA binding protein 5	20	BAV
HOXA1	Homeobox A1	7	BAV
JAG1	JAGGED1	20	Alagille syndrome; tetralogy of Fallot
KCNJ2	Potassium inwardly rectifying channel J2	17	Andersen syndrome
NKX2.5	NKX2-5	5	Hypoplastic left heart syndrome 2
NOS3	Endothelial nitric oxide synthase	7	BAV
NOTCH1	NOTCH1	9	BAV
PDIA2	Protein disulphide isomerase A2	16	BAV
Transforming growth factor beta receptor 1	Transforming growth factor- $\beta$ receptor type 1	9	Loeys-Dietz syndrome/sporadic BAV
Transforming growth factor beta receptor 2	Transforming growth factor- $\beta$ receptor type 2	3	Loeys-Dietz syndrome/sporadic BAV
UFD1L	Ubiquitin fusion degradation 1 like	22	Functional BAV and AA
UFD1L	Ubiquitin fusion degradation 1 like	22	BAV

BAV: bicuspid aortic valve; AA: aortic aneurysm.

*Reprinted with permission from Oxford University Press [19].*

### 1.2.3 Embryology

The endocardial cushion forms the left and right ventricular outflow tracts and the intrapericardial components of the aorta and the pulmonary trunk. The semilunar valve leaflets are cavitated from the valve cushions as shown schematically and in a histological section in Figure 5a and 5b [20, 21]. The exact process that leads to BAV formation is not entirely clear. In Syrian hamsters (see photo on the cover), fusion of valve cushions (RN) is a key factor [22], but the degree of fusion (phenotype) varies within the same genotype [23]. In mice, dysfunction of Rho kinase in neural crest cells located in the valve cushions leads to fusion or misplacement of the valve cushions, resulting in a variety of BAV phenotypes [24], whereas nitric oxide knockout mice have RL BAV [18] (phenotypes of BAV, as seen in Figure 2).

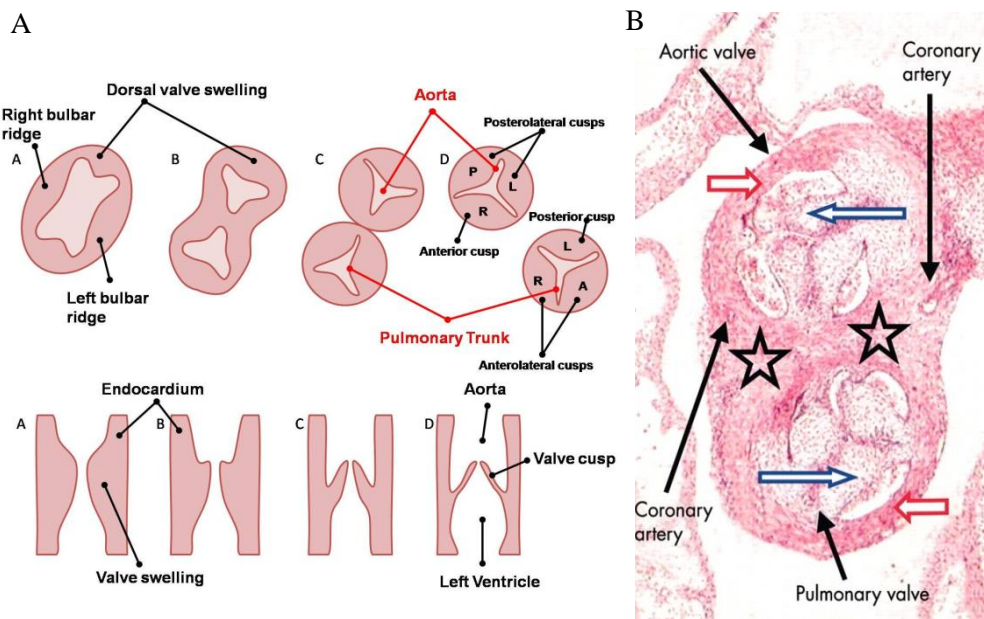


Figure 5. Atrioventricular-valve formation gestation week 5-7(a). Human embryo at Carnegie stage 22 (day 55), ☆ = fused proximal cushions, ⇨ = walls of sinus, ⇦ = valvular leaflets (b). Reprinted with permission from Dr. Mark Hill [21] and the BMJ Publishing Group [20].

### 1.3 Mechanical environment of the aortic valve

In a normally functioning aortic valve, the pressure gradient opening the valve during systole is only a few millimeters of mercury causing a laminar shear on the ventricular side of the valve. Along the aortic wall, the pressure difference causes reverse flow, resulting in vortices in the sinuses of Valsalva behind the aortic valve leaflets that help to close the leaflets in diastole and facilitating coronary flow (Figure 6a). The pressure difference between the aortic root and the left ventricle in diastole is about 80 mmHg at rest (Figure 6b) [25]. In a normally functioning BAV an eccentric jet creates higher shear stresses on both the ventricular and the aortic side of the leaflets [26]. In addition folding/unfolding of valve tissue to match the imperfect valve geometry in BAV generates high mechanical stress on the leaflets throughout the cardiac cycle [27].

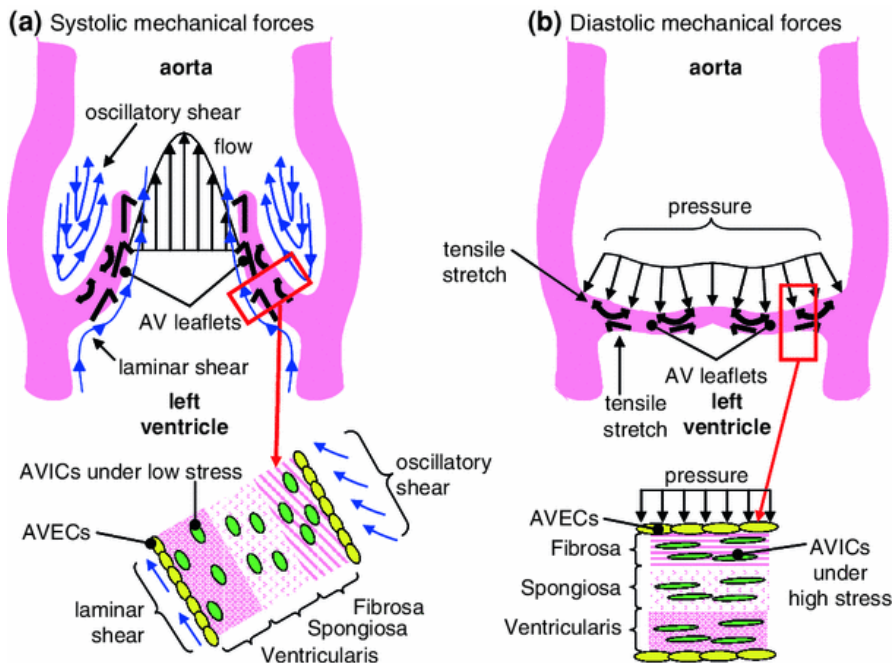


Figure 6. *Mechanical stimuli experienced by the AV endothelial cells (AVEC) and interstitial cells (AVIC) during a cardiac cycle: systolic mechanical forces (a) and diastolic mechanical forces (b). Reprinted with permission of the Biomedical Engineering Society [25].*

This might contribute to the faster progression of aortic valve disease in BAV, as repeated stretching of aortic valve tissue induces apoptosis and calcification processes [28]. Aortic stenosis (AS) generates a high pressure gradient in systole and thus high shear stresses, whereas aortic regurgitation (AR) means a high velocity flow over the valve in both systole and diastole, leading to a vicious circle of increased shear stress in both phases.

## **1.4 Clinical manifestations of bicuspid aortic valve disease**

While the BAV is usually an isolated defect, it may also coexist with other congenital cardiovascular malformations [29]. The most common of these is aortic coarctation (CoA), a narrowing of the proximal descending aorta, typically located near the attachment of ligamentum arteriosum. The localized constriction is made up of smooth muscle cells, fibrous and elastic tissue similar to that found in ductus arteriosus [30]. Although >50% of patients with CoA have BAV, it is much more uncommon with CoA in the BAV population [31]. Other associated congenital malformations include ventricular septal defects, patent ductus arteriosus, atrial septal defects and displaced coronary ostia. In Turner's syndrome (X0), BAV is noted in up to 30% of cases, while about 10% of patients with Williams syndrome have BAV.

Although BAV patients constitute only 0.5-2% of general population, > 50% of patients requiring valve replacement for aortic stenosis have BAV and present at a younger age compared with TAV [16]. Data show that at least 25% of BAV patients will require aortic valve and/or ascending aortic surgery within 20 years of diagnosis [32]. Only 30% of BAV cases seen in autopsy studies have no signs of aortic valve dysfunction [2]. The risk for endocarditis has been estimated to be as high as 30% in older case series [33], but in more recent studies the risk has been estimated to be much lower at 0.2-2% [32, 34].

The relative risk for ascending aortic dissection in BAV compared with TAV was eight times in a recent large cohort study [31]. Although this represents a much lower lifetime risk of dissection than in patients with Marfan syndrome, BAV will still be responsible for a greater number of dissections because of a

much higher prevalence of BAV in the population [31]. However, it is important to note that, despite these associated comorbidities, life expectancy in asymptomatic BAV patients is not shortened in comparison with the general population [32, 34].

## **1.5 Aortopathy associated with BAV**

The vessel wall in the arteries is composed of three different layers. The inner layer, called tunica intima, consists of the endothelium. The intermediate layer is called the tunica media and is formed mainly by smooth muscle cells and connective tissue (elastic and collagen fibers). In the aorta, elastic fibers are predominant, moderating the high flow and high blood pressure the aortic wall is subjected to. The outer layer is called the tunica adventitia and is formed by connective tissue and fibroblasts.

In the media, the extracellular matrix (ECM) plays an important role in maintaining tissue elasticity and linking vascular smooth muscle cells and elastin/collagen fibrils. Vascular smooth muscle cells produce and maintain the ECM [35] and have the same embryological origin in the aortic root, ascending aorta and the aortic arch, in addition to the pulmonary trunk constituting the classic anatomic boundaries of BAV disease [35-37]. In BAV patients, the ECM structure is altered in the ascending aorta [38], associated with increased activity of proteolytic enzymes [39]. In dilated ascending aortas of BAV patients the collagen orientation is the same, collagen turnover is increased and collagen cross-linking lower compared with TAV [40, 41]. Collagen stiffness is higher and the grade of inflammation is lower in dilated ascending aortas of BAV compared with TAV [40, 42]. There seems to be two different pathways, that differ in genes as well as RNA and protein expression, to ascending aortic dilation in BAV and TAV [43].

Ascending aortic dilation occurs more frequently and at a younger age in BAV than TAV patients. The prevalence of the aortic root and ascending aortic dilation in BAV varies from 15-88%, depending on the definition and age of the

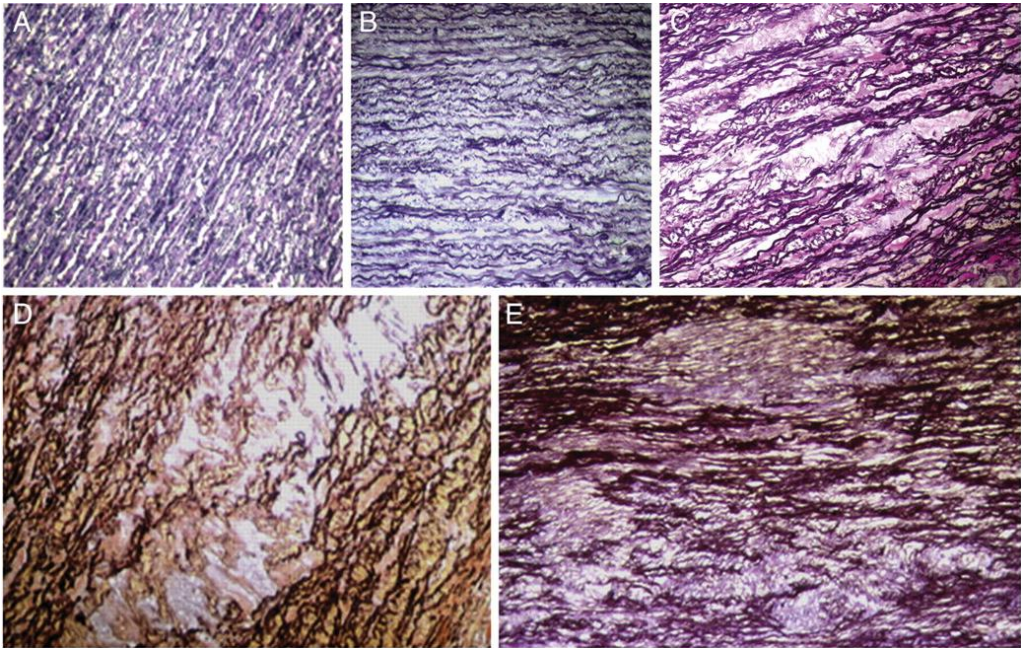


Figure 7. *Increasing grades of aortic medial disease in BAV (Weigert's elastic stain–Van Gieson stain). A: normal, B: grade 1, C: grade 2, D: grade 3, E: grade 4/4. Reprinted with permission from Oxford University Press [44].*

population studied [37, 45, 46]. The histopathological picture (Figure 7) shows media degeneration with the loss of smooth muscle cells, degraded elastic laminae, fibrosis and collapse of the elastic plates [47], leading to decreased elasticity.

Whether the aortopathy in BAV is caused by a genetic predisposition and/or a result from altered blood flow is still debated, with proponents of both theories.

### 1.5.1 Genetic theory

There are many reports about the heritability of the BAV itself. First-degree relatives of BAV patients have wider ascending aortas [48], and children with BAV without significant valve dysfunction have impaired elastic properties of the ascending aorta [49]. Furthermore, in patients with BAV, the presence of media degeneration in the pulmonary trunk has been described [50], a region that can hardly be affected by flow disturbances in the aorta. On the other hand, although ascending aorta has the same embryological origin [36] as the aortic valve it remains uncertain if the aortopathy is inherited in the same way.



### **1.5.2 Flow/shear stress theory**

MRI studies have described abnormal systolic helical flow patterns in the ascending aorta of BAV patients, regardless of valve stenosis or regurgitation, with different flow jets for different phenotypes of BAV [51]. It has been shown that altered helicity of the flow can be seen in the entire thoracic aorta in BAV patients [52]. Wall shear stress in the ascending aorta is dependent on flow patterns, secondary to the valve morphology [53].

One influential report showed that BAV patients with moderate dilation of the ascending aorta have a higher risk of aortic complications, even after aortic valve replacement [54], compared with TAV patients. This has not been replicated in more recent studies [55]. The descending aorta seems spared from aneurysms and/or dissection in BAV patients [56].

A combination of genetic, flow and environmental factors is probably responsible for BAV aortopathy [19]. Hitherto, the majority of reports about aortopathy in BAV have only investigated the ascending aorta. Since there is a lack of knowledge of the morphology and function of the descending aorta in BAV, we have focused on this topic in our studies.

## **1.6 Elastic properties of the aorta**

The aorta and its major branches act as an elastic reservoir for cardiac pulsations, converting stroke volumes into a steady flow. Indices of local arterial elasticity take into account the diameter or area change from diastole to systole (strain), the force (pulse pressure) needed for the given diameter or area change (stiffness), or the diameter or area change as a result of pulse pressure (distensibility). The carotid–femoral pulse wave velocity (PWV) is widely used and accepted as the gold standard for assessment of regional and global stiffness [57].

Arterial stiffness may be altered by changes in the composition of the aortic wall regarding smooth muscle cells, extracellular matrix and fibrosis, as well as the elastin and collagen content [58]. Pathophysiologically, increased stiffness leads to a change in pulse reflection, increasing systolic blood pressure and afterload on the left ventricle, as well as myocardial oxygen demand. The same

mechanisms reduce diastolic blood pressure, therefore, also the coronary perfusion pressure.

Arterial stiffness has been associated with known cardiovascular risk factors, such as obesity, smoking, hypercholesterolemia [59-61] and with hypertrophy and diastolic dysfunction of the left ventricle [62-64]. A number of studies have reported the independent predictive value of arterial stiffness for coronary heart disease, cardiovascular events, cardiovascular mortality and all cause mortality [57].

In BAV, abnormalities in the aortic wall, including histopathological changes, alterations in the metabolism, biology, and gene expression in smooth muscle cells, affect the stiffness as well as other functional measures (section 1.5).

### 1.7 Noninvasive imaging of aortic function

Local aortic stiffness has been evaluated using different techniques, such as transthoracic echocardiography (TTE), transesophageal echocardiography (TEE), transabdominal ultrasound, magnetic resonance imaging (MRI) and computed tomography (CT) [65-71] (Table 2).

M-mode (TTE and TEE) and echo tracking ultrasound, the prevailing ultrasound techniques for elasticity measurement, are based on the assumption that the diameter changes measured represent the motion of the whole circumference of the vessel. Tissue Doppler, although having the advantage of a very high frame rate (> 130–180 Hz), is an angle-dependent technique and only allows the near and far wall to be investigated. CT and MRI have limitations in spatial and temporal resolution and may require nephrotoxic contrast agents.

Table 2. *Indices of local arterial elasticity.*

	Definition	Techniques
<b>Strain</b>	$100 * (\Delta \text{Aortic Diameter} / \text{Diastolic Aortic Diameter})$	CT, MRI,TTE,TEE,US
<b>Stiffness</b>	$\ln(\text{SBP} - \text{DBP}) / (\Delta \text{Aortic Diameter} / \text{Diastolic Aortic Diameter})$	CT, MRI,TTE,TEE,US
<b>Distensibility</b>	$\Delta \text{Aortic Area} / (\text{Diastolic Aortic Area} * \text{Pulse Pressure})$	CT, MRI,TTE,TEE,US



## 1.8 Two-dimensional strain imaging

Strain is a dimensionless parameter representing the deformation of an object relative to its original shape. In an ultrasound image there are natural acoustic markers, referred to as speckles. By identifying these speckles throughout the cardiac cycle, the relative displacement between the speckles can be calculated. Speckles can be followed (tracked) in any direction within the imaging plane (but not in the z-plane) (Figure 8). The temporal resolution is defined by the frame rate (images/second), optimally 70-80 frames/s [72].

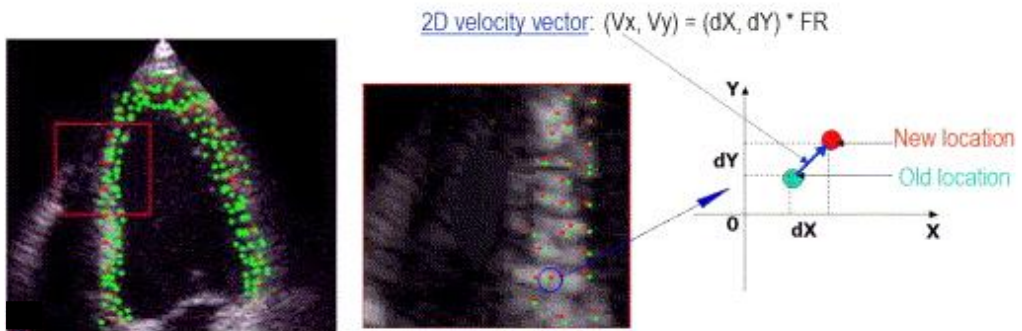


Figure 8. *Principle of speckle tracking used in 2-D strain assessment. Reprinted with permission from Elsevier [72].*

Two-dimensional strain is feasible for circumferential deformation parameters in contrast to the Doppler-based methods [73]. Two-dimensional strain has been validated in animal models using sonomicrometry and MRI [74, 75]. Velocity Vector Imaging (VVI) is a commercially available software combining speckle tracking and tissue border detection [76] (Figure 15).

VVI with ultrasonography [77] and MRI [78] has been used recently to assess the elastic properties of the carotid artery in different populations. VVI has also been used to study the wall motion of both the ascending and descending aorta in other patient groups [79, 80]. The proximity of the esophagus to the descending aorta makes it feasible to acquire TEE images with high temporal and spatial resolution.

## 1.9 Intima-media thickness

Intimal thickening occurs early in the atherosclerotic process but is also seen as a response to non-laminar flow with intimal hyperplasia, migration of smooth muscle cells and monocytes [81, 82].

There is no generally accepted *in vivo* method to measure only the intima. However, the thickness of the intima-media complex (IMT) can be measured with ultrasound and correlates well with histology (Figure 9). It is associated with cardiovascular risk factors [83-85] and increased IMT has been shown to result from flow induced remodeling [86]. Non-laminar flow has been described in the descending aorta in BAV [52], but the IMT of BAV patients in this region has not been studied.

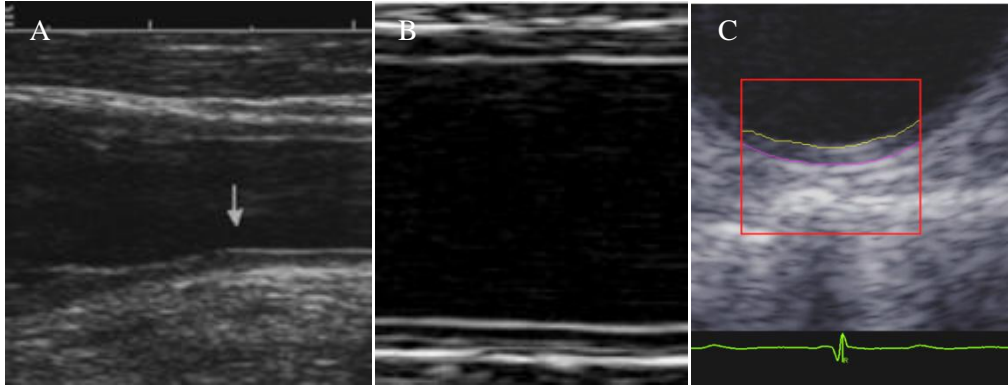


Figure 9. *Schematic drawing of the artery wall and its layers. Reprinted with permission from John Wiley and Sons [87].*

In 1986, Pignoli published the first study on ultrasound measurements of intima plus media, where they validated the double-line pattern seen on *in vivo* vessel ultrasound, with pathological specimen of the abdominal aorta [88]. Computerized measurement of the carotid IMT (CIMT) along a 10 mm-wide segment was introduced in the early 1990s [89, 90], and this is now available in most vascular ultrasound equipments using a variety of algorithms [91, 92].

CIMT is associated with cardiovascular risk factors and is an established marker of subclinical atherosclerosis [93]. It is also used for risk stratification of individuals and as an endpoint in intervention studies [94]. There is a covariance between CIMT and IMT in different vessels including the abdominal aorta [95]. Significant correlations between CIMT and descending aortic plaques, descending aortic intima-media thickness (AoIMT), aortic wall volume and coronary artery disease have been reported [83, 96, 97]. Statin treatment may

decrease not only CIMT but also AoIMT [98]. The examples of ultrasound imaging of IMT in different vessels are shown in Figure 10.



A: Diameter ( $\emptyset$ ) = 7mm, 7 MHz; B:  $\emptyset$  = 1.5mm, 55 MHz; C:  $\emptyset$  = 24 mm, 7MHz.

Figure 10. A: *Intima-media thickness in the carotid artery*, B: *radial artery* and C: *descending aorta*. Reprinted with permission (A and B) from John Wiley and Sons and Elsevier [84, 87] and (C) from Study IV.

In the descending aorta IMT has been measured previously using calipers [99], while backscatter analysis has been used for tissue characterization of plaques [98] from TEE images. Aortic wall volume [96] and wall thickness measurements by MRI have been reported [100]. CT is a sensitive method for detecting calcifications in the thoracic aorta [101]. Hitherto, there is only one report on using a semiautomatic edge-detection program for measuring IMT in the abdominal aorta [102]. TEE allows, like ultrasound investigations of the carotid artery, measurements along a 10-mm wide segment with excellent measurement error ( $<0.01$  mm) using 125 measuring points [87].



## 2 AIMS

The overall aim of this thesis was to study the factors influencing dilation of the ascending aorta and vascular structure and function in the descending aorta in patients with BAV, using novel noninvasive ultrasound methods.

Specific aims:

1. To explore whether a specific BAV phenotype or valve pathology is associated with a specific morphology of the ascending aorta (Study I).
2. To evaluate the feasibility of a new ultrasound-based speckle tracking method for studies of elastic properties of the descending thoracic aorta in patients with aortic valve disease (Study II).
3. To compare the elastic properties of the descending thoracic aorta in BAV and TAV, with reference to the type of aortic valve disease and known cardiovascular risk factors (Study III).
4. To study whether the intima-media thickness of the descending thoracic aorta is associated with aortic valve morphology (BAV/TAV), type of valve disease, known cardiovascular risk and genetic factors (Study IV).



### 3 PATIENTS AND METHODS

#### 3.1 Patients

All patients included in the studies of this thesis were recruited from the Advanced Study of Aortic Pathology (ASAP). This prospective study recruited 600 consecutive patients, (provided they were willing to participate and free of coronary artery disease according to coronary angiography) undergoing elective cardiac surgery because of aortic valve disease and/or pathology of the aortic root and/or ascending aorta at the Cardiothoracic Surgery Unit at the Karolinska University Hospital during 2007-2013 (Figure 11).



Figure 11. *Exclusion criteria were: age < 18 years; inability to give informed consent; significant coronary artery disease (i.e., significant stenosis on coronary angiogram); other concomitant valve surgery indicated; acute intervention indicated; previous cardiac surgery or blood-borne infection. \*These patients were excluded after inclusion due to logistic reasons (n = 4), discovery of existing exclusion criteria (n = 7), other medical reasons (n = 6), and patient withdrawal (n = 10).*

The preoperative screening included medical history, cardiovascular risk factor profile, medication, blood work and echocardiography. Patients who needed further evaluation of the aorta also underwent a preoperative CT scan.



### 3.2 Study population of the thesis

As shown in Figure 12 the patient population of studies II and III consisted of subpopulations of the patients in Study I, whereas Study IV included the first 400 operated ASAP patients, in whom AoIMT measurement was feasible.

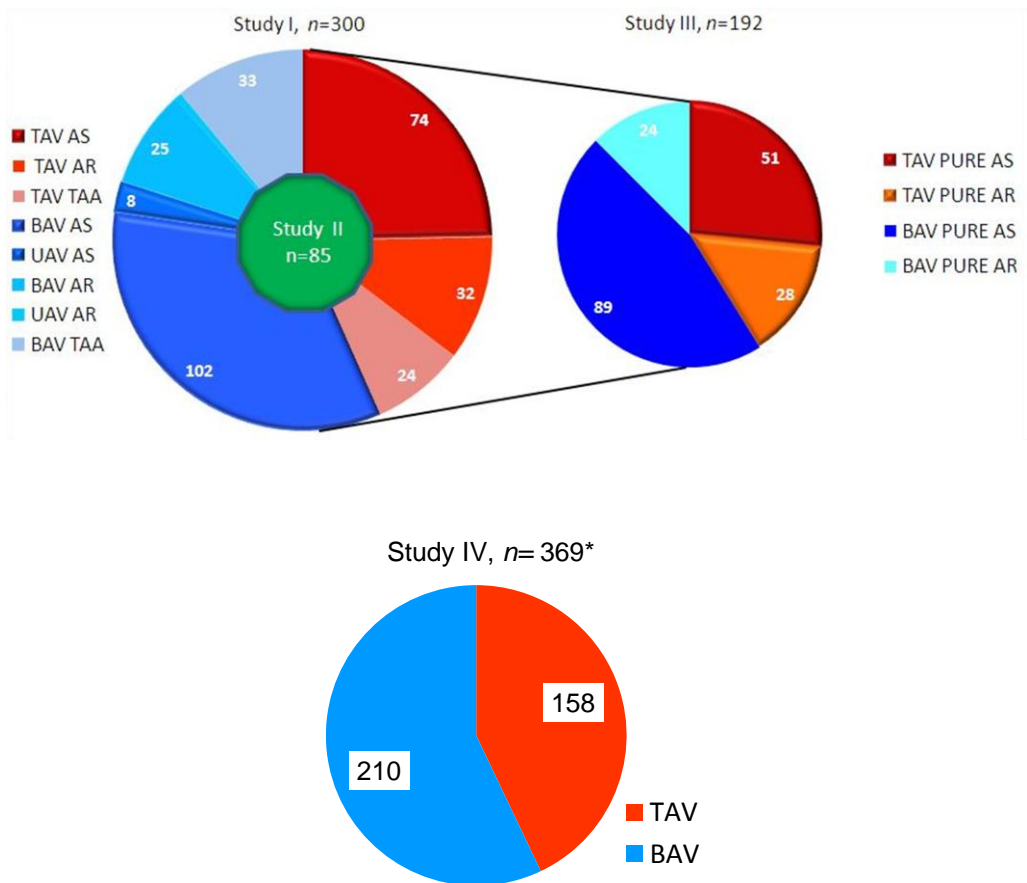


Figure 12. In Study I, the first 300 consecutive patients were included. Study II included the first 85 consecutive patients where VVI measurements were feasible. Study III included patients with 'true'/isolated valve lesions from the first 300 patients and where VVI measurements were feasible. Study IV included finally 369\* of 400 patients, where measurement of intima-media thickness in the descending aorta was feasible. \*One valve was unclassified.

### **3.3 Methods**

#### **3.3.1 Surgical inspection of BAV/TAV**

All patients were operated on through a midline sternotomy using cardio-pulmonary bypass with a centrifugal pump. The morphology of the aortic valve was evaluated by visual inspection of the valve during surgery. Based on the appearance, the valve was classified according to the number of cusps and commissures. Three cusps and three commissures denoted a ‘tricuspid’ valve. Two cusps and two commissures denoted a ‘bicuspid’ valve (if a remnant commissural raphe was present) or ‘true bicuspid’ valve (if no raphe was present). A single cusp and a single commissure denote a ‘unicuspid’ valve (UAV). The BAV was further classified according to which cusps were fused: right- and left-coronary cusps (RL), right- and non-coronary cusps (RN) or left- and non-coronary cusps (LN) (Figure 2).

The morphologic phenotype of the BAV was also classified using TEE. Eleven patients were classified by the surgeon as having UAV. However, on a close transesophageal echocardiographic image they showed two raphe extending toward the aortic wall. These valves were all BAV type 2 according to the classification of Sievers and Schmidtke [103], and there was no UAV with one commissure and no raphe on TEE. Therefore, we included these patients in the BAV group in studies II, III and IV.

#### **3.3.2 Transthoracic echocardiography**

All patients were examined by transthoracic echocardiography before surgery, using a Philips iE33 ultrasound scanner (Philips Medical Systems, Bothell, WA). Two-dimensional echocardiography [104] and Doppler measurements were performed according to the standards set out by the American Society of Echocardiography (ASE). In patients with aortic stenosis (AS), systolic transvalvular velocity was measured by continuous wave Doppler. The peak transvalvular pressure gradient ( $P_{\max}$ ) was calculated by applying the Bernoulli equation, whereas the mean transvalvular pressure gradient ( $P_{\text{mean}}$ ) was calculated by averaging the instantaneous gradients over the ejection period. The stroke volume and aortic valve area (AVA) were calculated according to the continuity equation [105].

Definitions and echocardiographic criteria of valve pathology used in study I to IV are shown in Table 3.

Aortic regurgitation (AR) was evaluated using the pressure half-time, jet density, diastolic flow reversal in the descending aorta, AR color flow jet area, and vena contracta. In studies II, III and IV, AR was classified into AR grades 0 to 3 (0, none; 1, mild; 2, moderate; 3, severe) according to the ASE guidelines [106]. In Study I a grading 0-4 was used, subclassifying severe AR into two groups.

Table 3. *Criteria of valve pathology used in studies I to IV.*

<b>Definitions of valve pathology</b>	<b>Study I</b>	<b>Study II</b>	<b>Study III</b>	<b>Study IV</b>
<b>AS</b>				
Severe AS: $P_{\max} > 50$ mmHg and/or <sup>†</sup> $P_{\text{mean}} > 40$ mmHg and/or $AVA < 1.0$ cm <sup>2</sup> , regardless of AR grade	x <sup>†</sup>			x
Main clinical diagnosis AS		x		
Pure/isolated AS: $P_{\text{mean}} > 40$ mmHg and/or $AVA < 1.0$ cm <sup>2</sup> and AR grade $\leq 1$		x <sup>#</sup>	x	
<b>AR</b>				
AR grade 1-4 of 4, not fulfilling the AS criteria	x			
Main clinical diagnosis AR		x		
Severe AR: AR grade = 3 of 3, not fulfilling the AS criteria				x
Pure/isolated AR: AR grade 3 and $P_{\text{mean}} < 20$ mmHg		x	x	

<sup>†</sup> In Study I only; <sup>#</sup>AVA not included in definition in Study II.

The included patients who did not fulfill the criteria of severe valve pathology or ascending aortic aneurysm based on the results of TTE performed within the ASAP study protocol, were allocated to a separate group (MIX) in *Study IV*.

### 3.3.3 Transesophageal echocardiography

In all patients TEE was performed under general anesthesia before surgery by experienced echocardiographers (n=5) in the operating room using a Sequoia c512 ultrasound scanner (Siemens Medical Systems, Mountain View, CA) with a transducer frequency up to 7 MHz.

The descending aorta was scanned in the short-axis view at three predefined distances from the teeth (30, 35 and 40 cm), with the 35-cm level representing approximately the level of the left atrium in the majority of patients. Segments with aortic plaque were excluded from the analysis. The descending aortic diameter was measured from leading edge to leading edge on short-axis M-mode recordings in diastole. All TEE examinations of the aorta were analyzed offline by one experienced echocardiographer (JP).

Two-dimensional measurements were obtained from the left ventricular outflow tract (LVOT) at systole, while dimensions of the aortic annulus, sinus of Valsalva (SV), sinotubular junction (STJ), ascending aorta 40 mm above the annulus, ascending aorta at the maximal observed diameter, and the aortic root height (the distance from the annulus to the STJ level) were measured in diastole according to the standards of the ASE in all patients [107] (Figure 13).

In *Study I*, an **aneurysm of the aorta** was defined as  $SV > STJ$  and a maximal diameter of the aorta  $> 40$  mm (BAV) or  $> 45$  mm (TAV), regardless of the location of dilation.

**Ectasia of the aorta** was defined as:  $SV < STJ$  and always associated with dilation of the ascending aorta.

**Normal aorta** was defined as:  $SV > STJ$  and maximal diameter of the aorta  $< 40$  mm (BAV) or  $< 45$  mm (TAV).

In *Study IV*, the aortic aneurysm was defined as a main diagnosis if the ascending aortic diameter  $> 45/50$  mm (BAV/TAV respectively), without fulfilling the AS or AR criteria.

In *Studies II, III and IV*, the ascending aortic diameter was included as a continuous variable in the multivariable analyses.

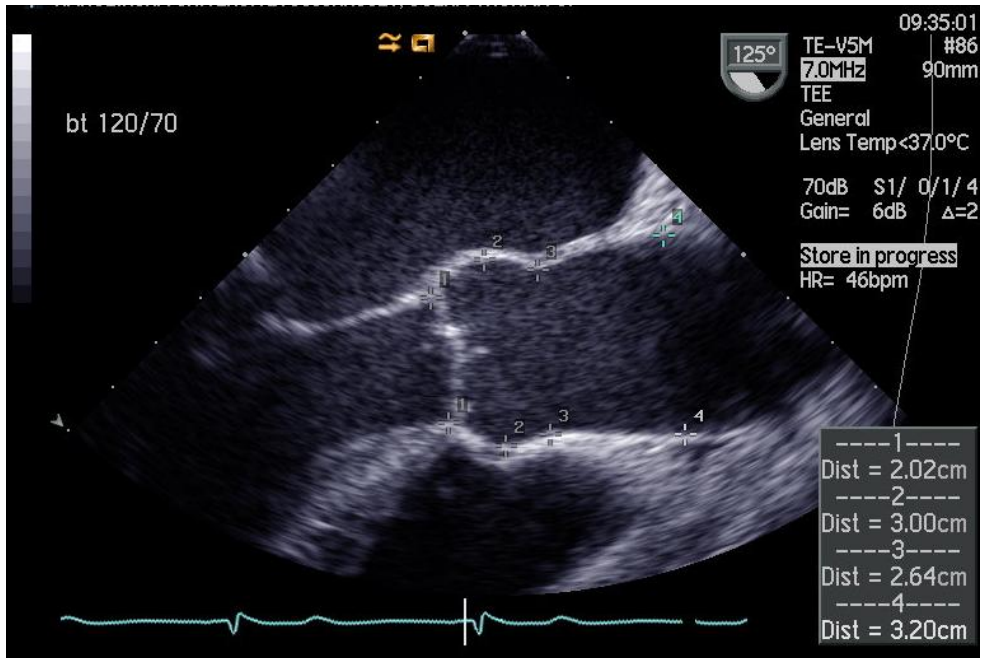


Figure 13. TEE image of the aortic root and the ascending aorta. Measurements of the aortic annulus (1), sinus of Valsalva (2), sinotubular junction (3) and the ascending aorta (4). The aortic root height is the distance from the annulus to the sinotubular junction [Study I].

### 3.3.4 Velocity Vector Imaging of the descending aorta

Recently, velocity vector imaging (VVI), a novel method based on ultrasonic 2-D images [76], has been developed and shown to be feasible for the assessment of cardiac mechanics. VVI involves speckle tracking combined with tissue-blood border detection and the periodicity of the cardiac cycle using RR-intervals (Figure 14). The tissue velocity is displayed as a vector projected on a 2-D echocardiographic image where the vector shows the direction of the movement while the length of the vector is related to the velocity of the tissue (Figure 15). VVI permits angle-independent measurements of tissue velocity and deformation – strain as well as rotational displacement. Because VVI tracks moving tissue, the area change can be calculated frame-by-frame automatically along the entire circumference of the cavity [76].

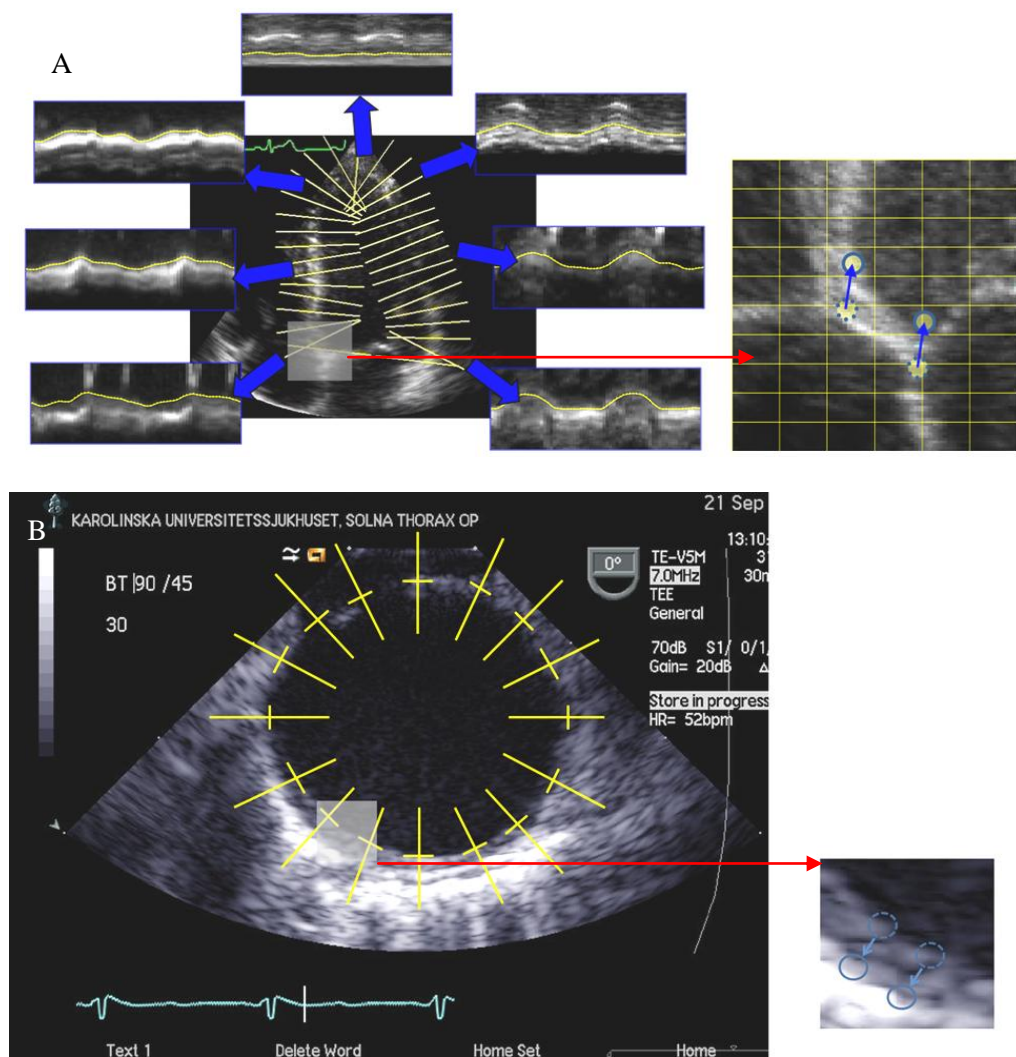


Figure 14. A: Principle of VVI border detection and speckle tracking for assessment of the left ventricular deformation, courtesy of Siemens Medical Systems. B: Schematic picture of speckle tracking and border detection algorithms adopted for the descending aorta.

All analyses in Study II and III were performed offline on a dedicated workstation Syngo US WP 30 VVI (Siemens Medical Systems). The best-quality TEE loops of the descending aorta (by visual assessment), preferably at the 35-cm level, were chosen and the blood-intima interface was traced manually. If tracking of the blood-intima interface was unsatisfactory, according

to indication by the software or visual assessment, a new tracing was performed or another loop (at the same level) was chosen. Means of 2-4 cardiac cycles from the two levels with the best image quality were reported (Figure 15).

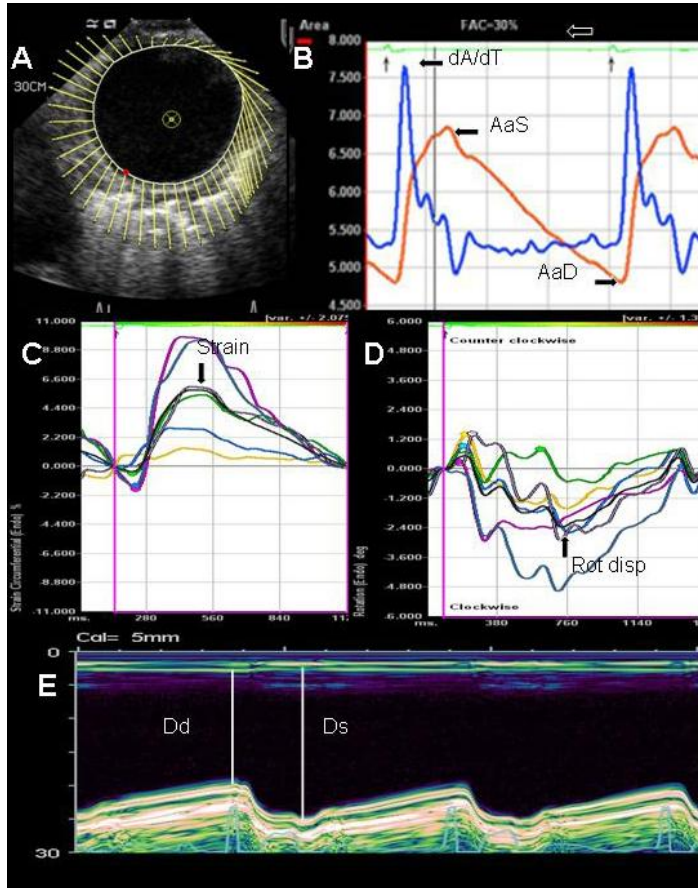


Figure 15. A: VVI tracking of the descending aorta; B: maximal systolic area (AaS), minimal diastolic area (AaD), area change over time ( $dA/dt$ ); C: maximal systolic circumferential strain (VVI strain); D: minimal systolic rotational displacement (VVI rot). The results were automatically calculated and the relevant values of each variable were depicted from the respective curves. E: The maximal systolic diameter (Ds) and minimal diastolic diameter (Dd) were measured along the M-line, leading edge of the near wall intima-lumen echo to the leading edge of the echo from the far wall lumen-intima interface. Reprinted with permission from Elsevier [Study II].

### 3.3.5 Elasticity indices and calculations of arterial and valvulo-arterial compliance

In Study II and III vascular variables were calculated from the ultrasound recordings of the descending aorta. The M-mode was recorded in short-axis view of the descending aorta, paying particular attention to placement of the cursor in the center of the vessel, perpendicular to the aortic wall. The maximal systolic diameter (Ds) and minimal diastolic diameter (Dd) were measured along the M-line, the leading edge of the near wall intima-lumen echo to the leading edge of the echo from the far wall lumen-intima interface, at the same levels as the VVI analyses [87]. All measurements were averaged over 3-4 cardiac cycles.

Blood pressure was measured invasively in the radial artery simultaneously with the acquisition of the TEE images.

Using Ds and Dd obtained from M-mode (Figure 15) the following indices of aortic elasticity were calculated [108]:

$$\text{Distensibility} = ((Ds^2 * \pi / 4) - (Dd^2 * \pi / 4)) * 10^7 / (Dd^2 * \pi / 4) * PP * 1333 \text{ kPa}^{-1} 10^{-3}$$

$$\text{Stiffness} = \ln (SBP/DBP) / ((Ds-Dd)/Dd)$$

where SBP and DBP refer to radial systolic and diastolic blood pressures (mmHg), respectively and pulse pressure (PP) = SBP-DBP;

VVI distensibility was calculated as  $= (As-Ad) * 10^7 / Ad * PP * 1333 \text{ kPa}^{-1} 10^{-3}$ .

VVI stiffness was calculated with the same formula as for the M-mode measurements, using diameters derived from the aortic area; systolic diameter =  $\sqrt{(4As/\pi)}$  and diastolic diameter =  $\sqrt{(4Ad/\pi)}$  [108].

Total systemic arterial compliance (SAC) in Study III was calculated as the stroke volume index obtained from echocardiography divided by the pulse pressure [109], and valvulo-arterial impedance (Zva) as  $(P_{\text{mean}} + SBP)/\text{stroke volume index}$  [110].



### 3.3.6 Intima-media thickness

Measurements of intima-media thickness in the descending aorta (AoIMT) in Study IV were performed off-line using the Syngo Arterial Health Package, a semi-automated edge detection program (version 3.5, Siemens Medical Systems), allowing detection of the echogenic lines of the intima-media complex in a 10-mm-wide segment (Figure 16). The best-quality end-diastolic image with good perpendicular alignment was selected and a region of interest (ROI) was placed manually over the far wall of the descending aorta in a short-axis view. The tracings of intima-blood and media-adventitia borders were adjusted manually when needed. The mean AoIMT was measured from two consecutive heartbeats.



Figure 16. *Intima-media thickness of the descending aorta in a short-axis view; two consecutive heartbeats in diastole [Study IV].*

### 3.3.7 Genotype analyses

Genotypes were extracted from genotype data previously obtained using the Illumina Human 610W-Quad BeadArrays (Illumina Inc, San Diego, CA) at the SNP Technology Platform at Uppsala University [111]. We studied the rs200991 in the HIST1H2BN locus and the rs4888378 in the BCAR1-CFDP1-TMEM170A locus, previously identified as determinants of CIMT study, in five

independent European cohorts [112]. The Genome Studio software from Illumina was used for genotype calling and quality control.

### **3.3.8 Blood analyses**

The plasma concentrations of creatinine, hs-CRP, HDL, LDL and serum concentrations of cholesterol and triglycerides were estimated by the Karolinska University Hospital laboratory using standard methods.

### **3.3.9 Statistical analysis**

Analyses were performed using the commercially available software Statistica 8.0/9.0 (StatSoft<sup>®</sup>, Inc., Tulsa, OK) and SPSS Statistics<sup>®</sup> (version 22, IBM, Armonk, NY). Normally distributed data were presented as the arithmetic mean and standard deviation and skewed variables as median and 25–75<sup>th</sup> percentile (studies I, II and III). In Study IV, all data were presented as median and 25–75<sup>th</sup> percentile. For comparisons between groups, Student's *t* test, ANOVA or Mann–Whitney *U* test were used for analyses of independent samples according to the distribution (studies I and II). In studies III and IV, skewed variables were log transformed before analyses. The chi-squared test was used to analyze variables on a nominal scale. In the case of a significant interaction, simple main effects tests were examined; that is, the effects of one factor while holding the other factor fixed. Otherwise, contrasts between the levels of the main factors were performed. Inter- and intraobserver reliabilities were analyzed by the intraclass correlation coefficient (ICC), which represents the portion of the total variance caused by the variance between subjects. The standard error of measurement (SEM) was also calculated, as well as the coefficient of variation (CV, %). To compare the VVI and M-mode methods, a one-way repeated measures ANOVA was performed. Correlations between the methods were estimated using the Pearson product-moment correlation coefficients. Limits of agreement according to Bland and Altman were also calculated and presented graphically [113]. Univariate and forward stepwise multiple linear regression analyses were used to evaluate predictors of different VVI measures and AoIMT. In multiple regressions, the variables were selected for inclusion in the model based on clinical grounds and significant univariate relations. Co-linearity statistics were analyzed to assess the relationships between the variables included in the

models. Only two-sided tests were used. Results were regarded as significant when  $P < 0.05$ .

### **3.4 Ethical considerations**

All studies were approved by the Regional Ethics Review Board (in Stockholm) and informed consent was obtained from all patients. All patients were scheduled for cardiac surgery, based on clinical decision making before inclusion in the study. TTE is a noninvasive investigation, with no radiation and no known risks. TEE is a semi-invasive investigation that has become a vital part of perioperative monitoring and is performed routinely in all patients undergoing cardiac surgery at the Karolinska University Hospital. The TEE transducer is placed in the esophagus by the anesthesiologist after general anesthesia and is kept there throughout the operation. TEE monitoring is considered to be of low risk for the patients [114, 115], and the clinical benefits outweigh the risks.



## 4 RESULTS

The results of the four studies in this thesis are presented in sections according to the main topics such as morphology and function of the aortic valve and the aorta, AoIMT and reproducibility. Unpublished data is reported when applicable.

### 4.1 Morphology of the aortic valve and aorta

#### 4.1.1 Aortic valve morphology

In our study I, more than 50% of the patients undergoing surgery of the aortic valve and/or ascending aorta had bicuspid aortic valve (BAV  $n=160$ , TAV  $=130$ , UAV $=10$ ) as shown in Figure 17A. BAV patients in Study IV were younger than TAV patients ( $P<0.001$ ) at the time of operation (Figure 17B). The majority of BAVs were of the RL phenotype; there was no LN malformation in our BAV patients (Figure 22).

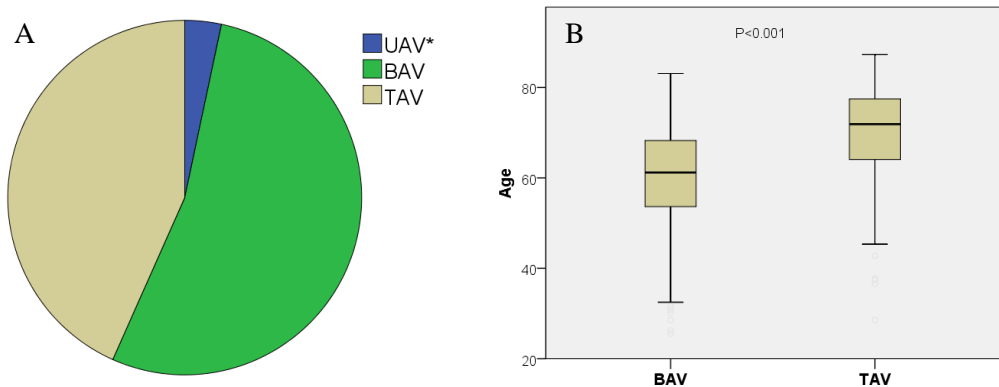


Figure 17. A: Distribution of valve morphology in consecutive patients without coronary artery disease, requiring aortic valve and/or ascending aortic surgery, \*UAV were considered BAV type 2 according to Sievers [Study I]. B: Patients with BAV ( $n=210$ ) were approximately 10 years younger than TAV ( $n=158$ ) [Study IV].

### 4.1.2 Morphology of the ascending aorta

When the size threshold for an aneurysm in BAV/TAV was set to  $> 40/45$  mm, respectively, the prevalence of aneurysms in the ascending aorta was three times more common in BAV than in TAV patients (Study I). When the size threshold was set to  $> 45$  mm for BAV and TAV aneurysms in the ascending aorta, the prevalence was twice as common in BAV patients (Figure 18). BAV patients had larger annular and LVOT dimensions compared with TAV patients, regardless of aortic root morphology.

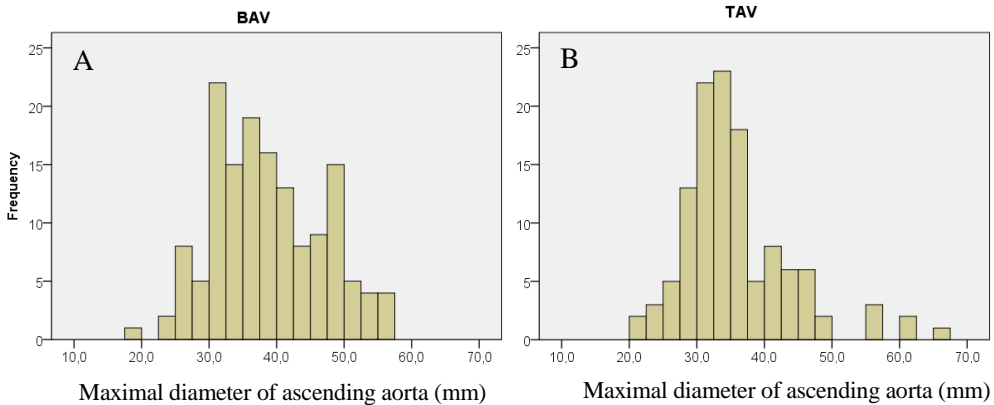


Figure 18. *Distribution of patients according to the diameter of the ascending aorta in the entire study population. A: In BAV (excluding Sievers' type 2) 36% of the patients had a diameter  $> 40$  mm and 24%  $> 45$  mm. B: In TAV 12% of the patients had a diameter  $> 45$  mm [Study I].*

All types of ascending aortic dilation (aneurysm + ectasia,  $> 45$  mm) were more common in BAV compared with TAV, in a ratio of 1.5:1 ( $P < 0.05$ ).

### 4.1.3 Type of valve pathology and dilation of the ascending aorta

In patients with aortic stenosis, there was a highly significant difference in the ascending aortic dimension between the BAV and TAV groups. Ascending aortic aneurysms were virtually nonexistent in TAV patients with AS ( $P < 0.001$ ) (Figure 19), all ascending aortic dilations (aneurysm + ectasia) showed the same pattern.

The prevalence of ascending aortic aneurysms in AR patients was similar in the BAV and TAV groups.

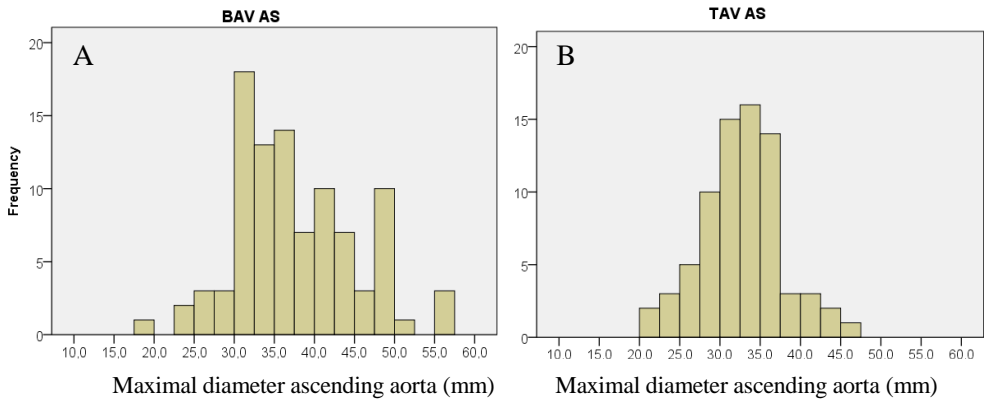


Figure 19. Distribution of patients with aortic stenosis (AS) according to the diameter of the ascending aorta. A: In BAV (Seivers' type 2 excluded) 29 % had a diameter >40 mm and 16 % >45 mm. B: In TAV only 1% had a diameter >45 mm [Study I].

#### 4.1.4 Type of valve pathology and dilation of the descending aorta

The descending aortic diameter showed a significant positive correlation with age ( $r = 0.625$ ;  $P < 0.001$ ). TAV patients had a larger descending aorta, related to age since patients with TAV were significantly older than those with BAV.

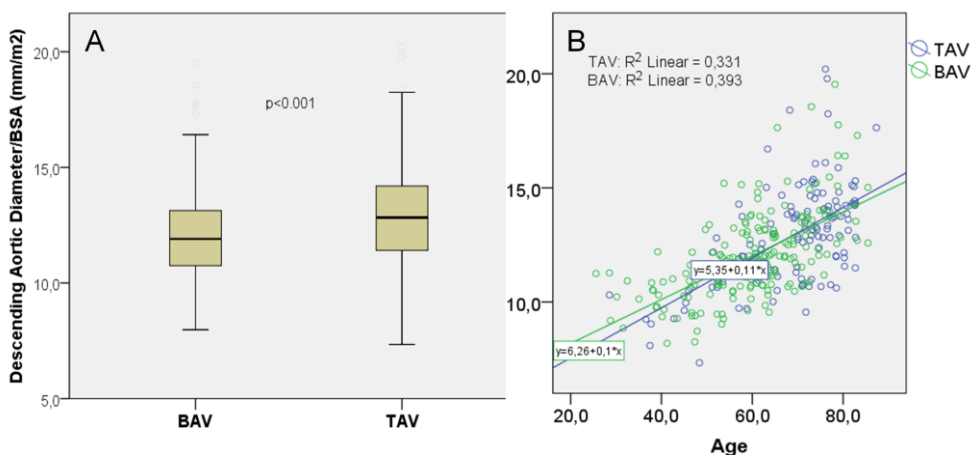


Figure 20. Diameter of the descending aorta in BAV and TAV normalized for BSA (A) and its correlation with age (B) [Study IV].

#### 4.1.5 Aortic root morphology in relation to types of bicuspid aortic valve

In study I three distinct aortic morphological patterns were observed [116] as defined in the methods (Figure 21).

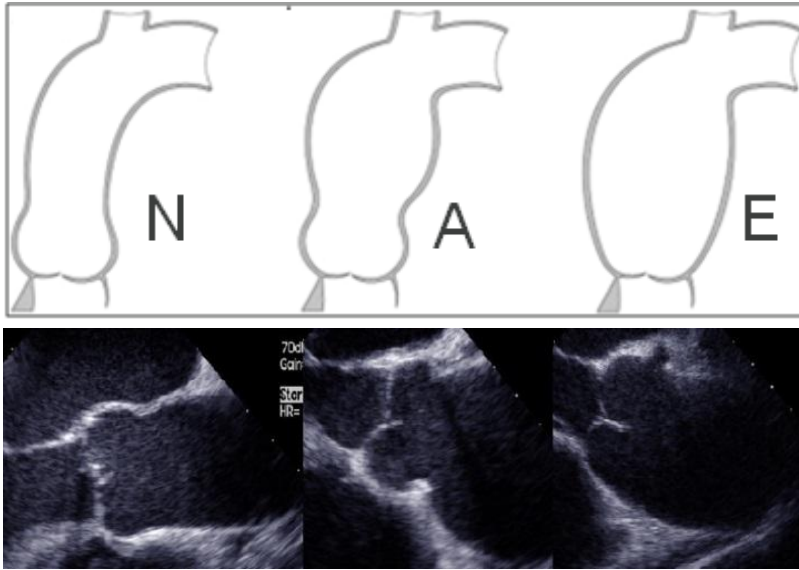


Figure 21. *Schematic illustrations (upper panel) and TEE examples (lower panel) of normal aorta (N); aneurysm of the ascending aorta (A); ectasia of the aorta (E). Upper panel reprinted with permission from Elsevier [Study I].*

We did not find any association between BAV phenotypes and the specific morphology of the aortic root or the ascending aorta, i.e. aneurysm, ectasia or normal. Ectatic aortas with true BAV were significantly smaller compared with RL and RN phenotypes ( $P < 0.01$ ). There were no other significant differences in aortic dimensions between phenotypes of BAV within the respective aortic morphology (Figure 22).

There were no significant differences in the descending aortic dimension between the phenotypes of BAV within the isolated valve pathologies (Table 5).



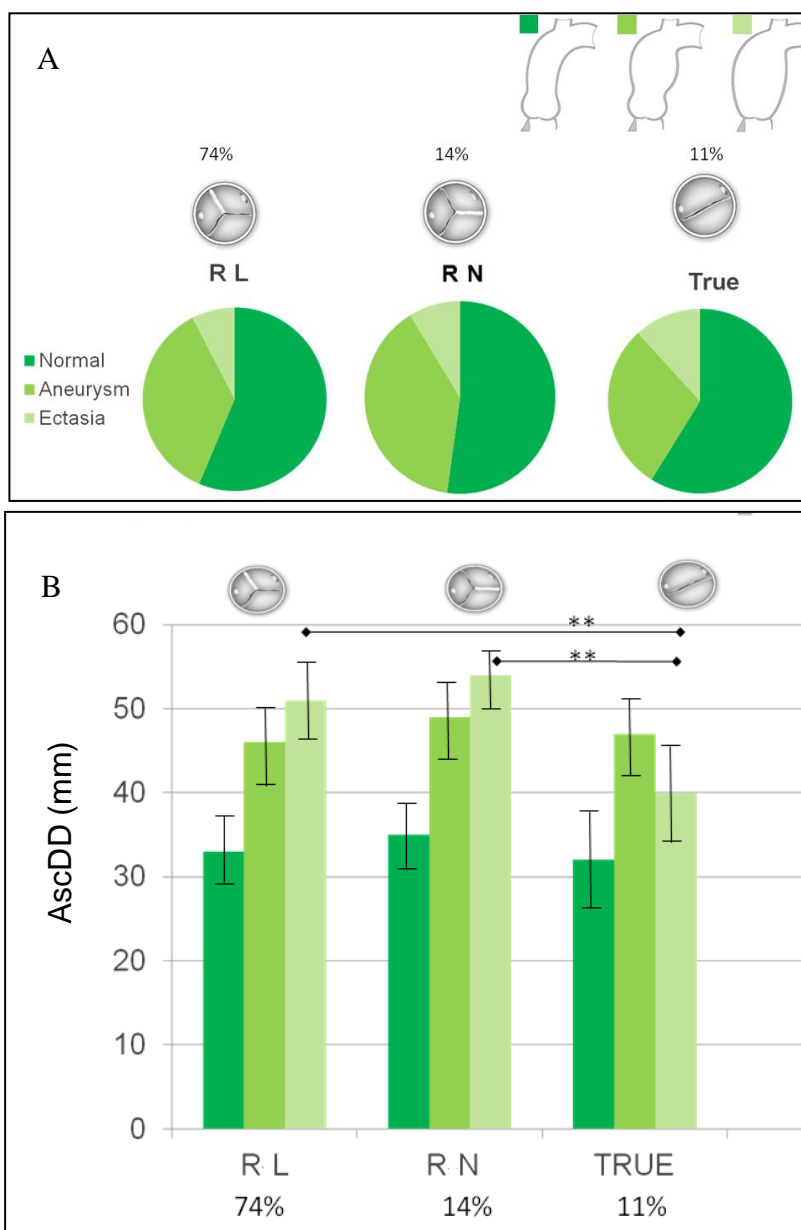


Figure 22. A: BAV phenotype (right-left (RL), right-non-coronary (RN) and True) and distribution of morphology of the aortic root/ascending aorta showed no significant differences, B: BAV phenotype and the diastolic diameter of the ascending aorta (AscDD).  $P < 0.01$  indicated by \*\*. BAV phenotypes are shown from surgeon's view [Study I].

## 4.2 Vascular function

In study II and III we used Velocity Vector Imaging analysis software to assess the elastic properties of the descending aorta. To validate VVI for short-axis measurements of the aortic mechanics, an experimental dynamic set up consisting of an aorta gel phantom connected to a programmable pulsatile flow pump (Figure 23) was built in cooperation with Matilda Larsson, Ph.D., Department of Medical Engineering, School of Technology and Health, the Royal Institute of Technology (KTH), Stockholm, Sweden. The strain measurements by VVI were compared with sonomicrometry. Strain calculated by VVI was lower compared with sonomicrometry ( $P < 0.001$ ); however, there was a strong correlation ( $r = 0.90$ ) between the two methods (unpublished data).



Figure 23. *Experimental setup with the Sequoia c512 ultrasound scanner, the flow pump and the phantom (courtesy of Matilda Larsson).*

## 4.2.1 Feasibility of VVI by TEE for the elastic properties of the descending aorta

We used M-mode, a one-dimensional measurement of vessel diameter (Figure 15 E) that is a well-established method for stiffness measurements in different vessels, for comparison with VVI (Study II). Stiffness and distensibility were calculated as described in the methods section. There was a strong and significant correlation between the indices of aortic function obtained by M-mode and VVI ( $P<0.001$ ). The Bland–Altman plots showed that the differences were independent of the mean values, and that there was a systematic bias between the methods (Figure 24).

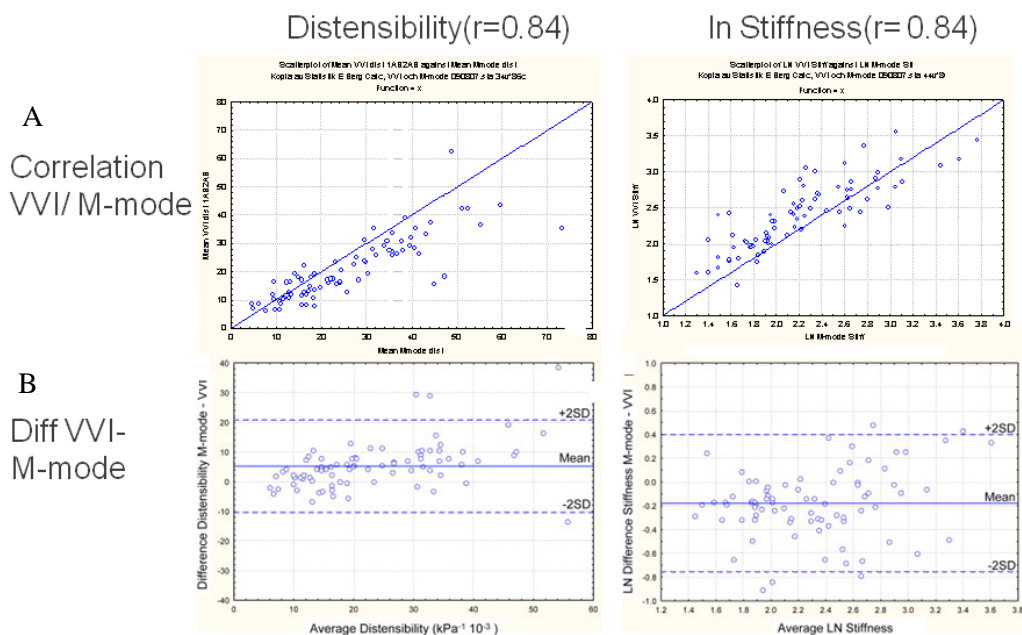


Figure 24. Correlations (A) and Bland–Altman plots (B) comparing distensibility and stiffness obtained using VVI and M-mode. Reprinted with permission from Elsevier [Study II].

## 4.2.2 Comparison of the descending aortic elasticity in patients with AS and AR

In study II and III we compared elasticity indices in AS and AR groups. AR patients had a significantly higher dA/dt and a higher VVI strain than patients with AS. VVI rotational displacement differed significantly between the groups; however, a large standard deviation made this variable less useful. The VVI distensibility was higher and the stiffness was lower in patients with AR than in those with AS (Figure 25).

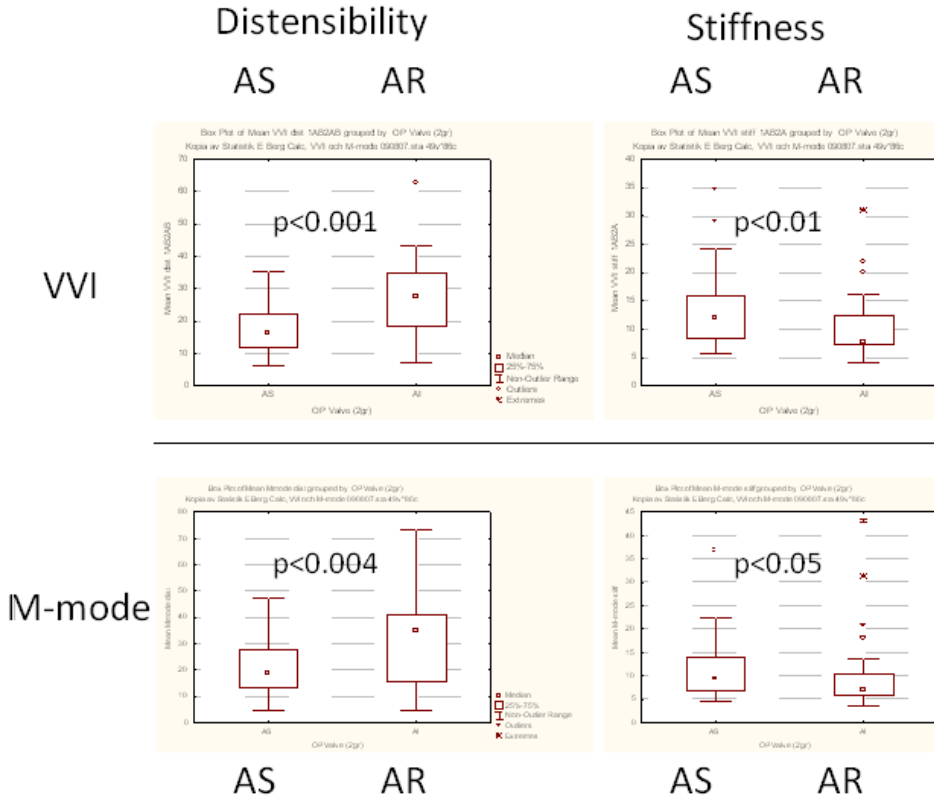


Figure 25. *Stiffness obtained by using VVI and M-mode in AS and AR [Study II].*

In patients with isolated/pure AR ( $n=52$ ) and AS ( $n=140$ ) in study III, we found significantly higher VVI distensibility in AR ( $31 [22-29] \text{ kPa}^{-1}10^{-3}$ ) compared with AS ( $17 [12-24] \text{ kPa}^{-1}10^{-3}$ ),  $P<0.001$ . Stiffness was higher in AS ( $12 [8.2-16]$ ) than in AR ( $7.5 [6.1-9.6]$ ),  $P<0.001$ . VVI strain was lower in AS ( $3.4\% [2.3-4.9]$ ) than in AR ( $8.6\% [6.3-13]$ ),  $P<0.001$ .

### 4.2.3 Comparison of the descending aortic elasticity in patients with BAV and TAV

As there was a significant difference in aortic elasticity between AS and AR (Figure 25), we first divided the material according to the type of valve pathology to compare BAV and TAV (Study III). To further purify the groups, we defined isolated AS and AR (Methods, Table 3).

BAV patients were younger than TAV patients (AS, 65 vs. 74 years; AR, 49 vs. 63 years; both  $P<0.001$ ). Because it is known that age is a major determinant of aortic elasticity, we performed a subgroup analysis of age-matched AS and AR patients. We found a significantly lower strain in the BAV group regardless of aortic valve disease (Table 4).

Table 4. *Variables of aortic function in patients with BAV and TAV, and aortic stenosis (AS) or aortic regurgitation (AR), matched for age.*

	Isolated AS			Isolated AR		
	BAV	TAV	P	BAV	TAV	P
<b>n</b>	<i>n</i> =31	<i>n</i> =31		<i>n</i> =12	<i>n</i> =12	
<b>Age (years)</b>	71±8	71±8	ns	57±9	57±10	ns
<b>VVI strain (%)</b>	2.7 [1.7-4.7]	3.5 [2.7-4.4]	<0.05	6.9 [5.5-8.0]	13 [9.2-15]	<0.01
<b>VVI stiffness (index)</b>	14 [9-18]	12 [10-15]	ns	7.6 [7.0-11]	6.4 [5.5-9.3]	ns
<b>VVI distensibility (kPa<sup>-1</sup>10<sup>-3</sup>)</b>	14 [10-24]	16 [13-19]	ns	26 [20-32]	36 [26-43]	ns

*Data are expressed as mean ± SD (for normally distributed parameters) and as median [interquartile range] (for skewed data) [Study III].*

Multivariate analysis of the entire patient population of isolated AS and AR showed that BAV was associated with lower strain and distensibility in the AR group, but higher distensibility in the AS group. Age was the main predictor of the descending aorta elasticity indices.

#### 4.2.4 Comparison of the descending aortic elasticity in patients with phenotypes of BAV

In study III we also compared aortic elasticity of the descending aorta between phenotypes of BAV in the AR and AS cohorts. Patients with Sievers' type 2 BAV were excluded from this analysis. There were no significant differences between any of the specific types of BAV for any elasticity index in the AS group ( $P>0.05$ ).

Table 5. *Elasticity indices and aortic diameters in relation to the phenotypes of BAV in AS and AR.*

<b>Isolated AS</b>	<b>RL (n=64)</b>	<b>True (n=8)</b>	<b>RN (n=14)</b>
Strain (%)	4.0 [2.3-5.3]	4.6 [1.1-6.2]	3.2 [2.0-4.8]
Stiffness (index)	10.3 [7.3-15.3]	7.9 [7.0-13]	11.5 [7.5-16.0]
Distensibility ( $\text{kPa}^{-1}10^{-3}$ )	19.6 [11.8-27.6]	21.1 [16.2-27.2]	16.8 [12.4-25.6]
AscDD (mm)	37 [32-45]	34 [33-38]	39 [33-49]
AscDD/BSA ( $\text{mm}/\text{m}^2$ )	20 [17-24]	18[16-20]	23[17-26]
DesDD (mm)	24 $\pm$ 3.8	24 $\pm$ 2.9	24 $\pm$ 3.0
DesDD/BSA ( $\text{mm}/\text{m}^2$ )	13 $\pm$ 1.8	13 $\pm$ 1.4	13 $\pm$ 2.6
<b>Isolated AR</b>	<b>RL (n=17)</b>	<b>True (n=2)</b>	<b>RN (n=2)</b>
Strain (%)	8.3[6.5-14.1]	10.2	8.6
Stiffness (index)	6.9[5.0-8.0]	6.7	7.5
Distensibility ( $\text{kPa}^{-1}10^{-3}$ )	32[23-47]	35	42
AscDD (mm)	38[31-40]	38	38
AscDD/BSA ( $\text{mm}/\text{m}^2$ )	18 [16-19]	18	20
DesDD (mm)	23 $\pm$ 3.2	27	24
DesDD/BSA ( $\text{mm}/\text{m}^2$ )	11 $\pm$ 1.6	12	12

*AscDD= Ascending aortic diastolic diameter; AscDD/BSA = Ascending aortic diastolic diameter/body surface area; DesDD = Descending aortic diastolic diameter; DesDD/BSA = Descending aortic diastolic diameter/BSA. Data are expressed as mean  $\pm$  SD and as median [interquartile range] when applicable. [Study III].*

### 4.2.5 Arterial compliance and valvulo-arterial impedance

Total systemic arterial compliance in the AS group did not differ between patients with BAV and TAV, and it was preserved in both groups. Patients with BAV had similar valvulo-arterial impedance as TAV patients. The median values of both groups were in the moderately increased range.

Table 6. *Arterial compliance and valvulo-arterial impedance in age matched patients with aortic stenosis, with reference to BAV and TAV [Study III].*

	AS		
	<b>BAV</b> <i>n=31</i>	<b>TAV</b> <i>n=31</i>	<b>P</b> <b>BAV vs. TAV</b>
Total systemic arterial compliance (ml·m <sup>-2</sup> ·mmHg <sup>-1</sup> )	0.90 (0.80-1.14)	0.87 (0.65-1.11)	ns
Valvulo-arterial impedance (mmHg·ml <sup>-1</sup> ·m <sup>-2</sup> )	3.8 (3.0-4.7)	3.8 (3.3-4.3)	ns

### 4.3 Intima- media thickness of the descending aorta

In study IV we investigated the intima-media thickness of the descending aorta. AoIMT in the entire patient population correlated significantly with age and hsCRP. AoIMT was significantly thinner in the BAV group than in the TAV group ( $P<0.001$ ), (Figure 26).

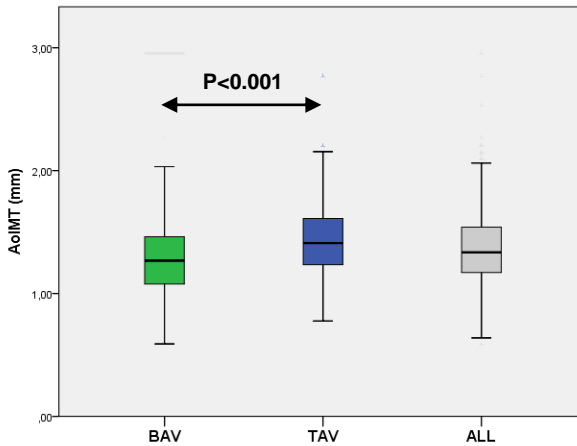


Figure 26. Intima-media thickness of the descending aorta (AoIMT) in BAV and TAV groups and the whole study population (ALL), mean  $\pm$  SD [Study IV].

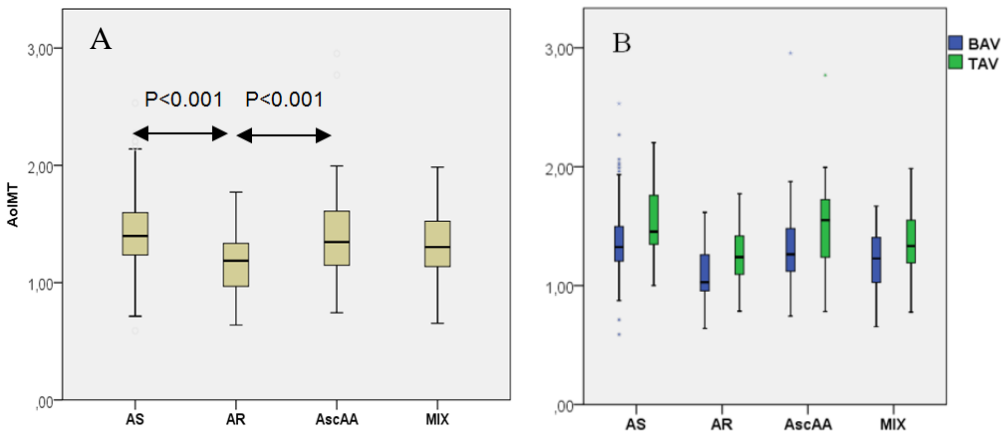


Figure 27. A: Box plot of AoIMT in aortic stenosis (AS), aortic regurgitation (AR), ascending aortic aneurysm (AoAA) and MIX pathology. B: and in addition valve morphology BAV/TAV, mean  $\pm$  SD [Study IV].



In Study IV, the patients were classified according to the TTE protocol as having AS ( $n = 203$ ), AR ( $n = 77$ ) and ascending aortic aneurysms (AscAA,  $n = 55$ ). Thirty one patients could not be classified into any of the above groups (MIX,  $n = 31$ ). AoIMT was thinner in those with AR than in those with AS ( $P < 0.001$ ) and in AscAA ( $P < 0.001$ ). There were no significant differences between the MIX group and the other groups. Patients with AS and TAV displayed a thicker AoIMT than those with AS and BAV ( $P < 0.001$ ). The same pattern was seen in the AR group ( $P < 0.01$ ) (Figure 27).

### **4.3.1 Genetic variability and AoIMT**

The rs4888378 SNP in the BCAR1–CFDP1–TMEM170A locus and the rs200991 SNP in the HIST1H2BN locus were studied in 338 patients. These SNPs have been identified previously as determinants of CIMT. We only found differences between the genotype groups for the rs200991 SNP, although not significant for the whole study population. Our study population had very few patients with an AA allele configuration (allele frequency 0.15), as expected according to Hardy–Weinberg equilibrium and in previous reports.

Analyzing BAV and TAV separately, only the patients with TAV showed a difference between the rs200991 alleles. Patients with a CC allele configuration displayed a trend toward a thicker AoIMT vs. the AC allele. There were no significant differences in any of the other patient variables between the AA, AC and CC groups.

### **4.3.2 Determinants of intima-media thickness in the descending aorta**

In our multivariate model, we chose variables of clinical relevance or variables shown to be associated with carotid intima-media thickness or AoIMT in previous studies. The multivariate analysis of the whole group showed that AoIMT was associated with age and male gender. When the BAV and TAV subgroups were analyzed separately, age was still the main predictor of AoIMT in both groups. The rs200991 SNP genotype was a significant factor ( $P < 0.05$ ) for the TAV group of patients, and the creatinine level was significant for the BAV group ( $P < 0.05$ ).

## 4.4 Reproducibility

### 4.4.1 Reproducibility of aortic root measurements

Intra- and interobserver variability of the 2D variables was assessed in 16 consecutive study patients, with measurements performed on selected cine loops. The intra- and interobserver variability measured as CV for LVOT was 1.8% and 1.2 %, SV 3.6% and 3.4%, STJ 3.0% and 2.5%, maximal diameter of ascending aorta 2.7% and 2.9 % respectively (unpublished data).

### 4.4.2 Reproducibility of VVI variables

The intra- and interobserver variability of measurements was determined for the VVI variables (As, Ad, dA/dT, VVI strain and VVI rot, Figure 15) in 32 patients (Table 4). The variability of the VVI was low with high ICC, low CV, and strong correlations, except for rotational displacement, which showed a high CV.

Table 7. *Intra- and interobserver variability of the VVI variables.*

	Intraobserver variability			Interobserver variability		
VVI variables	ICC	SEM	CV (%)	ICC	SEM	CV (%)
Aortic area, systole	0.985	0.123	2.5	0.973	0.169	3.4
Aortic area, diastole	0.981	0.127	2.9	0.970	0.166	3.8
dA/dt	0.979	0.443	10.5	0.955	0.676	15.3
Strain	0.986	0.441	8.39	0.980	0.533	9.99
Rotational displacement	0.564	0.459	52.5	0.645	0.358	43.5

*ICC = Intraclass correlation coefficient; SEM= standard error of mean; CV= coefficient of variation. Reprinted with permission from Elsevier [Study II].*

Interobserver variability of the M-mode variables was assessed in 40 patients; it was excellent for the systolic and diastolic diameters, with high ICC (0.97-0.99) and low CV (2.5-3.8%) [Study II].

### 4.4.3 Reproducibility AoIMT

The intra- and interobserver variability of measurements was determined for AoIMT in 27 patients; the coefficients of variation were about 10% [Study IV].

## 5 DISCUSSION

The association between BAV and dilation of ascending aorta is well established. However, whether the aortopathy in BAV is due to a genetic predisposition and/or because of altered blood flow is still debated [19]. The vascular smooth muscle cells have the same embryological origin in the aortic root, ascending aorta and the aortic arch, in addition to the pulmonary trunk constituting the classic boundaries of BAV disease [36]. In the ASAP population we studied ascending aortic dilation in BAV and TAV patients with regards to valve pathology in terms of AS/AR as well as different phenotypes of BAV. We also studied dimensions, intima-media thickness and function of the descending aorta. We employed the new VVI technique for studies of aortic function. Finally we investigated if SNPs with a known association to CIMT were also associated to AoIMT.

### 5.1 Morphology

#### 5.1.1 Aortic valve morphology

More than 50% of the first 300 consecutive patients undergoing aortic valve or aortic surgery within the ASAP study had BAV. This is in strong contrast to a reported prevalence of only 0.5-2% in the general population. The discrepancy is explained by an earlier development of valve lesions [117, 118], Figure 28, and a larger tendency for aortic dilation requiring surgery in BAV than in TAV. Therefore, historically BAV has been described as diseased [9]. Long-term studies of normally functioning BAVs have shown a markedly increased risk for valve replacement and/or aortic surgery [32, 34]; one study reported as high as 70 times higher risk compared with TAV patients [32]. An explanation for the early development of lesions in BAV seems to be abnormal shear stress leading to valve calcification and malfunction [26]. However, even in older patients undergoing aortic surgery, BAV is seen frequently. Our findings are in line with previous reports [16, 119], and confirm that BAV is a common reason for aortic valve procedures in all age groups.

### Calcific Aortic Stenosis and Congenital Bicuspid Aortic Valves

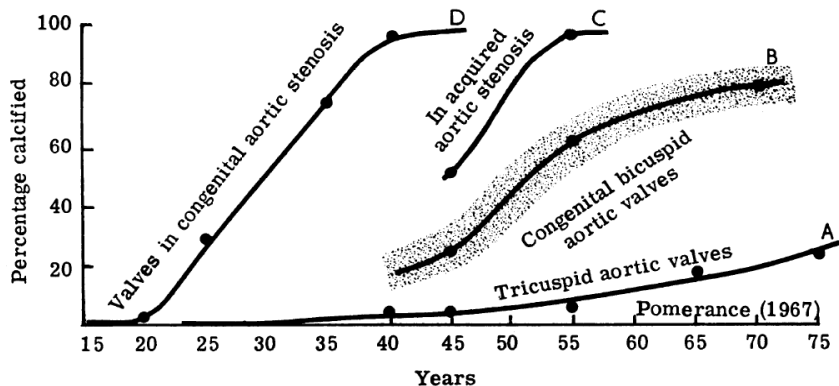


FIG.—Incidence of calcification with increasing age. (A) In normal aortic valves with three cusps (based on 805 cases of Pomerance (1967)). (B) In congenital bicuspid aortic valves. The shaded area defines the probable limits of accuracy. (C) In the aortic valves of acquired aortic stenosis. (B) and (C) are based mainly on Wauchope (1928), Bacon and Matthews (1959), and A. Pomerance (personal communication). (D) In the valves of congenital aortic stenosis. Based mainly on Campbell (Table VI) and Baker and Somerville (1964).

Figure 28. Incidence of aortic valve calcification with increasing age. Reprinted with permission from the BMJ Publishing Group [120].

The classification of valve morphology is central to this thesis. The position of leaflets (anterior/posterior; right/left), the size of the cusps, the number of sinuses and the positions of coronary ostia are included in different classification systems [2-4]. We adopted a widely used classification based on the number of leaflets and on which cusps were fused [116]. Surgical inspection has the advantage of direct evaluation and contact with the valve and it was chosen as reference standard. On the other hand, TEE can picture the moving valve during a cardiac cycle. Histopathological examinations of valve morphology would have been favorable [121], but this was not possible as the explanted valves were used for other analyses [122, 123]. Overall, there was good agreement between TEE and the surgical classification (sensitivity 92% and specificity 94 % with a kappa value of 0.86) [124]. The relative distribution of the morphologically distinct patterns in BAV observed in our study was similar to earlier reports.

### **5.1.2 Dilation of the ascending and descending aorta in BAV**

Dilation of the ascending aorta is associated with BAV. Two studies published in the early 2000s reported a high risk for aortic dissection and aneurysms in BAV patients with a moderately dilated ascending aorta [54, 125]. The guidelines existing at the time the ASAP study was carried out stated a lower threshold for ascending aortic replacement in BAV patients [126]. This was the rationale for the different threshold diameters used for the classification of aneurysms in the BAV and TAV groups ( $> 40$  and  $> 45$  mm, respectively) in Study I.

As shown in Figure 18, ascending aortic aneurysms were twice as common in BAV patients even when the same classification cutoff was used. Optimal timing of aortic replacement has been debated, with authors arguing for replacement of the aorta when  $>40$  mm in BAV patients [127], while others have argued that BAV and TAV patients should be treated the same way [128]. Two recent cohort studies have shown a low incidence of aortic complications in BAV patients, although higher than in TAV patients, and the same survival rate in each group [31, 34]. One study showed no difference in aortic complications in BAV and TAV patients with a moderately dilated aorta [129]. Recently, in new guidelines the lower threshold has been maintained for surgery of the ascending aorta in BAV patients undergoing aortic valve replacement for severe aortic valve disease, but not in patients being considered for a prophylactic procedure [130].

There has also been a discussion that risk of ascending aortic dilation varies between phenotypes of BAV, especially when valve disease is present [131]. The ectatic aortic root phenotype is considered a special subtype of aortopathy that is more frequently associated with AR [132], but we did not find such a relation. Schaefer et al used the same classification of BAV as us, and found that patients with RL BAV had a normal aortic root morphology, whereas RN BAV was associated with ascending aortic aneurysms [116]. In BAV, proponents of the hemodynamic theory state that the jet from cusps with restricted motion will cause shear stress on the aortic wall, causing dilation [5, 51, 133]. There are reports showing different histological patterns in the areas of the aorta where the flow jet from the restricted cusp hits the wall, compared with other regions of the ascending aorta used as control samples [134]. In our studies (I and III), we found only minor differences in the distribution of aortic root morphology and

ascending aortic dimensions between different phenotypes of BAV (Figure 21 and Table 5). This does not support the hemodynamic theory of aortopathy in BAV being related to a certain pattern of cusp fusion.

Using a different classification of BAV based on the location and orientation of the commissure [2], Kang et al found that aortic dilation was more common in BAV phenotype with right-left orientation of the commissural line (RN or LN phenotype in our study) and that AR was dominant in the anterior-posterior phenotype (RL in our study) [135]. Our grouping of BAV types according to cusp fusion was supported by embryology [18].

Dilation of the ascending aorta in aortic valve disease has been described as poststenotic, caused by turbulence of the bloodstream leading to vibrations and structural fatigue of the vessel wall [136]. In contrast, other papers found no correlation between aortic gradient and ascending aortic diameter [137], and several reports have shown ascending aortic dilation only in BAV patients with AS [137-139]. Study I confirmed the absence of more than mildly dilated ascending aorta in TAV AS patients. In AR patients, aortic dilation was present regardless of valve type. There are reports of AR being a determinant of sinus of Valsalva/aortic root dilation [45]; this was not confirmed in our Study I.

In our study population, TAV patients had significantly larger diameters of the descending aorta compared with BAV patients. The size of the descending aorta was highly correlated to age and the difference in diameter was age-dependent (Figure 20). This has been confirmed in other studies, which showed that descending aortic dimensions did not differ between BAV and TAV patients [140], and that they were age-dependent [141]. In ASAP patients where the ascending aorta was replaced concurrent descending aortic dilation was more common in TAV patients [56]. This is in line with the finding that the same molecular mechanisms seem to be present in aneurysm formation in the whole aorta in TAV [56].

## 5.2 Aortic function

### 5.2.1 Descending aortic function in BAV and TAV

Age was the main univariate determinant of the elastic indices in our patient population. There was a 10-year age difference between our BAV and TAV patients, explained by the fact that BAV patients require valve and/aortic surgery earlier [16, 119]. This may also lead to difficulty in performing age-matched comparisons between BAV and TAV.

In the multivariate analysis, age was still dominant. BAV was associated with lower strain and distensibility in the AR group, but higher distensibility in the AS group. Distensibility represents the area change for the pressure increment, whereas stiffness is the ratio of the logarithm (systolic/diastolic blood pressure) relative to diameter change. Reduced elasticity of arteries with age leads primarily to an increase in the systolic blood pressure and, consequently, to increased pulse pressure. For example, starting from a baseline level of 130/80 mmHg (pulse pressure 50 mmHg), a rise in pulse pressure of 10 mmHg translates to a 20% increase, but the ratio of SBP/DBP (to 140/80) increases only 8%. For the SBP/DBP ratio to increase by 20% the pulse pressure must rise by 30 mmHg. In the calculation of stiffness, the logarithm of the ratio is used, further reducing the influence of the pulse pressure change. Furthermore, in the distensibility equation the changes in the vessel size influence the result more since the radius of the vessel is squared. This might explain why only distensibility, but not stiffness, correlated significantly in the multivariate analysis of the AR group.

There was also an interaction between valve morphology and age for stiffness, showing that the measured variable was not the same in younger and older subjects. No interactions were found for strain or distensibility.

Regarding the descending aortic function in phenotypes of BAV, we did not find any significant differences (Table 5). The subgroups were very small, especially for AR, limiting the possibility of performing adequate statistical analyses.

### **5.2.2 Ascending aortic function**

Most reports on BAV aortopathy involve the ascending aorta [19, 31, 131, 142]. A comparison between functional parameters in the ascending and descending aorta would be of interest, and we initially considered analyzing the ascending aortic elasticity using VVI in short-axis 2-D loops. However, in our experience, it is somewhat difficult to obtain good quality 2-D short-axis images of the ascending aorta with TEE, and we found that the VVI analysis of the proximal ascending aorta was not feasible because of the longitudinal movement of the vessel synchronous with the heartbeat.

Dilation of the ascending aorta is an accepted marker of aortopathy in BAV [142-144]. Therefore, we incorporated the ascending aortic diameter in our analyses as a ‘proxy’ parameter of function in Studies III and IV. In Study III, the ascending aortic dimension was related negatively to elasticity, suggesting that the same process is involved in the ascending and descending media. This could also be explained by a larger buffering capacity in a dilated ascending aorta leading to a smaller impact of a given stroke volume on the descending aorta.

### **5.2.3 Is BAV associated with systemic arteriopathy?**

Our data do not permit strong, generalized conclusions because the association between descending aortic elasticity and BAV in our patients with severe valvular disease was weak. Nevertheless, there are reports of an increased frequency of intracranial aneurysms [145] and increased global aortic stiffness in asymptomatic BAV patients [67], supporting the hypothesis that the connective tissue disorder and a more diffuse arteriopathy might be generalized, involving the descending aorta and other vascular territories in the BAV population. Furthermore, it is difficult to attribute degeneration of the media [50] and dilation in the pulmonary trunk [140] in BAV patients to the aortic valve flow jet.

## **5.3 Intima- media thickness of the descending aorta**

In the ASAP study population, the descending aortic IMT (AoIMT) was mainly determined by age and to some degree by male gender. This is not surprising as



age is the main determinant of CMT in young [146], as well as in middle-aged persons [147]. There are also known associations between CMT and AoMT [83], thoracic aortic calcium [101], plaques [97] and descending aortic wall volume [96]. Male gender is also a known determinant for CMT [147] and although the females were older, male gender was still a significant positive determinant for AoMT in Study IV.

We found no correlation between aortic valve morphology (BAV/TAV) and AoMT, group differences being explained by age. The altered flow from BAV did not affect AoMT. On the other hand a majority of our patients had significant aortic valve dysfunction which probably affected the flow pattern in the descending aorta also in the TAV group.

For comparison some other medical conditions are of interest. Similar to our findings in BAV, SLE is not associated with thicker AoMT but with stiffer aortas [148]. Patients with familial hypercholesterolemia have thicker thoracic aortic walls [96], and statin treatment reduces the AoMT as well as lipoprotein levels [98]. The ASAP patients generally displayed lipoprotein and triglyceride levels within normal limits; we found no correlation between AoMT and these factors. The correlation between AoMT and coronary atherosclerosis [83] could not be examined in our studies because of the exclusion criteria. The population in Study IV represents a low-risk stratum regarding atherosclerosis burden, especially in the elderly AS patients.

One way to identify the genes involved in human disease is to conduct genome-wide association studies. This method searches the genome for small variations, or SNPs, if functional, could be of higher frequency in people with a specified condition than in people without. It is possible to look at hundreds of thousands of SNPs at the same time using gene chips. The human genome consists of about 10 million SNPs [149]. Seven SNPs have been associated with CMT and/or carotid artery remodeling [112, 150, 151]. In Study IV, we evaluated two of these SNPs as possible determinants of AoMT, available on the Illumina Human 610W-Quad BeadArrays platform that was used in the ASAP study.

The ASAP population has about the same age as the populations in the genetic studies of CMT, but with a favorable risk-factor profile regarding atherosclerosis. All ASAP patients were free of coronary stenosis. In addition

BAV patients displayed a lower prevalence of myocardial infarction and hypertension, as well as a lower mean age than TAV patients. Furthermore, BAV AS is the result of an altered mechano-biological environment [25] with high shear [26] and mechanical[27] stresses implying a different process in BAV and TAV leading to the same AS phenotype. These factors might explain why the rs200991 SNP genotype was significant only for the TAV group in Study IV; however, with low power for a genetic study. Gertow et al [112] reported a positive association for the A allele with the CIMT values, whereas we found a negative association with AoIMT. This is somewhat puzzling and additional larger studies are needed to confirm our findings.

## **5.4 Methodology**

### **5.4.1 Strain imaging**

VVI incorporates speckle tracking and border-detection tracking, following tissue from frame to frame using algorithms for spatial coherence [76, 152]. The relative displacement of speckles provides an angle-independent measure of tissue deformation enabling calculation of area change, strain and other parameters.

As in all 2-D imaging, there are problems with lateral resolution at the border of the ultrasound sector and displacement of speckles out of the imaging plane. VVI is dependent on good image quality throughout the cardiac cycle. VVI analysis incorporates the entire aortic circumference, accounting for local variations in wall motion and deformation. This is in contrast to M-mode and echo tracking, which have been the prevailing methods for local elasticity measurements.

We do not have a clear explanation of the inhomogeneous pattern of movement demonstrated in Figure 29. MRI of the descending aorta showed a similar pattern of sector velocities with the smallest displacement nearest the spine [153]. Interestingly, the inhomogeneous deformation pattern has also been reported in the carotid artery [78], implying that it is not an isolated phenomenon that is dependent on the anatomic structures surrounding the descending aorta.

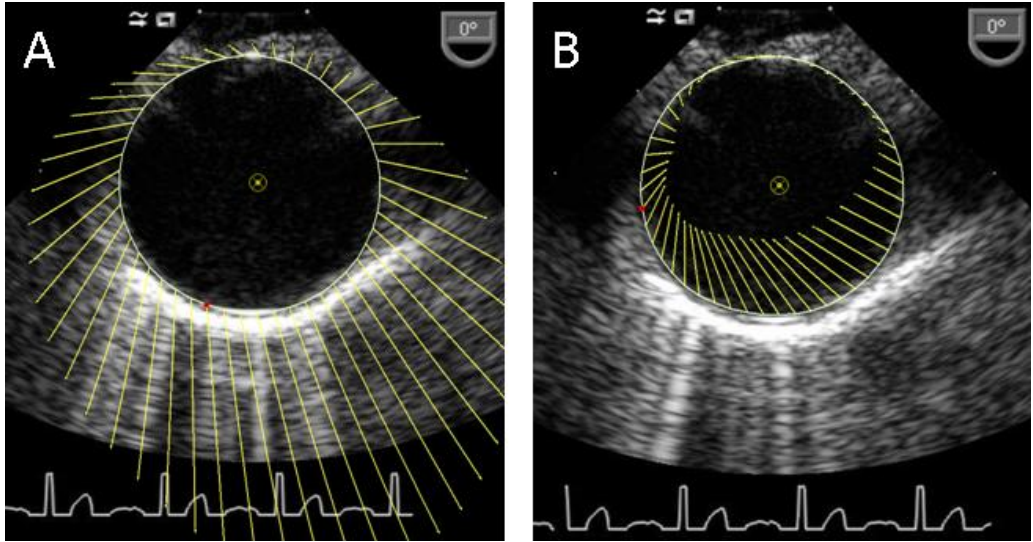


Figure 29. *Inhomogeneous deformation pattern of the descending aorta measured by VVI. A: systole; B: diastole. Reprinted with permission from Elsevier [Study III].*

We also validated VVI in an aortic phantom mimicking TTE measurement elasticity in the descending aorta and found a strong correlation between strain measured by VVI and sonomicrometry (unpublished data).

In Study II, we showed low variability for the majority of the VVI variables, except for the rotational displacement. VVI-derived strain, distensibility and stiffness correlated strongly with the corresponding parameters derived by M-mode. VVI-derived strain in the descending aorta is correlated with PWV [79] and the histological findings of collagen and elastin [154]. VVI seems to be a feasible method for the measurement of local aortic elasticity.

### 5.4.2 Intima-media thickness of the descending aorta

Intima-media thickness outside of the carotid territory is not a clinical marker with established normal values. Different IMT variables have been reported (max or mean IMT), based on M-mode or 2-D images. Looking at the guidelines for CIMT, it is recommended that measurements of IMT should be averaged over a 1-cm segment and not determined by a ‘simple point-to-point

measurement' [93]. There are many reasons for this, including non-uniform thickening of the arterial wall, lower spatial resolution and low reproducibility.

Regarding the resolution, the pixel size in 1-dimensional imaging is about 0.1 mm [93], whereas the measurement error measuring along a 10-mm-wide segment is much lower, at  $<0.01$  mm [87]. This is possible because of the high number of independent measuring points. Referring to the CIMT guidelines [93], true longitudinal imaging demonstrating double lines on the near and far wall simultaneously is the preferred method. This was not possible in the majority of our patients so we decided measure AoIMT of the far wall in the transverse plane. Until now, there has been only one study of aortic IMT using semiautomatic edge-detection methods in a 10-mm-wide vessel segment in long axis, but this was performed in the abdominal and not in the thoracic aorta [102]. Therefore, our study adds some new insights into the vascular structure in BAV.

The intra- and interobserver variability for IMT measurement in short axis view was in terms of CV 10-11%. The measurements were performed on cine loops manually set to end-diastole, defined using the ECG recorded on the pictures. Compared with studies of CIMT, this CV may seem rather high. One explanation could be that the measurement was not always performed on the same frame. Some authors have reported a CV of 5-6% when examining the same still frame, but 13% between two separate imaging procedures [84]. For semiautomatic edge-detection methods in the long axis of the abdominal aorta the CV was 9-12 % [102], and the circumferential approach alone is not a likely sole reason for the rather high CV. Image resolution, distance to transducer, transducer frequency, transducer construction, and imaging protocol are other factors that might differ between TEE investigation of the aorta using a small sector probe and carotid investigation using a considerably larger linear probe.

## 5.5 Limitations

The study population of this thesis consisted of patients with aortic valve disease and/or ascending aortic aneurysm, requiring surgery. Our patients had no significant coronary artery disease on angiography. There was no age-matched control group. This means that the results are not generalizable to patients with normally functioning bicuspid aortic valves or patients with BAV and coronary artery disease. The studies were cross-sectional so there are no follow-up data at this point.

TTE exams were performed in an outpatient setting, whereas TEE exams were performed during general anesthesia prior to the planned surgical procedure. The anesthesia and other medications given in connection with the preoperative preparation without doubt affected the pre- and afterload. Even though the absolute values for the elasticity indices calculated might have been affected, we were able to compare BAV and TAV patients under similar conditions.

Another limitation concerning the calculations of vascular indices was that we used radial blood pressure instead of central aortic pressure. However, in a limited number of patients, where the aortic pressure was measured invasively and simultaneously, the agreement with radial pressure was within 2 mmHg [155]. The patient population in Study IV was small for a genetic study, leading to a low statistical power.

## 6 FURTHER PERSPECTIVES

The cause of ascending aortic dilation in BAV will probably continue to be debated. Mahadevia et al showed that 3-D blood flow in the aorta differed significantly between phenotypes of BAV and that the eccentric flow pattern predicted the morphology of the aortic root and ascending aorta [133]. From the ASAP data, proteomics and RNA expression have shown different biological pathways leading to TAA in BAV and TAV patients [43] and a flow-mediated impairment of wound healing in BAV patients [156]. On the other hand, the aortopathy is prevalent in first-degree relatives of BAV patients [48] and degenerative changes have been demonstrated in the pulmonary trunk of BAV patients [50]. There is probably no single factor explaining the aortopathy in BAV patients, rather a complex interaction between genetic predisposition, hemodynamic factors, epigenetics and environmental factors [19].

In the general population, dilation and aneurysm of the ascending aorta are approximately three times more common than in the descending aorta [141, 157]. The repair of a descending aortic dissection is only called for when organ ischemia occurs or in connective tissue disorders [158], and the surgical repair of descending aortic aneurysms and dissections is complex. BAV is not associated with a higher prevalence of descending aortic dilation [56, 159] or dissection [160, 161]. The clinical implications of our findings, that BAV aortopathy extends beyond the ascending aorta, remain to be shown. Altered descending aortic function may be due to alterations by genetic or hemodynamic [52] factors, however, further studies are needed in order to understand the mechanisms.

As TEE is a semi-invasive method, it is unlikely to be applied for the sole purpose of measuring AoIMT. However, AoIMT can provide additional information about the atherosclerotic burden in patients undergoing TEE for other indications. Regarding SNPs correlated with AoIMT, larger studies are needed to draw firm conclusions.

## 7 CONCLUSIONS

- BAV phenotypes were not associated with any specific morphology (aneurysm, ectasia or normal) of the aortic root or the ascending aorta. Ectatic aortas with true BAV were significantly smaller compared with RL and RN phenotypes. In patients with AS only BAV was associated with a dilated ascending aorta (Study I).
- VVI was feasible for studies of aortic strain and function, with acceptable measurement variability. VVI provides new insights into aortic wall deformation (Study II).
- In BAV patients functional abnormalities were not limited to the ascending aorta, but present also in the descending aorta. BAV was associated with lower strain and distensibility in the AR group, but higher distensibility in the AS group compared to TAV. Age was the main predictor of function of the descending aorta (Study III).
- Intima-media thickness of the descending aorta was not affected by aortic valve morphology (BAV/TAV), and age was the main determinant of AoIMT. Genetic markers (SNPs) known to influence IMT in the carotid artery seem to correlate to IMT in the descending aorta in patients with TAV (Study IV).





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