Faculteit Geneeskunde en Gezondheidswetenschappen

Aortic dilation in Turner Syndrome: quest for the missing link

A clinical contribution to the understanding of the pathogenesis of aortic dilation in Turner syndrome

Katya De Groote

Thesis submitted to fulfill the requirements for the degree of Doctor in Medical Science

Ghent, 31 May 2016

Supervisor: prof. dr. Daniël De Wolf

Second supervisor: prof. dr. Jean De Schepper

Composition of the PhD Jury:

Prof. Dr. Johan Vandewalle	President	Ghent University
Prof. Dr. Bert Callewaert		Ghent University
Prof Dr. Michel Deneyer		Vrije Universiteit Brussel
Prof. Dr. Katrien François		Ghent University
Prof. Dr. Peter Gheeraert		Ghent University
Prof. Dr. Dirk Matthys		Ghent University
Prof Dr. Bert Suys		KU Leuven
Prof Dr. Guy T'sjoen		Ghent University

Cover illustration by Annemarie Iserbyt

PREFACE

The Quest

In mythology, a ***quest*** is a journey towards a goal, frequently serving as a symbol. By the quest, the hero aims to obtain an object, something or someone that fulfills a lack in her life. Obtaining this object requires great exertion on the part of the hero and the overcoming of many obstacles.

In narratology, the monomythe (or the hero's journey) is the common template of a broad category of tales that involve a hero who goes on an adventure, in a decisive crisis wins a victory, and then comes home changed or transformed. The hero’s story is described in different stages.

The Departure

The hero lives in the ordinary world and receives a call to go on an adventure. The hero is reluctant to follow the call, but is helped by a mentor figure.

The Initiation

The hero traverses the threshold to the unknown or "special world", where she faces tasks or trials, either alone or with the assistance of helpers. The hero eventually reaches "the innermost cave" or the central crisis of her adventure. Here she must undergo "the ordeal" where she overcomes the main obstacle or enemy, undergoing "apotheosis" and gaining her reward.

The Return

The hero again traverses the threshold between the worlds, returning to the ordinary world with the treasure she gained, which she may now use for the benefit of her fellow man. The hero herself is transformed by the adventure and gains wisdom or spiritual power over both worlds.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ACE	angiotensin converting enzyme
AoAsc	ascending aorta
AoHP	highest position of the transverse aortic arch
AoRt	aortic root
AoT	transverse diameter of the aortic arch
ARB	angiotensin receptor blocker
ASI	aortic size index (diameter ascending aorta / BSA)
BAV	bicuspid aortic valve
BMI	body mass index
BSA	body surface area
BP	blood pressure
CHD	congenital heart disease
CW	continuous wave
DBP	diastolic blood pressure
DHT	diastolic hypertension
DSD	disorder of sexual development
EMS	external masculinization score
ETA	elongated transverse arch
GH	growth hormone
HLHS	hypoplastic left heart syndrome
HRT	hormone replacement therapy
IVF	in vitro fertilization
MIP	maximum intensity projection
MRI	magnetic resonance imaging
NA	not available
LSVC	persistent left superior caval vein
PAPVC	partial anomalous pulmonary venous connection
PW	pulsed wave
PWV	pulse wave velocity
RAA	renin-angiotensin-aldosterone
SBP	systolic blood pressure
SHT	systolic hypertension
SMR	standardised mortality rate (ratio observed to expected deaths)
STJ	sinotubular junction
TAV	tricuspid aortic valve
TS	Turner syndrome

CHAPTER I

DEPARTURE

-

The call to the adventure

An American endocrinologist, Henry Turner (1892-1970), described in 1938 “a syndrome of infantilism, congenital webbed neck, and cubitus valgus” [154]. Although we currently know that the pathology is much more complex than described in his first publication, this clinical entity entered history as Turner syndrome (TS).

In the last decades, the knowledge about TS increased dramatically. In this introduction, the incidence and the complexity of the genetic and clinical picture of Turner syndrome will be described with focus on its cardiovascular aspects.

I.1 Turner genotype and phenotype

I.1.1 Turner genotype

TS is a chromosomal disorder occurring only in females. It is caused by a partial or a complete absence of an X chromosome. This may occur within the presence of a structurally abnormal X chromosome (isochromosome or ring chromosome) or the presence of Y-chromosome material. The genotype of TS is therefore very diverse complicating research in this patient group. Classification is often presented in 4 major groups presented in table 1.

Table 1: Turner genotype

Monosomy 45,X	
Isochromosome 46,X,i(X)	
Deletion 46,X,del(X)	
Mosaic forms consisting of 45X with another cell line	46,XX 46,X,i(Xq) 46,X,del(X) 46,XY or other abnormal Y material 47,XXX 46,X,r(X)

Data on the prevalence of the different genotypes in live born girls are diverse and difficult to compare due to the different categorisations used. In all datasets, the largest subgroup consists of monosomy 45,X found in 36 to 45% of TS patients [106, 141, 149]. Another important but highly heterogeneous genotype subgroup is formed by the mosaic forms that express both a normal 46,XX and an abnormal cell line (table 1). The degree of mosaicism is expressed as the number of normal 46,XX to the number of abnormal cells (i.e. 46,XX [45]/45,X [55]). The relation between both cell lines in patients is not constant and varies according to the number of meioses counted, the tissue studied and with age [38]. In nature, a true monosomy is not compatible with life and all 45,X individuals with monosomy are probably low graded mosaics [74]. For the purpose of this thesis however, monosomy 45,X remains valid as a subgroup.

I.1.2 Turner phenotype

Table 2: Turner phenotype

System	*Abnormality*
Dismorphic features	Webbed neck
	Shield chest with widely spaced nipples
Gonadal	No pubertal development
	Infertility
	Chronic estrogen deficiency
Endocrine	Short stature
	Hashimoto thyroiditis and hypothyreosis
	Diabetes mellitus type 1 and 2
	Dyslipidemia
	Decreased bone mineral content
Cardiovascular	Structural congenital heart disease (left sided)
	Progressive dilation of the ascending aorta
	Hypertension
	ECG abnormalities
Gastrointestinal and hepatic	Celiac disease
	Elevated hepatic enzymes
	Inflammatory bowel disease
Renal	Horseshoe kidney
	Abnormal positioning or duplication
Skeletal	Cubitus valgus
	Scoliosis
	Madelung deformation
	Short 4th metacarpal
	Genu varu
Nose, ear, throat	Distortion of the Eustachian tube
	Neurosensorial hearing loss
	Micrognathia
	High arched palatum
	Abnormal dental development
Ophthalmologic	Epicanthus
	Strabismus
	Ptosis
Dermatological	Increased skin ridge count
	Lymphedema
	Multiple pigmented naevi
	Nail hypoplasia
	Vitiligo
	Alopecia
	Low posterior hair line
Neurological	Emotional immaturity
	Specific learning problems
	Poor visuospatial organisation
	ADHD
	Autism and mental problems

The most distinctive clinical features of Turner syndrome are small stature and premature ovarian failure but patients also present a broad spectrum of other diseases with a major impact on morbidity and mortality.

All systems that can be affected are listed in table 2. Although the phenotype can vary highly in severity, most TS patients develop problems in several systems and benefit from a multidisciplinary follow-up.

I.1.3 Association between genotype and phenotype in Turner syndrome

It is not defined yet which chromosomal regions and genes account for the physical characteristics of TS, but there seems to be some association between the genotype and phenotype in TS [149]. Girls with monosomy 45X are more likely to present with congenital lymphedema. TS patients with mosaic 45,X/46,XX have a higher chance for normal menarche and fertility while those with 45,X/46,XX/47,XXX tend to have a taller final height. The presence of an isochromosome X increases the risk for hypothyroidism and inflammatory bowel disease while a marker or ring chromosome is more likely to be associated with mental retardation. TS girls with a 46,X/46,XY genotype are at an increased risk for germ cell tumours [28].

One of the subgroups of the TS genotype is formed by sex chromosome mosaicism (45,X/46,XY and variants). Apart from females TS patients, this genotype is also found in apparently normal males presenting at infertility clinics, in boys consulting for short stature or in neonates born with genital ambiguity [22, 151]. Some males patients with a mosaic 45,X/46,XY show stigmata typically associated with TS like short stature, renal pathology and coarctation [151].

Some data suggest a correlation between the Turner genotype and the cardiac phenotype. The most severe cardiac phenotypes are found in TS patients with a monosomy 45,X. They more frequently have congenital heart disease [109, 135, 136, 140] and develop more severe aortic dilation [135] than those with other genotypes. The high prevalence of BAV and coarctation in TS patients with an Xp deletion, suggests that haploinsufficiency for genes that are located on Xp contribute to an abnormal aortic valve and aortic arch development [8]. This phenomenon could also explain the increased incidence of BAV and aortic pathology in males with 45,X/46,XY.

Left sided heart defects like bicuspid aortic valve and coarctation are more frequently found in TS patients with a webbed neck compared to those without [72, 94, 140]. The webbed neck is a remnant of the central fetal lymphedema that is often noted prenatally in TS foetuses. It can present as a severe cystic hygroma or as an increased nuchal translucency. There are several hypotheses to explain this association between abnormal fetal lymphedema and the congenital heart defects found in TS. Clark hypothesises that centrally localized distended lymphatic sacs compress the developing aortic root, resulting in specific left-sided defects, including hypoplastic left heart, BAV, and coarctation due to low flow, and specific right-sided defects such as persistent left superior caval vein, anomalous pulmonary venous return, and dilated right atrium, due to back pressure from obstruction to forward flow [26]. In favour for this hypothesis is the well known association between

fetal increased nuchal translucency and congenital heart defects that is also found in non-TS foetuses [75]. An alternative explanation for the association between neck webbing and BAV in TS could be the haploinsufficiency for an X-chromosome gene that causes central fetal lymphedema and aortic heart defects independent of each other [140].

Some X-linked genes have been put forward as being possibly involved. Vascular endothelial growth factor (VEGF) is a specific growth factor for endothelium that also plays a role in the signalling involved in embryonic endocardial-to-mesenchymal transformation of the endocardial cushions. Overexpression of VEGF during fetal life is associated with fetal hydrops and with the development of congenital heart defects [16].

I.1.4 Timing of diagnosis of Turner syndrome

The phenotype of TS patients is highly variable and may change over time as some diseases only appear later in life. In many patients, the diagnosis of TS is delayed until adult age. The age at the diagnosis of TS depends on the phenotype and the clinical presentation.

Prenatal diagnosis of TS is mostly made “accidently” after amniocentesis for increased nuchal translucency, abnormal triple testing or advanced maternal age. Prenatal genetic testing can also be performed after the prenatal detection of a congenital heart defect (CHD). In case of an incidental finding of mosaic 45,X/46,XX or 46,X/46,XY without abnormalities on prenatal ultrasound, the initial phenotype at birth is often normal [149].

One fifth to one third of TS girls receive their diagnosis in the neonatal period because of puffy hands and feet, webbed neck or the presence of left sided CHD. Another third is diagnosed in childhood on investigation for short stature. Most other patients present in adolescence or adulthood because of absent or arrested puberty or infertility.

I.2 Incidence of Turner syndrome

I.2.1 Incidence of Turner syndrome in live born girls

Turner syndrome is a rare disease with an estimated incidence of 1/2000 [116] to 1/5000 [77, 96] live born girls. This might however be an underestimation as the phenotype can be mild and patients may remain clinically undiagnosed. Moreover, older genetic techniques were insufficient to detect low grade mosaicism categorising some TS patients as “normal 46,XX”.

I.2.2 Incidence of Turner syndrome in the prenatal setting

Data on prenatal genetic testing in a large non-selected population are lacking and the exact incidence of TS in early fetal life remains currently unknown. The incidence in foetuses is much higher than in the live born population as only a very small proportion of all foetuses with TS is born alive. Studies in foetuses with early spontaneous fetal death show a monosomy 45X in 2% of the cases [53]. In pregnancies that were terminated because of severely increased nuchal translucency or hygroma colli, 1% of the foetuses had TS [40]. Based on this data, Hook et al. calculated that 1 to 1.5% of all clinically recognised pregnancies starts as a monosomy 45X but 99% of the foetuses does not survive [73].

I.3 Cardiovascular pathology in Turner syndrome

Cardiovascular pathology is highly prevalent in TS and adds significantly to the morbidity and mortality of the syndrome. In this section, the cardiovascular pathology related to TS is described using the most common classification. Congenital structural heart defects, dilation and dissection of the aorta as part of a generalised vasculopathy, hypertension and ECG abnormalities and sympathic dysfunction are discussed and a comparison is made between TS and the general population.

As described further in this chapter, CHD, hypertension and aortic dilation contribute to the risk of aortic dissection. Timely diagnosis, treatment and follow up of these pathologies help to reduce the occurrence of this often lethal complication. The following section therefore describes extensively their clinical presentation and the current treatment strategies.

I.3.1 Congenital structural heart defects

Structural congenital heart defects (CHD) are frequent in TS and mainly involve the left side of the heart. In table 3, the prevalence of the most frequent abnormalities in TS are listed and compared to data of the general population [6, 39, 48, 55, 72, 81, 94, 109, 136].

Table 3: prevalence of CHD in TS compared to the general population

Heart defect	TS (%)	General population (%)
Bicuspid aortic valve	14 – 40	1 - 2
Coarctation of the aorta	4 – 15	0.04
Elongated transverse aortic arch	31 – 49	Non existing
Bovine arch	6 – 29	13
Aberrant right subclavian artery	6 – 8	0.5 – 2.5
Persistent left superior caval vein	8 – 13	0.3 – 0.5
Partial anomalous pulmonary venous connection	4 – 16	unknown
Hypoplastic left heart syndrome	4 – 5	0.0002 – 0.0003

The reported prevalence of structural heart disease in literature is highly variable and partially depends on the imaging technique used (MRI or ultrasound). Echo windows in TS can be very poor due to obesity and an increased anteroposterior diameter of the thorax. MRI can overcome these difficulties, thus leading to a higher reported prevalence of bicuspid aortic valve, aortic coarctation and partial anomalous pulmonary venous connection.

I.3.1.1 *Bicuspid aortic valve*

Definition

Bicuspid aortic valves (BAV) can either have 2 cusps (structurally bicuspid) or 3 cusps of which 2 are completely or partially fused (functionally bicuspid). When the right and left coronary cusp are fused the valve has an anterior-posterior configuration on transthoracic echocardiography. When the non-coronary cusp is fused with the left or right coronary cusp, the BAV has a left-right configuration [32] (figure 1).

In TS, only 1 in 3 BAV is structurally bicuspid. The others are functionally bicuspid. In the majority of cases BAV have an anterior-posterior configuration on transthoracic echocardiography [140].

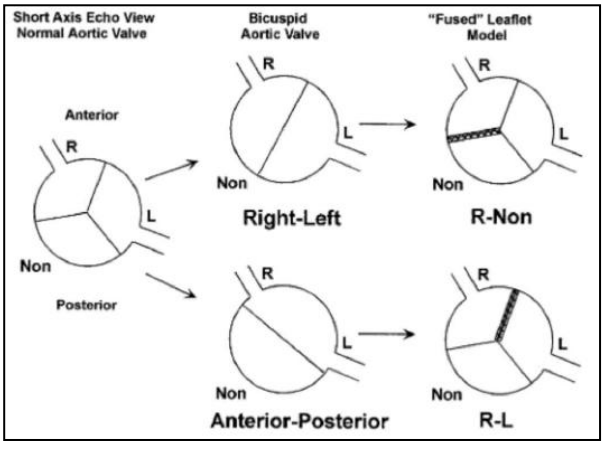

Figure 1: two-dimensional cross-section of the aortic valve on echocardiography with a schematic representation of the various abnormal aortic valve morphologies. L=left; R=right [32].

Prevalence in TS

The reported prevalence of BAV in TS is high, ranging from 14 to 40% [39, 48, 55, 81, 94, 109, 136]. The highest numbers are found is studies were imaging was performed using MRI [81, 109].

Clinical significance

BAV is highly prevalent in TS but clinically important dysfunction of the aortic valve is rare [109, 124, 140]. Severe insufficiency is found in 4 to 15% of TS patients [124, 140] and although TS patients show an increased peak velocity across the aortic valve, moderate to severe stenosis is only found in 2% [124, 140]

BAV is often associated with structural abnormalities of the aorta such as dilation of the aortic sinus, sinotubular junction and ascending aorta (independent of the presence of aortic valve stenosis) (25 - 46%) [71, 109, 140], coarctation of the aorta (22%) [8] and elongated transverse arch (50%) [109].

Treatment

A BAV only needs treatment when its is dysfunctional. In general, the therapeutic options are similar to the aortic valve pathology in other patient groups. When the aortic valve is severely stenotic but without significant insufficiency, the valve can be ballooned percutaneously. If there is a clinically important valvular insufficiency, surgical treatment is required. When the aortic valve has a reasonable size and is not severely dysplastic, conservative valvuloplasty is preferred. In TS patients with a concomitant dilation of the aortic sinus, the root is replaced by a vascular graft with re-implantation of the coronary arteries (David procedure). If the aortic valve is too dysplastic to repair, surgical replacement is necessary. In adults, replacement of the abnormal aortic valve with a mechanical valve is an option. If there is an associated dilation of the aortic root and the ascending aorta, the aortic valve, root and ascending aorta are replaced with a composite graft with re-implantation of the coronary arteries (Bentall procedure). The use of mechanical valves and vascular graft interposition is avoided in children. In this young age group, the diseased aortic valve is replaced with the patient's own pulmonary valve and a pulmonary allograft is used to replace the patients' pulmonary valve (Ross procedure).

Comparison with the general population

In the general population, BAV is found in 1 to 2% and is more prevalent in males than in females. Fusion of the right-coronary and the left-coronary leaflet is the most common type (59-70%), both in isolated BAV and in BAV associated with congenital heart disease. Fusion of the right-coronary and non-coronary cusp is found in 28 - 37% while fusion of the left-coronary and non-coronary cusp is rare (1-4%) [24, 46].

I.3.1.2 *Coarctation of the aorta*

Definition

Coarctation of the aorta is a stenosis of the descending aorta, which is typically located at the insertion of the arterial duct distal to the left subclavian artery (figure 2).

Prevalence in TS

The reported prevalence of aortic coarctation in TS ranges between 4 and 15% [39, 48, 55, 72, 81, 94, 136]. The highest numbers are found in studies using the MRI technique [72, 81, 109].

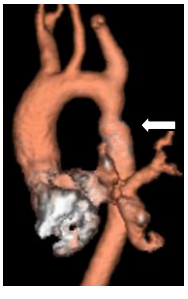

Figure 2: coarctation in an 11 year old TS girl (MRI reconstruction image)

Clinical significance

The clinical significance of coarctation depends on the severity of the stenosis. If the coarctation is tight, patients present in the first days of life with severe cardiac decompensation after closure of the arterial duct (duct dependent circulation) and medical treatment is an emergency. Less severe stenosis often remains undetected until later in life. Adolescents and young adults present with upper limb hypertension and severe headache. In adult TS patients, coarctation can be an incidental finding on routine MRI [48, 81].

Coarctation of the aorta is frequently associated with BAV (21%) [8] and is often seen in association with elongation of the transverse arch (ETA) [72, 81, 109].

Even after successful treatment, patients with coarctation of the aorta remain at increased risk for hypertension later in life. The incidence of hypertension in non-TS patients treated for coarctation increases with age going from 1.36% in the paediatric group to 45.7% in patients above 30 years of age [164].

Treatment

Treatment of coarctation in TS is similar to that of coarctation in the general population and depends on the severity of the coarctation and the age at presentation. Severe neonatal coarctation is a medical urgency requiring prompt administration of IV prostaglandins to open the arterial duct and to relax the coarctation region followed by surgical resection of the narrowed aortic region. Significant coarctation that presents later in life can be treated either by balloon dilation, percutaneous stenting [167] or surgical resection. Surgical treatment in TS more often requires the more complex subclavian flap technique (instead of the straight forward end-to-end anastomosis), but long term results are similar to those obtained in non-TS patients [31].

Comparison with the general population

In the general population, the reported prevalence of aortic coarctation is 4.4/10 000.

I.3.1.3 *Other structural abnormalities of the aortic arch*

Definition

Apart from aortic coarctation, the aorta of TS patient can show other structural abnormalities:

Elongation of the transverse arch (ETA): combination of late takeoff of the left subclavian artery behind the trachea and a box shaped appearance of the aortic arch with an acute angle along the lesser curvature of the aortic isthmus (figure 3).
Bovine arch: common origin of the brachiocephalic trunk and the left common carotid artery.
Right arteria lusoria: abnormal origin of the right subclavian artery that does not arise from the brachiocephalic trunk but originates distal to the left subclavian artery and courses to the right behind the oesophagus.

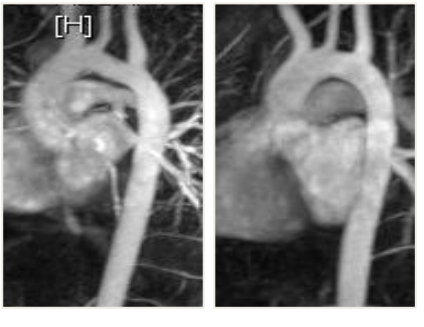

Figure 3: in the left panel elongation of the transverse arch (ETA) compared to a normal aortic arch in the right panel

Prevalence in TS

All reported prevalences are derived from MRI studies [72, 81, 109]:

ETA: 31 to 49%

Bovine arch: 6 to 29%

Right arteria lusoria: 6 to 8%

Clinical significance

Structural abnormalities of the aorta have little clinical significance. An aberrant right subclavian artery is usually asymptomatic, but can cause dysphagia and dyspnea or chronic coughing in some patients [112]. ETA is isolated in 50% of the cases but can be associated with other structural heart defects like coarctation of the aorta or BAV [72, 81, 109].

Comparison with the general population

ETA is a typical finding in TS and is not described in the general population. The overall prevalence of bovine aortic arches in the general population is 13% (8% in Caucasians and 25% in individuals of African origin) [91]. A right arteria lusoria is reported in 0.5 to 2.5% [112].

I.3.1.4 *Persistent left superior caval vein*

Definition

A persistent left superior caval vein (LSVC) is a remnant vessel that is present as a counterpart of a normal right-sided superior caval vein in early embryological development and that normally disappears during fetal life. It drains venous blood to the right atrium through the coronary sinus.

Prevalence in TS

Although it is possible to visualize LVCS on ultrasound, most cases in TS are reported in MRI studies with a prevalence of 8 to 13% [72, 81].

Clinical significance

In general, LSVC has no clinical significance and does not require medical treatment. In patients with TS, it is often associated with ETA or right arteria lusoria [72].

Comparison with the general population

In the general population, LSVC is found in 0.3 to 0.5% [150].

I.3.1.5 *Partial anomalous pulmonary venous connection*

Definition

In partial anomalous pulmonary venous connection (PAPVC) one or more pulmonary veins drain abnormally to the right instead of to the left atrium.

Prevalence in TS

PAPVU is reported in 4 to 16% of TS patients with higher numbers in studies with MRI compared to imaging by ultrasound [6, 72, 81, 101]. Abnormal drainage of the left pulmonary veins is the most common form in TS.

Clinical significance

The clinical significance of PAPVC depends on the number of abnormal veins and the amount of blood that drains to the right atrium. In most cases, PAPVC is an incidental finding without clinical repercussion. If several veins drain abnormally to the right atrium, patients exhibit signs of right sided volume overload with exercise intolerance as the major clinical complaint. If not recognised, it can eventually evolve towards pulmonary hypertension.

Treatment

The treatment of PAPVC is similar in TS compared to the general population. Surgical re-routing of the abnormal veins to the left atrium is considered when there are clinical or radiological signs of volume overload.

Comparison with the general population

There are no data on the prevalence of PAPVC in the general population. Abnormal drainage of the right sided pulmonary veins is 10 times more frequent than an abnormal drainage of the left sided pulmonary veins [2, 146]. The most common type is PAPVC to the superior caval vein (74%) of which 87% is associated with a sinus venosus atrial septum defect [2].

I.3.1.6 *Hypoplastic left heart syndrome*

Definition

Hypoplastic left heart syndrome (HLHS) is the most extreme expression of left sided pathology. Although structural defects can vary, the overall characteristic is the severely underdevelopment of the left ventricle, the mitral valve and the aortic valve with no or insufficient forward flow through the aorta. HLHS is probably one of the reasons for the high proportion of spontaneous prenatal demise in TS foetuses.

Prevalence in TS

HLHS is found in 13.2% of TS cases that are detected prenatally. Most pregnancies end in a termination of pregnancy arte provocatus or in spontaneous intra uterine demise [147]. In TS babies who are born alive a prevalence of 4 to 5% is reported [31, 159].

Clinical significance

If not detected prenatally, HLHS presents during the first days of life with severe shock when the arterial duct closes. Initial stabilisation requires urgent administration of IV prostaglandins. If not treated adequately, the situation quickly deteriorates towards death.

Treatment

The treatment of HLHS is similar in TS compared to the general population and consists of surgical palliation in 3 stages:

- Norwood procedure in the neonatal period
- bidirectional cavopulmonary anastomosis at the age of 3 to 6 months
- total cavopulmonary anastomosis after the age of 3 years.

Although the outcome after Norwood procedure improved dramatically over the last decade, this optimism does not apply to TS patients where the outcome remains very poor (mortality rate 75 to 90%) compared to other children without TS [31, 95].

Comparison with the general population

In the general population, HLHS is found in 2 to 3/10 000 live births with a male:female ratio of 1.5/1. The reported prevalence is probably underestimated because of the indeterminate rate of spontaneous intra uterine demise and elective termination of pregnancy.

I.3.2 Dilation and dissection of the aorta and generalised vasculopathy

I.3.2.1 *Definition of normal aortic growth and evaluation of aortic diameters*

Aortic growth and aortic dimensions in children

In children, the diameter of the aorta changes with age and growth. Therefore aortic dimensions are interpreted using z-scores that not only include the absolute aortic diameters but also the height and the weight (BSA) of the child. Until recently, the Sluysmans z-scores were the most frequently used in children [145].

$$Zscore\ AoAsc = \frac{AoAsc - 1.68 * \sqrt{BSA}}{0.192 * \sqrt{BSA}}$$

Measurements are made in systole from inner-to-inner wall. The BSA used in this formula is calculated according to Haycock [69] and takes into account body weight and height.

Recently new z-scores were published that not only take include the weight and the height (BSA) but also the gender and the age of the patients [18].

$$Zscore\ AoAsc_{females} = \frac{\log_{10}(AoAsc) - (1.006 + 0.172 * \log_{10}(age) + 0.087 * BSA)}{0.0450}$$

Measurements are made in diastole from leading edge to leading edge. The BSA used in this formula is calculated according to Haycock [69] and takes into account body weight and height..

Aortic growth and aortic dimensions in adults

In normal females the diameter of the aorta increases progressively with advancing age with an average of 0.9±0.4 mm/decade at the site of the aortic sinus and 1.2±0.5 mm/decade at the ascending aorta (BSA adjusted values) [18].

Prospective longitudinal data on the natural history of aortic growth in TS are scarce. There is only one study that repeated MRI imaging in a group of 102 adult TS women. This study showed an aortic growth rate in TS patients of 0.1-0.4 mm/year which exceeds the growth velocity of normal females [110]. The ascending aorta in TS women with a BAV grew faster that than in those with a TAV [110] . Also in non-TS patients, the presence of a BAV is associated with accelerated aortic growth with a mean increase in diameter of 0.77 mm/year [45].

I.3.2.2 *Aortic dilation*

Definition

Aortic aneurysm is an abnormal dilation or bulging of the aortic wall that involves the three layers of the vessel wall (figure 4).

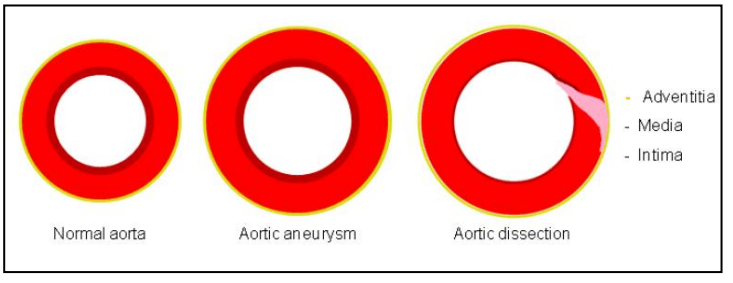

Figure 4: schematic presentation of a normal aortic wall (left), aortic aneurysm (middle) and aortic dissection (right)

Ascending aortas with a z-score ≥2 (above the 95th percentile) are considered dilated. TS women are generally small and therefore it is now advised to use the aortic size index (ASI) that normalizes aortic diameters for BSA [98] for adult TS patients. ASI>20mm/m² corresponds to the 95th percentile and ASI>25 mm/m² to the 99th percentile. An ascending aorta/BSA >20mm/m² is considered dilated, >25mm/m² is considered a risk for dissection. Although these definitions are initially calculated based on an MRI images, they are also applied in clinical practice to echo derived measurements.

Prevalence in TS

In a large paediatric TS population, ascending aorta dilation (z-score ≥2) was seen in 30% and aortic root dilation (z-score ≥2) in 20% of TS girls. The proximal aortic diameters were even larger in the 26% of patients with a BAV [93]. Similar data (10% of patients with z-score of 1.96 and 20% of patients with z-score of 2) were found in an MRI study in a smaller group of TS girls [21].

In adulthood, enlarged aortic dimensions are frequently found at the aortic root and the ascending aorta. Although dilation is often associated with CHD, abnormally large aortic diameters are also found in adult TS patients without a heart defect. In relatively young TS patients without CHD or BAV, aortic root dilation is seen in 11% [135]; the prevalence is higher, up to 42%, in the total group [42, 81]. Ascending aortic dilation is reported in 8% of TS patients without CHD or BAV [135] and is higher (20 to 24%) in the total group of adult TS patients [71, 98, 109].

Clinical significance and treatment

Aortic aneurysms are a major risk factor for aortic dissection. There are no prospective data on the surgical treatment of aortic aneurysms in TS and it currently remains unclear which aortic diameter urges prophylactic replacement. In clinical practice, treatment strategy is therefore based on theoretical assumptions. Aortic dilations with an ASI≥20 mm/m² require close follow-up and treatment of associated risk factors for dissection as hypertension or coarctation. In most centres,

an ASI ≥25mm/m² is considered an increased risk for aortic dissection and this diameter is used as a cut off for prophylactic aortic surgery [10, 19, 98, 156]. The role of medical treatment in the prevention of rapid aortic growth in TS remains unknown. There are no data to neither support nor reject the usefulness of medical treatment with betablockers or angiotensin converting enzyme in TS.

I.3.2.3 *Aortic dissection*

Definition

Aortic dissection is a progressive tear in the aortic wall. The intima of the aorta tears and blood surges trough the tear, creating a new false lumen and dissecting the media from the adventitia (figure 4). Dissections that affect the thoracic and abdominal aorta are usually classified according to the Stanford or DeBakey classification (figure 5)[70]. The Stanford classification categorises dissections depending on whether the ascending aorta is involved or not. The Debakey classification categorises dissections based on the origin of the tear and the extend of the dissection (ascending aorta, descending aorta, or both).

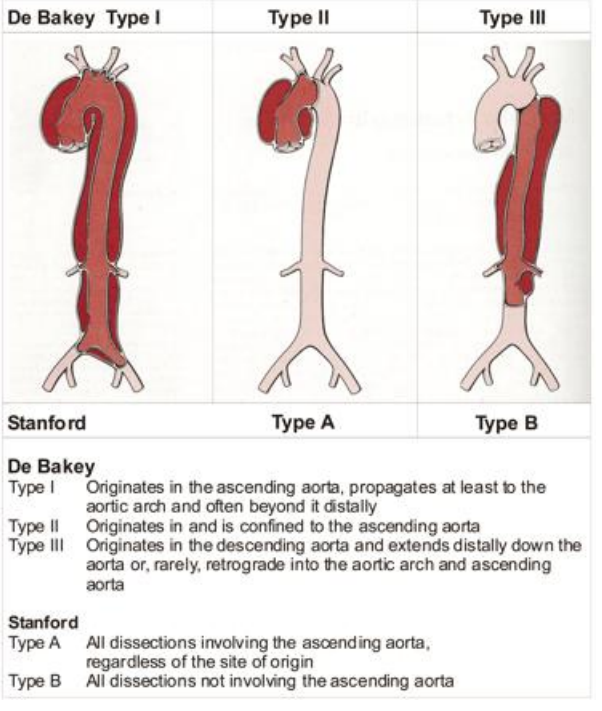

Figure 5: types of aortic dissection, classified according to Stanford and Debakey [117]

Incidence in TS

There are little prospective data on aortic dissections in TS patients. The reported incidence of aortic dissection in TS patients is 36/100 000 patient years or 1,4% [61]. Dissection of the aorta occurs at the relative young mean age of 30 to 35 years with a range of 18 to 61 years [20, 61] but dissection is also reported in childhood [148]. In more than 2/3 of the cases, the aorta dissects at the level of the ascending aorta (Stanford type A dissection) while type B dissections are less common [19].

Clinical significance

The clinical presentation of an acute aortic dissection is that of an acute thoracic syndrome. The symptoms are highly variable and can mimic those of other more common conditions like acute coronary syndrome or arrhythmia. Aortic dissection can present with congestive cardiac failure, syncope, acute stroke, acute myocardial ,infarction, ischemic peripheral neuropathy, paraplegia and cardiac arrest or sudden death. Although not always present, most patient report severe pain in the chest, the back and/or the neck [144].

Preliminary data of the International Turner Syndrome Aortic Dissection Registry show that nearly all TS patients developed clinical symptoms of dissection, chest pain being the most common. Other frequently reported signs were neck or back pain, dyspnea, nausea or vomiting and dizziness. The initial presentation was sudden death in 9 of the 20 TS patients. Mortality after dissection was high with a survival rate of only 37% [19]. Symptoms of an acute aortic syndrome are often not recognised or ignored by the young TS patients which leads to a late or underdiagnosis and probably explains the high mortality of dissection in this group.

Treatment

Treatment of an acute Stanford type A dissection is a medical emergency since patients are at a high risk for life-threatening complications such as malperfusion syndrome, tamponade, massive aortic regurgitation or myocardial infarction. The type of the surgical procedure depends on the involvement of the aortic valve and root. Both are spared if possible. In case of an ascending aortic aneurysm without involvement of the aortic valve and root, the aneurysmal part of the aorta is replaced by a Dacron tube. In case of associated aortic root involvement, also the root is placed by a vascular graft with re-implantation of the coronary arteries (David procedure). If the aortic valve can't be repaired, the aortic valve, root and ascending aorta are replaced with a composite graft with re-implantation of the coronary arteries (Bentall procedure). Aneurysms of the descending aorta are treated with endovascular therapy if feasible, given the morbidity associated with open procedures. [15]

In the general population, patients with an uncomplicated type B dissection are mostly treated medically. Surgical or endovascular intervention is reserved for patients with a complicated course. It is unclear whether this strategy also applies for TS patients [144].

Risk factors for aortic dissection

Risk factors for aortic dissection are the presence of a CHD (coarctation, BAV) or arterial hypertension. The majority of TS with aortic dissection have one or more risk factors, but dissection is also described in TS patients without a cardiac malformation, aortic aneurysm or hypertension [19, 20, 61].

The impact of pregnancy on the risk for aortic dissection remains the subject of discussion. Retrospective study of published case reports suggests that pregnancy in TS increases the risk for aortic dissection and report a maternal demise of 2% [79]. On the other hand, in a large Swedish study in 124 childbearing TS patients, there was no maternal death [67]. The incidence of aortic aneurysm was similar in the TS group with or without pregnancy (2.4 versus 2.2%). Two of the 3 TS patients who developed an aneurysm during pregnancy or in the postpartum period had a concomitant cardiac problem.

As pregnancy increases the risk for aortic dissection in TS patients with pre-existing CHD or aortic dilation, rigorous preconceptional cardiovascular screening and counselling is mandatory. Pregnancy is contraindicated in TS patients with previous cardiac surgery, aortic coarctation, severe dilation of the aorta defined as ASI ≥25 mm/m² or uncontrolled hypertension. [17]

Comparison with the general population

In the general population dissection occurs with an incidence of 2.9/100 000 patient years [102] at a mean age of 63 years [66]. Like in TS, Stanford type A dissections are the most frequent (70-75%) [88, 102]. Men are more at risk for aortic dissection than women [66]. Mortality after acute type A dissection in the general population varies between 10 and 35% for patients who are surgically treated [144].

I.3.2.4 *Vasculopathy beyond the aorta*

Apart from the aorta, abnormally large diameters are also found in the carotid, subclavian and brachial arteries of TS women [109, 126]. In a group of young TS patients, the stiffness of the carotid artery was increased compared to controls, suggesting a vasculopathy that reaches beyond the aorta [90].

Although not studied in a large population, the vasculopathy in TS seems to extend into the brain. The odds to die from a cerebrovascular accident are increased 4 fold in TS [141]. Large datasets or reviews on cerebrovascular pathology in TS are lacking but several case reports describe severe cerebrovascular accidents in young TS patients: atherosclerosis, congenital carotid hypoplasia, dissection of the internal carotid artery with cerebri media infarction and Moyamoya disease [47, 50, 76, 76, 78, 83, 111, 123, 152].

I.3.3 Arterial hypertension

Definition of normal and increased blood pressure

Blood pressure in children varies with age. Blood pressure percentiles for sex, age and height are available for in office [115] and 24 hour ambulatory [165] measurements. Blood pressure values above the 95th percentile indicate hypertension [115], values between the 90th and 95th percentile pre-hypertension.

According the the European Society of Cardiology blood pressure in adults is considered too high if in office blood pressure exceeds 140/90 mmHg or if the mean 24 hour blood pressure exceeds 130/80 mmHg [97]. However, in the TS literature, different definitions of hypertension are used, complicating comparison of data [37].

Prevalence in TS

Hypertension is a common problem in TS. It is reported in 13 to 58% of adult [4, 42, 62, 71, 86, 126, 134, 160] and in 25% of the paediatric [1, 49] TS patients. The large variance in the reported prevalances can probably be explained by differences in race and lifestyle and by the different definitions of hypertension used in the various study protocols. Insufficient nocturnal dipping is a common finding on 24 hour ambulatory blood pressure monitoring both in adults [51, 63] and children [1, 49, 114]. Although some groups found that TS patients preferentially suffer from isolated diastolic hypertension, this is not confirmed by others [37].

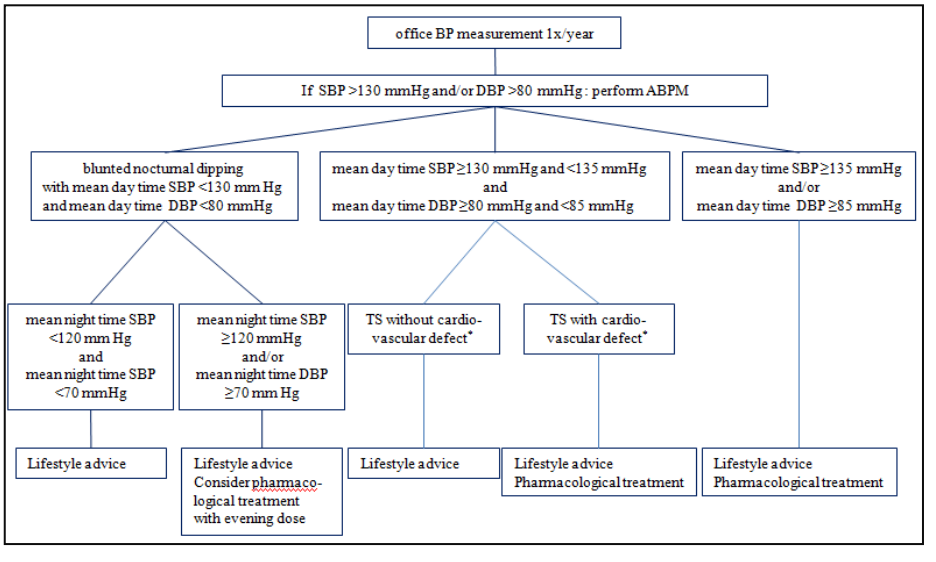

Figure 6: algorithm for the follow-up and treatment of hypertension in TS patients. Adapted from K De Groote et al. [37]. * Cardiovascular defects: bicuspid aortic valve, aortic coarctation or dilation of the ascending aorta >20 mm/m².
TS patients with additional cardiovascular risk factors are treated according the international guidelines on arterial hypertension [97]

Clinical significance

Hypertension is a well known risk factor for cerebrovascular accidents and aortic dissection and contributes to the increased morbidity and mortality of TS. The relative risk for hypertension related morbidity is 2.9 (95% CI 1.9–6) [60] and death related to hypertensive disease is six times higher than in the general population (SMR 6.0, 95% CI 1.2–17.5) [141].

Treatment (figure 6)

Hypertension contributes highly to the negative prognosis of TS and therefore needs rigorous follow-up and treatment. Cut off values for the initiation of medical treatment and blood pressure targets in TS remain a subject of debate as interventional studies in this population are laking. In view of the increased risk for aortic dilation and the established detrimental effect of hypertension in the evolution to aortic dissection or rupture, lower blood pressure thresholds seem appropriate in TS patients with CHD [27, 155]. Treatment of hypertension includes adaptation of a healthier life style, anti-hypertensive medication and treatment of concomitant diseases as diabetes and thyroid dysfunction.

There are no data comparing different antihypertensive therapies in TS patients. Current treatment strategies are guided by clinical experience and common sense (table 4).

Table 4: antihypertensive treatment based on associated pathology

Indication	Initial treatment
Isolated hypertension	Beta-blocker If contra-indicated or not tolerated: ACE-inhibitor or angiotensin receptor blocker or calcium channel blocker
Associated dilation of the aorta	Beta-blocker If contra-indicated or not tolerated: angiotensin receptor blocker
Associated systolic ventricular dysfunction	ACE-inhibitor Angiotensin receptor blocker Beta-blocker Associate a diuretic if necessary Associate a mineralocorticoid receptor blocker if necessary
Associated left ventricular hypertrophy	ACE-inhibitor Angiotensin receptor blocker Beta-blocker Calcium channel blocker Associate a diuretic if necessary Associate a mineralocorticoid receptor blocker if necessary
Metabolic syndrome	ACE-inhibitor Angiotensin receptor blocker Calcium channel blocker
Pregnancy	Beta-blocker Methyldopa Associate long-acting calcium channel blocker if necessary

Most groups prefer the use of beta-blockers, given their favourable effect on aortic dilation in Marfan syndrome [11, 27, 155, 169] but it must be noted that a similar effect in TS is not proven. However, they have a positive effect on the sinus tachycardia that is frequently encountered in TS [58, 169]. Beta-blockers can increase the risk for glucose intolerance and are relatively contra-indicated in patients with asthma. As some studies show an increased RAA activation in Turner syndrome, ACE-inhibitors (ACE-I) or angiotensin receptor blockers (ARB) are a reasonable alternative [160]. Losartan was found to have an additive effect to beta-blocker therapy in the treatment of aortic dilation in Marfan syndrome patients [64, 105] but again this is not studied in TS patients. Both ACE-I and ARB have teratogenic properties and cannot be used during pregnancy or the IVF process.

A systematic review on the prevalence and aetiology of arterial hypertension in TS is added as an appendix to this PhD thesis (Chapter VI, pa 135). It describes the relation between blood pressure, aortic dilation and increased cardiovascular risk. Current treatment strategies are reviewed and an integrated practical approach for the diagnosis and treatment of hypertension in Turner syndrome applicable in daily practice is proposed.

I.3.4 ECG abnormalities and sympathic dysregulation

Definition

The ECG of TS girls differs from that of normal controls. They show a higher resting heart rate (91±15 bpm vs. 76±11 bpm), shorter PR (127±16 ms vs. 144±26 ms) and a longer QTc interval (431±22 ms vs. 407±21 ms) [12, 14, 33]. A QTc interval > 440 ms is found in 20 to 36% of TS girls [12, 34]. During exercise test and increasing heart rates, the QTc interval normalises [34].

Adult TS patients have higher resting heart rates [58, 169] with increased norepinephrine levels and similar epinephrine levels compared to normal controls [169]. Although the physical physiological response to exercise is normal in TS patients (normal increase in blood pressure and heart rate), the exercise-induced surge of epinephrine levels is lower in the TS patients [169]. These findings suggest a dysregulation of the sympathic nervous system [58, 169].

Prevalence

Only one study describes the prevalence of ECG abnormalities in young TS patients: a QTc interval that exceeds 440 ms was found in 35% [12].

Clinical significance

Although the QTc interval of TS patients is prolonged compared to normal controls, clinically significant “long QTc syndrome” is rare. There is no apparent relation between the ECG alterations and an increased risk for life threatening rhythm disorders in TS. However, literature describes one case of sudden death in a 17 year old with TS and a mildly prolonged QTc.

In a group of 40 TS women with a QTc interval >432 ms, 8 genetic mutations were found: 6 in KCNH2 and 2 in SCN5A. There is however no genetic explanation for the apparent co-segregation of long QTc mutations with the complete or partial loss of an X chromosome in TS.

Treatment

Clear guidelines are lacking, but it seems wise to avoid all QTc prolonging drugs in TS patients with a prolonged QTc interval on resting ECG. Treatment with antiarrhythmic drugs can be considered in selected cases [33]. Although there are no comparative studies on the use of drugs for long QTc in TS, the use of a betablocker seems appropriate. Data on the usefulness of routine 24 hour holter monitoring in TS patients are lacking.

Comparison with the general population

In the general population, the reported prevalence of long QTc syndrome is 1/2000 [142]. At least 14 mutations are known to cause the syndrome [113]. Apart from avoidance of QTc prolonging medication, treatment consists of anti arrhythmic drugs and in selected cases the implantation on an ICD *(*Implantable Cardioverter Defibrillator).

I.3.5 Cardiovascular disease in Turner, the role of the second heart field and the neural crest

Modern insights into the embryogenesis of the human heart help to explain the particular association of the cardiovascular abnormalities in TS [52]. Heart development is a complex process during which the heart transforms from a single tube towards a fully septated heart with four chambers and a separated outflow tract. Initially, the mesoderm of the first heart field gives rise to the primary heart tube that consists of a small atrial component connected to the sinus venosus, an atrioventricular canal, a ventricular inflow tract and a small outflow tract connecting to the aortic sac. The dorsally positioned mesoderm, the second heart field, reaches to the heart tube at both the arterial and the venous poles. At the venous pole, the posterior second heart field gives rise to the sinus venosus, the dorsal wall of both atria and the primary atrial septum. At the arterial pole, the anterior second heart field supplies the myocardium of the right ventricle and the right side of the septum. The anterior second heart field also contributes to the semilunar valves and the walls of the great arteries. At the distal end of the two main endocardial outflow tract ridges in the still unseptated arterial orifice level, two small intercalated cushions appear. During normal development, separation of the arterial orifice level results in three semilunar valve leaflets in the aortic and pulmonary orifices. Abnormal separation can lead to the occurrence of a bicuspid aortic valve. There are indications that the anterior second heart field is not only involved in the formation of the aortic valve but also of the wall of the ascending aorta.

For subsequent remodelling, septation, valve formation and coronary vascular development two other cell populations, the neural crest cells and the epicardium, are added to the heart. Aortic arch malformations are often linked to a deficient interaction of neural crest cells and the anterior second heart field. Neural crest cells also contribute to the development to the autonomic nervous system and cells that are important for the differentiation of the cardiac conduction system (CCS).

Based on this new insights, it appears that the complex cardiovascular pathology of Turner syndrome is related to an abnormal development of the anterior second heart field (explaining the presence of a bicuspid aortic valves and abnormal stiffness of the ascending aorta), an abnormal development of the neural crest cells (explaining the sympathic dysbalance and the conduction abnormalities) and a deficient interaction between the neural crest cells and the anterior second heart field (explaining the abnormal aortic arch morphology). Until now, it remain unknown which genes are involved in this process.

I.4 Endocrine pathology with an impact on cardiovascular health of Turner patients

I.4.1 Metabolic risk factors for cardiovascular health

TS patients exhibit a variety of endocrine and metabolic disorders that add to their increased cardiovascular burden [100]. Hyperglycemia, obesity and hyperlipidemia are highly prevalent in TS. They are well known risk factors for atherosclerosis, an established precursor of cardiovascular disease. Atherosclerosis presents in TS from a young age on [1, 4, 126] and adds significantly to the morbidity [60] and the mortality [141] of the syndrome.

I.4.1.1 *Impaired glucose metabolism*

Girls and women with TS are at an increased risk for impaired glucose metabolism and type 2 diabetes. Glucose intolerance presents in 15 to 50% [56] and diabetes type 2 in 10% of TS patients [57]. Both are found from childhood on [23, 120, 120]. The risk for glucose intolerance is the highest in patients with monosomy X0 and isochromosome Xq, suggesting haploinsufficiency of genes on Xp as a major cause [23]. The exact pathophysiology of the impaired glucose metabolism remains subject of debate. Both reduced insulin secretion and impaired insulin sensitivity are described [100].

I.4.1.2 *Obesity and unfavourable body composition*

Overweight and obesity form a major health problem in TS [43]. Adult and paediatric patients show an increased weigh and BMI, an increased waist circumference, a higher percentage of whole-body fat mass, a decreased lean body mass and increased visceral fat [59, 120, 128]. This unfavourable body composition contributes to the development of insulin resistance, diabetes type 2 and the metabolic syndrome.

I.4.1.3 *Lipid abnormalities*

Data on the lipid level of TS patients are inconsistent, both in the paediatric and the adult population [1, 43, 86, 139]. Although some describe a less favourable lipid profile with increased total cholesterol, increased triglycerides, increased low density cholesterol and decreased high density cholesterol, this is not confirmed by others.

I.4.1.4 *The metabolic syndrome*

Patients who exhibit at least 5 cardiovascular risk factors have the so called “metabolic syndrome” and carry the highest cardiovascular risk:

- Obesity, defined as a BMI above the 95th percentile for age and gender
- Systolic or diastolic blood pressure above the 95th percentile for age, height and gender
- Triglyceride level above the 95th percentile for age and gender
- High density cholesterol levels below the 5t^{h} percentile for age and gender
- Glucose intolerance or diabetes type 2

As described above, all these risk factors are highly prevalent in TS and present from childhood, making TS patients at an increased risk for the metabolic syndrome.

Because of the increased risk of for the before mentioned endocrine disorders, follow-up of TS patients include regular screening of weight, lipid profile, glucose metabolism and blood pressure. If risk factors are present, they should be rigorously treated. Preventive measures however remain of uppermost importance and include promotion of a healthy life style with sufficient physical activity and a healthy diet.

I.4.2 Premature ovarian failure and estrogen **replacement therapy**

Premature ovarian failure in Turner syndrome

TS is characterised by premature ovarian failure and sex hormone deficiency. The lack of estrogens and androgens highly contributes to the burden of TS as it causes abnormal bone maturation, osteoporosis, absent secondary female sex characteristics and infertility, abnormal cognitive development and cardiovascular disease.

Estrogen replacement therapy in Turner syndrome

Estrogen deficiency is responsible for the absent or arrested puberty in TS. Spontaneous initial pubertal development occurs in only 10 to 30% of girls with TS. The majority requires hormonal induction with increasing doses of estrogens and progesterone [11]. In adult life, sex hormone replacement therapy (HRT) is promoted to improve bone mineral density and overall body composition. Natural 17 β-oestradiol is the preferred type of estrogen. It can be administered both orally or transdermally [153].

The impact of hormone replacement therapy on the cardiovascular status

HRT could theoretically have a lipid lowering and anti-oxidant effect, enhance endothelial function, antagonise the renin-angiotensin-aldosterone system and improve insulin sensitivity. The cardioprotective effect of prophylactic HRT in postmenopausal women is however disappointing as it does not reduce the cardiovascular risk but increases the risk for breast cancer, venous thromboembolism and stroke.

The positive effect of HRT on the cardiovascular risk in TS remains subject of debate [153]. In young women with various causes of premature ovarian failure, physiological doses of HRT resulted in lower systolic and diastolic blood pressure, better renal function and less activation of the renin-angiotensin system [87]. Studies on short term HRT in TS also show a positive effect on 24 hours diastolic blood pressure [63, 108] but long term prospective studies are lacking. Few studies exist on the effects of estrogen replacement on the arterial wall. Mortensen et al. did not find any effect of HRT on arterial stiffness nor on lipid profile in TS [108]. Using increasing doses of 17β-oestradiol, Ostberg et al. found an increased HLDL and decreased LDL cholesterol with a significant reduction in carotid intima-media thickness but without significantly improving blood pressure or arterial stiffness [127].

I.4.3 Small stature and exogenous growth hormone treatment

Small stature in Turner syndrome

Haploinsufficiency of the SHOX genes is the primary cause of the reduced final height and abnormal body proportion of TS patients [84]. Altered IGF-I activity, caused by reduced bioavailability and/or reduced sensitivity, probably contribute to the phenomenon [163].

Growth hormone treatment in Turner syndrome

Treatment with supraphysiologic doses of exogenous recombinant human growth hormone (GH) is widely used to increase adult height of TS patients. If the diagnosis is made early, treatment is usually started at a young age [11].

The impact of growth hormone treatment on the cardiovascular system

Exogenous growth hormone treatment in adults causes sodium and water retention with increased levels of plasma renin activity and aldosterone levels leading to hypertension [7]. These changes are not observed in children [7]. In healthy adult volunteers, administration of supraphysiologic doses of GH, similar to those used in the treatment of TS children, results in a concentric hypertrophy with an increased left ventricular mass index and an increased cardiac output [25].

Prospective longitudinal data on the impact of GH treatment in TS are lacking. Cross-sectional data showed that long-term administration of GH does not induce left ventricular hypertrophy or alter systolic and diastolic function [99, 138, 157]. GH treatment has no effect on abnormal aortic growth as an MRI study showed similar aortic diameters in women with and without GH treatment in childhood [13]. The effect of GH on blood pressure remains less clear. In an Italian cross-sectional study, TS girls treated with high doses of growth hormone (1U/kg/week) had an increased systolic and a decreased diastolic blood pressure compared to age and BSA-matched healthy girls without GH treatment [138]. In a Dutch study comparing different regimens of GH registration, only TS girls receiving high doses (2.7 mg/m² BSA/day) of GH for several years, showed a decreased diastolic blood pressure while systolic blood pressure was not affected [157]. This positive effect of growth hormone on diastolic blood pressure was however not sustained after discontinuation [5]. In TS patients receiving standard doses of GH (1.3 mg/m²BSA/day and 2.0 mg/m² BSA/day) no changes in blood pressure were observed [157].

I.5 Non-invasive techniques for the evaluation of the aorta

Evaluation of the aorta in TS includes evaluation of the aortic valve, the aortic root, the ascending aorta, the transverse arch, the descending and the abdominal aorta (figure 6). The aorta can be evaluated noninvasively by ultrasound, magnetic resonance imaging (MRI) or computertomography (CT) scan.

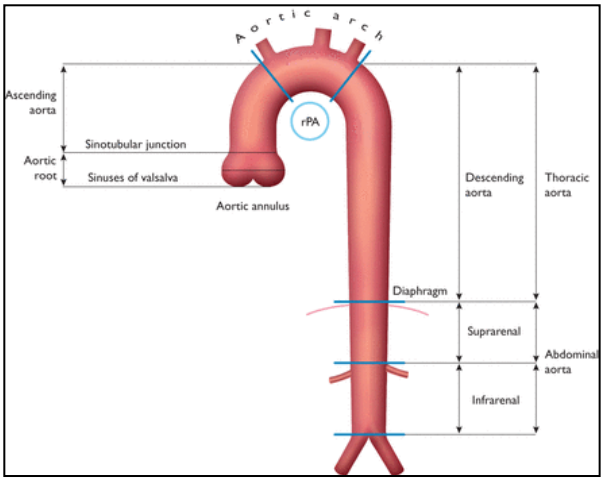

Figure 7: schematic representation of the aorta [44]

Ultrasound is a readily accessible, quick (real time assessment) and cheap technique with no adverse events. It is very well tolerated by patients of any age. The result from the ultrasound exam however depends highly on the experience of the observer and the body composition of the patient. In TS, ultrasound imaging can be hampered by an increased anteroposterior thoracic diameter ("barrel-shaped thorax") [72] and obesity. In a large group of 253 TS patients of variable age, echocardiography failed to visualise the aortic valve morphology in 6%, while visualisation was of moderate quality in another 4% [140]. Visualisation of the distal aorta is difficult and less reliable. Besides the evaluation of the aorta and great vessels, ultrasound allows identification of intracardiac lesions and evaluation of systolic and diastolic ventricular function.

Ultrasound can also be used to measure the elastic properties of the aortic wall. In clinical practice, pulse wave velocity (PWV) is routinely used as it the most important independent predictor of cardiovascular events in adults. The measurement is based on the time it takes for the pulse to travel from one place to another. The distance travelled by the pulse is measured with a measuring tape on the body of the patient. Other parameters of arterial stiffness that can be derived by ultrasound are distensibility and stiffness index of the ascending aorta. Both are based on the difference in arterial diameter during systole and diastole. The ascending aorta is visualized from

the parasternal axis in a horizontally plane and measurement of the aortic diameters is performed at the site of the right pulmonary branche thus enabling standardized imaging. In children, the ascending aorta can be visualized nicely in the majority of cases. Normal values for distensibility and arterial stiffness index, based on a large population of German children, are available [68]. In adults, horizontal visualisation of the aorta is more difficult and in TS images are additionally hampered by obesity and the barrel shape of the thorax. Normal values for echo derived distensibility and stiffness index in adults are lacking .

MRI is superior to ultrasound when it comes to visualisation of the entire aorta, but appreciation of the aortic valve morphology does fail in some cases (1-2%) [140]. MRI allows determining ventricular volume and ventricular mass in a reproducible way. In patients with poor transthoracic visualization, MRI is favourable for the diagnosis of PAPVC, LSVC and coarctation.

MRI is a useful tool for the evaluation of the elastic properties of the aortic wall. The use of MRI for measuring PWV has 2 major advances as compared to ultrasound. The distance travelled by the pulse can be exactly measured inside the vessel, taking into account abnormal aortic arch morphology. Secondly, the PWV can be measured between any 2 locations on the aorta, enabling determination of separate PWV values for different segments of the aorta. Distensibility and stiffness index can also be derived from MRI images. MRI allows good quality imaging in all patients but requires a long scanning time. Normal values are available both for children [161] and adults [80].

In contrast to ultrasound, MRI is an expensive technique and access is limited to specialised centres. In some specific cases, imaging MRI is not possible: in patients with pacemakers or implantable cardioverter defibrillators (ICD's), vascular clips, cochlear implants, neural stimulators, epileptic seizures, large tattoos, significant claustrophobia or morbid obesity. A complete MRI investigation is time consuming and requires a good cooperation of the patient. In young children, the technique is not applicable unless the patient is sedated.

CT imaging allows morphologic evaluation of the entire aorta and the aortic valve. It is also a valuable diagnostic tool for the diagnosis of PAPVC, LVCS and aortic coarctation. Moreover, the technique is superior to MRI when it comes to visualization of the coronary arteries.

In comparison with MRI, the scanning time of the CT is shorter, which makes an imaging study without sedation at an early age easier. However, CT imaging requires radiation and intravenous contrast administration. Furthermore, functional evaluation of left ventricle and aortic wall is not possible without an unacceptably high radiation dose. Although CT is less expensive than MR, the need for radiation is the main reason why CT is less frequently used in research protocols.

As is the case for most CHD, imaging techniques are complementary when it comes to a complete cardiovascular evaluation. The technical details on the specific measurements used in the research protocols are described in chapter II.

CHAPTER II

INITIATION

-

Trial and quest

II.1 Contribution of cardiovascular pathology to the mortality in Turner syndrome

Morbidity and mortality are increased in TS compared to the general population.

A large multicenter study in the UK described the most frequent causes of death in TS [141]. The authors calculated that the mortality in TS is 3-fold higher than in women of the same age category in the general population. Although mortality is raised for almost all major causes of death, cardiovascular pathology plays the most important role. The standardised mortality rates (SMR) for all major cardiovascular disease are listed in table 5.

Table 5: SMR for cardiovascular pathology in Turner syndrome

Pathology	SMR	95% CI
Aortic aneurysm	23.6	13.8 – 37.8
Congenital heart disease	20.7	11.8 – 33.7
Aortic valve disease	17.9	4.9 – 46
Hypertension	6.0	1.2 – 17.5
Cerebrovascular disease	3.9	2.6 – 5.6
Ischemic heart disease	2.8	1.9 – 3.8

SMR: standardised mortality rate (ratio of the number of observed to the number of expected deaths in a similar age group) for the whole TS cohort [141]

The risk for dying from an aortic dissection is 23.6 (CI 13.8-37.8) times higher in TS patients than in the general female population under the age of 85 years [141]. This risk is specifically increased in the younger age groups (278 times higher for the age group 15 to 44 years *vs.* 7.4 times higher for the age groups 45 to 84 years) [141].

Current guidelines on follow-up and treatment of TS therefore include regular cardiovascular examination and rigorous treatment of risk factors for aortic dissection [3, 11, 133]. Risk stratification for aortic dissection includes assessment of aortic valve morphology, coarctation, aortic diameters and blood pressure. However, retrospective analysis showed that 5 to 11 % of TS with dissection had neither a CHD nor hypertension [19, 20, 61].

II.2 Knowledge gaps in the pathophysiology of aortic dilation in Turner syndrome

Data on the histological structure of the aortic wall of TS patients, either with or without dilation, are scarce. Gravholt et al. reported data on the biopsy of 6 TS patients with aortic dissection that showed cystic media necrosis in 3, fibrous degeneration in 2 and increased amounts of acid mucopolysaccharides in 1 [61]. They also described an altered ratio between type I and type III collagen [61]. In the tissue of ascending aortic aneurysms associated with other pathologies like Marfan syndrome or in association with bicuspid aortic valves, elastic fibre destruction, mucoid accumulation, and smooth muscle cell apoptosis have been found. In the specimens of these patients dysregulation of TGF-ß retention and Smad2 signalling play a major role [54].

TGF-ß seems also involved in TS. In TS patients, serum TGF-ß1 levels are about 3-fold higher than in controls while TGF-ß2 levels are about 3.5-fold lower. There is no difference in TGF-ß levels between patients with or without aortic pathology, suggesting that all TS patients might have abnormal aortic wall properties [168].

Recently, research in TS patients suggested that aortic dilation is associated with an intrinsic abnormal elasticity of the aortic wall. In one publication, aortic dilation was inversely correlated with aortic distensibility in a group of TS of varying age but an abnormal distensibility was also found in 2 TS girls with normal aortic dimensions [143]. In another group of young TS patients, PWV and augmentation index were impaired compared to lean and obese non-TS patients. The authors did not find any correlation between the presence of vasculopathy and CHD, growth hormone treatment or the administration of estrogen [90]. In a group of young TS adults previously treated with growth hormone, aortic distensibility was decreased [158]. In contrast to the previous reports, a German study found similar aortic compliance in young TS patients compared to normal male and female controls [82].

Longitudinal data on the vascular wall changes as well as histological studies of the aortic wall in TS could help to understand the aetiology of aortic dilation. However, research in this field is hampered by the lack of animal TS models making clinical research of most importance.

II.3 Quest for the missing link: research questions for the PhD research

Aortic dilation is the most important risk factor for aortic dissection in TS but the exact aetiology of this process is not completely understood. In this thesis, different factors that could contribute to aortic dilation in TS were explored in a clinical research setting.

The following research questions were formulated:

1. **Is there a relation between aortic dilation and CHD and X chromosome haploinsufficiency?**
   Cardiovascular pathology in males and females with 45,X/46,XY mosaicism.
   Katya De Groote, Martine Cools, Jean De Schepper, Margarita Craen, Inge François, Daniel Devos, Karlien Carbonez, Benedicte Eyskens, Daniel De Wolf.
   PlosOne 2013; 8(2): e54977.

2. **Is there a relation between aortic dilation, aortic arch morphology and hypertension in adult TS patients?**
   Abnormal aortic arch morphology in Turner syndrome patients is a risk factor for hypertension.
   Katya De Groote, Daniel Devos, Koen Van Herck, Laurent Demulier, Wesley Buysse, Jean De Schepper, Daniel De Wolf.
   Heart and Vessels 2015; 30: 618-625.

3. **Is there a relation between aortic dimensions, aortic valve morphology and aortic wall stiffness in TS children and adults?**

   3.1. In prepubertal TS girls

   Increased aortic stiffness in prepubertal girls with Turner syndrome.
   Katya De Groote, Daniel Devos, Daniel De Wolf, Koen Van Herck, Saskia Van der Straaten, Ernst Rietzschel, Ann Raes, Kristof Vandekerckhove, Joseph Panzer, Hans De Wilde, Jean De Schepper.
   Journal of Cardiology, in press.

   3.2. In TS children and adults

   Proximal aortic stiffening in Turner patients is more pronounced in the presence of a bicuspid valve. A segmental functional MRI study.
   Daniel Devos, Katya De Groote, Danilo Babin, Laurent Demulier, Yves Taeymans, Jos Westenberg, Luc Van Bortel, Patrick Segers, Eric Achten, Jean De Schepper, Ernst Rietzschel.
   Currently unpublished, preliminary results.

II.4 Description of the populations studied

The table below describes for each study the TS population studied, the control group if included and the evaluation technique that was used.

Table 6: Description of the populations studied in the PhD thesis

Study	Recruitment	Study population	N	Age (y)	Controls (n)	Technique
1	Multicenter Belgium	Females 45,X/46,XY Males 45,X/46,XY	8 10	0 - 38	None	Echo and MRI
2	Ghent University Hospital	Adult TS with at least 1 routine MRI	74	18 -59	None	MRI
3	Ghent University Hospital	Prepubertal TS	15	6 - 13	Age matched healthy girls, stature <p25 (n=31)	Echo
4	Ghent University Hospital	TS with at least 1 MRI	55	13 - 59	Healthy women (n=31)	MRI

II.5 Description of the cardiovascular evaluation techniques used

The non-invasive ultrasound and MRI techniques used for the evaluation of the aorta in the clinical research protocols are described.

II.5.1 Evaluation of aortic morphology and aortic diameters

II.5.1.1 *Evaluation by ultrasound*

Echocardiography was performed with the use of a VIVID 7 ultrasound (GE Vingmed Ultrasound, Horten, Norway) equipped with a 3,5 MHz probe. Data were stored and used for off line analysis using EchoPAC version 110.1.0 software (GE Vingmed Ultrasound).

Aortic valve morphology was studied by 2D echo on the parasternal short axis and included evaluation of the number of cusps, the presence of a raphe and the functional opening. Aortic valve insufficiency and stenosis were quantified on the parasternal short and long axis using colour and CW Doppler.

Diameters of the aorta were measured from the parasternal long axis at the level of the aortic valve annulus, the aortic root and the ascending aorta (figure 8). Measurement of the transverse arch was obtained from the suprasternal notch. Normality of the data was determined by using the z-scores, as described earlier in the first chapter.

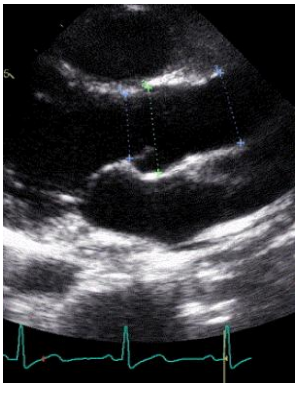

Figure 8: measurements of the aortic dimensions at the site of the aortic annulus (left) aortic root (middle) and ascending aorta (right)

II.5.1.2 *Evaluation by MRI*

Cardiac MRI was performed by an experienced radiologist on a 1.5T magnet (Siemens Avanto, Erlangen) and included the use of intravenous contrast medium (Gadobutrol at 1ml/10kg bodyweight).

Morphologic and functional evaluation of the aortic valve was done on cine images of the left ventricular outflow and aortic valve plane; quantification of stenosis or regurgitation was performed by means of phase contrast images.

Diameters of the aorta were measured on multiplanar reconstructed images perpendicular to the aortic lumen at the site of the aortic valve annulus, the aortic sinus, the sinotubular junction and the ascending aorta at the site of the right pulmonary artery, as well as at mid-arch between left carotid and subclavian ostium, and at proximal (at the level of the pulmonary artery bifurcation) and distal descending aorta (at the level of the diaphragm).

II.5.2 Evaluation of the elastic properties of the aorta

The elastic properties of the aorta are classically described with the terms elasticity and stiffness.

Distensibility or elasticity refers to the ability of a vessel to increase in volume without a significant increase in pressure. **Stiffness** or elastic resistance is the reciprocal and refers to the inability of a vessel to increase in volume.

II.5.2.1 *Evaluation by ultrasound*

Ultrasound evaluation of the vascular function was performed after minimal 20 minutes of rest. The data were acquired using a VIVID 7 ultrasound (GE Vingmed Ultrasound, Horten, Norway) equipped with a vascular transducer 12L set at 10 MHz . Data were stored and used for off line analysis using EchoPAC version 110.1.0 software (GE Vingmed Ultrasound).

Pulse wave velocity of the aorta

Pulsed Doppler signals were obtained consecutively at the right common carotid artery and the right femoral artery. The time interval (Δtime) between the arrival of the pulse at the carotid and femoral artery was calculated using the foot-to-foot velocity method [89] (figure 9). The distance between the carotid and the femoral artery ($D_{\text{carotid artery-femoral}}$) and between the carotid artery and the upper border of the sternum ($D_{\text{carotid artery-suprasternal notch}}$) was measured with a measuring tape. The distance (D) was calculated as the $D_{\text{carotid artery-femoral}} - D_{\text{carotid artery-suprasternal notch}}$. PWV was then defined as D/Δtime expressed in m/s.

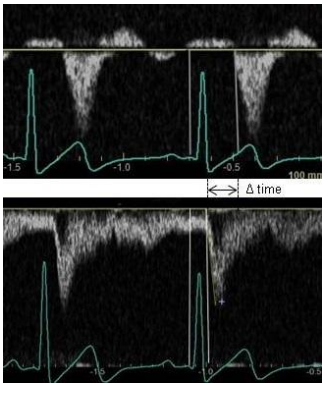

Figure 9: PW Doppler signal at the femoral artery (upper part) and the common carotid artery (lower part). At both signals, the time interval is measured between the onset of the QRS complex and the onset of the systolic PW Doppler signal. Δtime is calculated as the difference between both time intervals.

Distensibility of the ascending aorta

On the parasternal long axis, an M-mode image of the ascending aorta was recorded. Care was taken to place the aorta horizontally on the screen with the cursor perpendicular to the arterial wall at the level of the right pulmonary artery. On the M-mode recording, the diameter of the ascending aorta was measured from inner to inner surface during diastole and systole (figure 10).

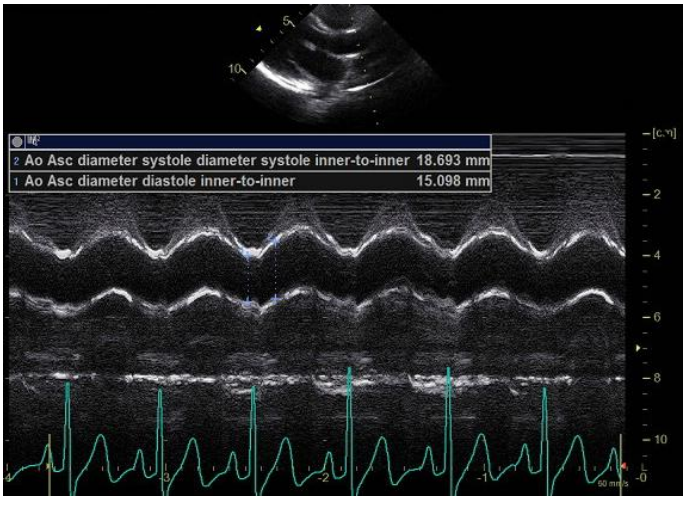

Figure 10: M-mode recording of the ascending aorta on the parasternal long axis. The diameter of the ascending aorta is measured from inner to inner surface in diastole and systole.

During the ultrasound exam, the blood pressure was taken 3 times and averaged for calculations. The arterial stiffness index (dimensionless) and the distensibility (in 10^{-3}kPa^{-1}) were calculated based on the ascending aortic diameter in systole and diastole and on the pulse pressure.

aortic stiffness index= $\frac{\left[\ln\left(\frac{blood\ pressure_{syst}}{blood\ pressure_{diast}}\right)\right]}{\left[\frac{diameter_{syst}-diameter_{diast}}{diameter_{diast}}\right]}$ (dimensionless) and

distensibility = $\left[\frac{\left[\left(\left(\frac{diameter_{syst}}{2}\right)^2*\pi\right)-\left(\left(\frac{diameter_{diast}}{2}\right)^2*\pi\right)\right]}{\left(\left(\frac{diameter_{diast}}{2}\right)^2*\pi\right)*\left(blood\ pressure_{syst}-blood\ pressure_{diast}\right)*1333}\right]*10^7$ (in 10^{-3}kPa^{-1})] [68].

II.5.2.2 *Evaluation by MRI*

Pulse wave velocity of the aorta

MRI derived PWV was measured for the thoracic aorta, the abdominal aorta and the entire aorta (figure 10). PWV was calculated as the ratio of distance Δx per time Δt, where Δx is the length of an aortic segment (thoracic, abdominal or entire aorta) measured on the MR image data along the centreline, and Δt is the time duration needed for the pulse to travel that length through the aorta [65].

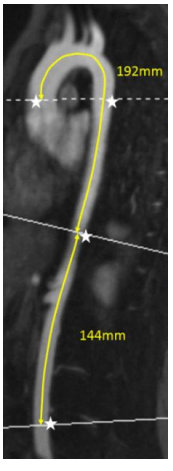

Figure 11: distance measurement along the aortic path (lines) and location of the aortic through-plane flow velocity measurements (white stars).

Distensibility of the ascending aorta

On the MRI images, maximum and minimum transverse aortic luminal areas were manually located and drawn on the cine series. Distensibility was calculated as:

$$Distensibility = \frac{area_{max} - area_{min}}{area_{min} * (blood\ pressure_{systole} - blood\ pressure_{diastole})}\ (1/1000\ mmHG)\ [122]$$

CHAPTER III

ORDEAL

-

Research papers

III.1 The relation between X-chromosome haploinsufficiency, aortic dilation and other cardiovascular pathology

Cardiovascular pathology in males and females with 45,X/46,XY mosaicism.

Katya De Groote, Martine Cools, Jean De Schepper, Margarita Craen, Inge François, Daniel Devos, Karlien Carbonez, Benedicte Eyskens, Daniel De Wolf.

PlosOne 2013; 8(2): e54977.

In this study, the relation between X haploinsufficiency, aortic dilation and CHD was explored. The paper describes a Belgian multicentre study that investigated cardiovascular abnormalities in 45,X/46,XY males compared to 45,X/46,XY females.

Patients with 45,X/46,XY mosaicism were selected from the Belgian Registry for Growth and Puberty problems and via the multidisciplinary clinic for disorders of sexual development. Eighteen patients were included: 8 raised as females (F) and 10 as males (M). All 18 patients underwent a complete cardiac examination with blood pressure measurement, ECG and echocardiography by a single observer. An additional MRI was performed in 12. In a second phase, clinical features and external masculinisation score (EMS) were retrospectively collected from the medical files.

Two patients had a history of a structural heart defect before inclusion: 1 F with coarctation and 1 M with a spontaneously closed VSD. During the study examinations, a bicuspid aortic valve was found in 8 (3F, 5M). Dilation of the ascending aorta was present in 4 M and was severe in 2 young boys (z-scores 4.48 and 6.19). QTc was prolonged in 3 F and 2 M. In male patients, cardiac pathology was more frequent in those who also had other TS related abnormalities.

We concluded that males with 45,X/46,XY mosaicism have similar cardiovascular pathology as 45,X/46,XY females. Dilation of the ascending aorta can be important, also in males. We advise cardiac screening and lifelong monitoring in all males with 45,X/46,XY mosaicism according to the existing guidelines for Turner syndrome.

III.2 The relation between abnormal aortic arch morphology, hypertension and aortic dilation

Abnormal aortic arch morphology in Turner syndrome patients is a risk factor for hypertension.

Katya De Groote, Daniel Devos, Koen Van Herck, Wesley Buysse, Jean De Schepper, Daniel De Wolf

Heart and Vessels 2015; 30: 618-625.

In this study the association between abnormal aortic arch morphology and hypertension in adult TS patients was explored. We retrospectively revaluated the MRI images of 74 adult TS patients who underwent a cardiac MRI for routine follow-up. Aortic arch morphology was initially appreciated for normality based on the curve of the transverse arch and the distance the left common carotid - left subclavian artery. We additionally used a new more objective method to describe aortic arch abnormality in TS by determination of the relative position of the highest point of the transverse arch (AoHP). Based on the blood pressure in the medical files, TS patients were assigned to the hypertensive group (in office blood pressure ≥ 140/ 90 mmHg, 24h ambulatory blood pressure ≥ 130/ 80 mmHg or use of antihypertensive treatment) or to the normotensive group.

Thirty-one (42%) TS patients were hypertensive. When we evaluated the aortic arch morphology by the curve of the transverse arch, 20 TS patients had an abnormal aortic arch. When applying the quantitative measurement of the relative position of the highest point of the aorta, 19 arches were abnormal. Based on logistic regression analysis we showed that hypertension is significantly and independently associated with age, BMI and abnormal arch morphology, with a larger effect size for the new AoHP method than for the classical method. TS patients with hypertension and abnormal arch morphology more often had dilation of the ascending aorta.

We concluded that there is a significant association between abnormal arch morphology and hypertension in TS patients, independent of age and BMI, and not related to other structural heart disease. We suggest that aortic arch morphology should be included in the risk stratification for hypertension in TS and propose a new quantitative method to express aortic arch morphology.

III.3 The relation between aortic dilation and abnormal aortic stiffness in paediatric and adult patients with Turner syndrome

In the 2 papers presented in this section, the relation between aortic dilation and abnormal aortic stiffness was explored in prepubertal TS girls and older TS patients.

The first paper reports a study on aortic diameters, distensibility and stiffness index of the ascending aorta and pulse wave velocity (PWV) of the aortic arch, measured by **ultrasound** in 15 **prepubertal** TS girls and 31 age and height matched controls.

The second part of this section reports **preliminary** results of a parallel study, in which **MRI** was used to measure pulse wave velocity (PWV) and distensibility of the thoracic and abdominal aorta in 55 **older** TS patients (13-59 years) compared to 31 healthy controls. This study is currently unpublished, and enrolment is ongoing.

III.3.1 The relation between aortic dilation and abnormal aortic stiffness, measured by ultrasound, in prepubertal patients with Turner syndrome

This paper reports a study on aortic diameters, distensibility and stiffness index of the ascending aorta and pulse wave velocity (PWV) of the aortic arch, measured by ultrasound in 15 prepubertal TS girls and 31 age and height matched controls.

The 6 TS girls with a bicuspid aortic had significantly larger ascending aortic diameters than the controls. Distensibility of the ascending aorta was lower and stiffness index was higher in the TS than in control group both for TS patients with a tricuspid aortic valve and a bicuspid aortic valve. The abnormality was however more pronounced for TS patients with a bicuspid aortic valve. PWV along the whole aortic arch was not different between groups. There was no correlation between the stiffness index and the z-scores of the ascending aorta.

We concluded that the stiffness of the ascending aorta is increased in prepubertal TS girls in patients with a bicuspid and a tricuspid aortic valve while dilation of the ascending aorta is more severe in patients with a bicuspid aortic valve. This suggests that the aortic wall abnormality in TS is inherent to the chromosomal disorder, that probably all TS patients are at an increased risk for severe aortic complications but that the risk is the highest for those with a bicuspid aortic valve.

III.3.2 The relation between aortic dilation and abnormal aortic stiffness, measured by MRI, in paediatric and adult patients with Turner syndrome

The second part of this section reports preliminary results of a parallel study, in which MRI was used to measure pulse wave velocity (PWV) and distensibility of the thoracic and abdominal aorta in 55 older TS patients (13-59 years) compared to 31 healthy controls. This study is currently unpublished, and enrolment in ongoing.

TS patients had significantly higher PWV in the thoracic but not in the abdominal aorta. The diameter of the ascending aorta was significantly larger in TS compared to the controls. The increase in PWV and ascending aorta diameters were more pronounced in TS patients with bicuspid aortic valves compared to those with tricuspid valves.

We concluded that adult Turner patients exhibit a predominant stiffening and dilation of the proximal aorta, especially if their aortic valve is bicuspid. These abnormalities are present from an early age, suggesting a limited effect of accelerated ageing.

CHAPTER IV

RETURN AND APOTHEOSIS

-

General discussion and future research

IV.1 Aims and conclusions of the clinical studies

Aortic dissection is a major problem in Turner syndrome and an important cause of early death [20, 61, 141]. Since the first case reports on aortic dissection in TS in 1986 [92], several researchers focused on aortic pathology in this patient group. Although the knowledge increased impressively, not all risk factors are clearly defined and there still remain gaps in the risk stratification of aortic dissection in TS.

This thesis aimed to add additional information on the aetiology of aortic dilation in TS. The following research questions were answered:

1) Is there a relation between aortic dilation and CHD and X chromosome haploinsufficiency?

In the first paper (III.1), a Belgian multicenter study compared cardiovascular abnormalities in 45,X/46,XY female Turner patients with those in 45,X/46,XY males. Cardiovascular pathology, including BAV and dilation of the ascending aorta, was found in both groups, suggesting that aortic dilation and the other cardiovascular pathologies in TS are probably related to the X chromosome haploinsufficiency.

2) Is there a relation between aortic dilation, aortic arch morphology and hypertension in adult TS patients?

In the second paper (III.2), a retrospective MRI study explored the relation between abnormal aortic arch morphology and hypertension. There was a significant association in adult TS patients between abnormal arch morphology on MRI and hypertension that was not related to structural heart disease. TS patients with hypertension and abnormal arch morphology more often had dilation of the ascending aorta. This association between abnormal arch morphology, hypertension and aortic dilation suggests the presence of a common aetiology. Evaluation of the aortic arch morphology should be included in the follow-up of TS patients and could help improving the risk stratification for aortic dissection.

3) Is there a relation between aortic dimensions, aortic valve morphology and aortic wall stiffness in TS children and adults?

The third and fourth papers studied the relation between aortic valve morphology, aortic dilation and abnormal aortic stiffness.

A prospective ultrasound study (III.3.1) compared aortic valve morphology, aortic dimensions and ascending aortic stiffness (stiffness index, distensibility and PWV) between prepubertal TS girls and healthy controls. The stiffness of the ascending aorta was increased in prepubertal TS

girls with a BAV and a TAV although the abnormality was more pronounced in those with a BAV. Severe dilation of the ascending aorta was only seen in TS girls with a BAV. There was no correlation between ascending aortic z-scores and stiffness index.

A parallel MRI study (III.3.2) measured aortic dimensions, PWV and distensibility of the thoracic and abdominal aorta in older TS patients compared to healthy controls. TS patients had significantly higher PWV in the thoracic but not in the abdominal aorta. The diameter of the ascending aorta was significantly larger in TS patients. The increase in PWV and ascending aorta diameters were more pronounced in TS patients with BAV compared to those with TAV.

Both studies show that TS patients have an abnormally stiff ascending aorta from a young age. This increased stiffness is also found in TS patients with a TAV and normal aortic dimensions.

IV.2 Research findings in relation to the current knowledge on risk factors for aortic dilation

Dilation of the aorta is a major risk factor for aortic dissection. Several pathological processes have been put forward as possible causal or aggravating factors for progressive aortic dilation. In the following section, current knowledge on these factors will be discussed and supplemented with the results of the PhD research papers.

Aging

TS patients suffer from typical ageing diseases like type 2 diabetes, dyslipidemia, decreased bone density and hypertension. The increased growth velocity of the proximal aorta described in TS patients [110] has also been assigned to an accelerated ageing process. Indeed, the association of enlarged aortic diameters and age have been confirmed in several publications [42, 81, 110, 124, 125] and dissection of the aorta occurs in TS at a much younger age than in the general population [20, 61].

Our study in young prepubertal girls confirmed the finding of others [21, 93] that large aortic diameters and abnormal aortic stiffness are already present in young children with TS, suggesting an intrinsic genetic pathology rather than a pure effect of accelerated ageing.

Presence of a bicuspid aortic valve

In the general population, BAV is associated with aortic dilation and an increased risk for aortic dissection [103, 166]. In BAV patients, the distensibility of the ascending aorta is impaired, both in adults [104] and in children [132]. This aortic wall abnormality is not only observed in BAV patients with aortic dilation but also in those with normal aortic dimensions [118, 119]. Histological studies in specimens of the human ascending aortic wall showed a dysregulation of TGF-ß retention and Smad2 signalling in syndromic and non-syndromic aneurysms [54]. Unfortunately, histological data in TS are very scarce.

BAV are present in 14 to 40% of TS patients [39, 48, 55, 81, 94, 109, 136] and are associated with enlarged aortic dimensions [39, 93, 98, 109, 110, 124, 125, 137, 140]. However, larger aortic diameters, abnormally increased aortic growth [93, 107, 135] and aortic dissections [20] are also found in TS patients with normal tricuspid aortic valves.

In our studies, aortic diameters of the TS patients were larger than those of controls while aortic dilation was more severe in TS patients with a BAV. Abnormal aortic wall stiffness on the other hand was present in TS patients with a BAV and a TAV, although it seemed more pronounced in the BAV group. This abnormal aortic wall property was present from early childhood on. This finding suggests that the abnormal stiffness of the ascending aorta in TS is not just secondary to the presence of a BAV but is inherent to the TS syndrome.

Future research comparing aortic dimension and aortic wall distensibility in non-syndromic patients with BAV and Turner patients could help to clarify the relative contribution of the intrinsic aortic wall property and the effect of BAV in TS.

Presence of an aortic coarctation

Coarctation of the aorta is found in 4 to 15% of TS patients [39, 48, 55, 72, 81, 94, 136]. It is associated with aortic dilation [110] and an increased risk for aortic dissection [19, 20, 61]. In the International Turner Syndrome Aortic Dissection Registry, 4 of the 20 patients with dissection had a history of aortic coarctation. Two of these patients had an associated aortic aneurysm but data on the aortic dimensions of the other 2 are missing. Until now it remains unclear whether an aortic coarctation can cause dissection if it is not associated with aortic dilation.

In the general population, aortic coarctation is associated with an increased stiffness of the proximal aorta even after a successful surgical repair [85, 130, 162]. In our prospective study in prepubertal girls, the number of TS patients with coarctation (n=2) was too low to investigate a possible additive effect of coarctation on aortic stiffness.

Abnormal aortic arch morphology

Surgical repair of an aortic coarctation often results in an abnormal arch geometry that is described as “gothic” or “crenel. This gothic shaped aortic arch morphology is associated with hypertension and an increased stiffness of the proximal aorta [129-131]. A similar abnormal arch morphology with a late takeoff of the subclavian artery and a box shaped appearance of the arch (ETA) is frequently found in TS patients [72, 81, 109] with and without coarctation. A borderline significant association between ETA and aortic dilation is reported in literature [109].

In our retrospective MRI study we found an abnormal arch morphology in nearly one third of the TS patients. In line with previous data [72], there was a relation between abnormal aortic arch morphology and hypertension, irrespective of coarctation or other concomitant CHD. Moreover, we found that TS patients with an abnormal arch morphology more often had dilation of the ascending aorta.

There are no data on the aortic arch morphology in TS children and it currently remains unclear whether ETA presents in childhood or whether it develops later in life. One could speculate that the aortic morphology is normal at birth and becomes more abnormal with age due to the increased stiffness of the ascending aorta that generates abnormal flow patterns in the aortic arch and creates aortic arch deformation. This phenomenon of abnormal flow could also explain the dilated head vessels that are frequently found in adult TS patients and that are associated with the presence of ETA [109].

Unfortunately, we did not measure aortic distensibility in the retrospective MRI study. We are therefore unable to determine the relation between increased aortic stiffness and aortic arch morphology. Future research should focus on the longitudinal follow-up of aortic arch morphology using the quantitative measuring technique we described. The additional repetitive measurement of aortic distensibility could help to understand the role of aortic stiffness in the development aortic arch deformation. Computational flow models would be helpful to study flow alterations in stiff aortic arches and their possible association with arch deformation.

Abnormal aortic stiffness

Increased stiffness of the ascending aorta was previously described in TS patients of varying age [90, 143, 158]. It has been suggested a predictive risk factor for aortic dilation in TS since aortic dilation was associated with a decreased distensibility in one study [143]. However, in that same study, abnormal distensibility was also found in 2 TS children with normal aortic dimensions [143]. In another group of young TS patients aged 9 to 20 years, abnormal arterial stiffness of the aorta and the carotid artery was found irrespective of associated CHD [90].

In our prospective study, we found an abnormally decreased distensibility and an abnormally increased stiffness index in the ascending aorta of young prepubertal TS girls with a BAV and a TAV. We could not find an association between abnormal wall properties and aortic dilation in this young group. Severe ascending aortic dilation was only present in young TS patients with a BAV.

Similar results for the elastic property were found in our prospective MRI study in adult TS patients. In these older patients aortic dilation was present in TS patients with a BAV and a TAV, although dilation was more pronounced in TS BAV patients.

These data suggest that abnormal wall properties of the ascending aorta are inherent to the genetic problem and present from a young age on. This abnormal aortic stiffness probably plays a key role in the risk for aortic dissection but not necessarily through the process of severe aortic dilation.

Large long term follow-up studies with focus on the evolution of aortic wall properties and the development of aortic dilation could help to understand the importance of abnormal stiffness in the evolution towards dilation and possibly dissection.

Hypertension

Abnormal arterial stiffness is the principal cause of increasing systolic pressure with advancing age and is associated with progressive arterial dilation in the non-TS population [29, 121]. In TS, hypertension is considered an important additional risk factor for aortic dilation and dissection. Several studies in TS patients confirmed a positive relation between arterial blood pressure and aortic dimensions [42, 71, 110] but data on arterial stiffness are lacking in these studies. It still remains to be clarified how abnormal arterial stiffness, hypertension and aortic dilation are

interrelated in TS. Theoretically, an intrinsic aortic wall abnormality could be the primary cause of both hypertension and dilation.

Hypertension is reported in half of the adult and in one fourth of the paediatric TS patients [37]. In our study population, hypertension was present in 42% of the adults TS patients. The young prepubertal TS patients were all normotensive in office, but 24 hour ambulatory blood pressure measurement showed mild hypertension in 33% and abnormal nocturnal dipping in 41%. This discrepancy between in office blood pressure measurements and 24 hours blood pressure recordings has been reported previously [1].

In our prepubertal TS girls, we could not find any difference in blood pressure measured in office compared to controls, although TS girls showed marked aortic wall abnormalities. Unfortunately, the limited number of patients did not allow studying the relation between an abnormal 24 hour ambulatory blood pressure recording and abnormal aortic wall properties. Longitudinal follow-up of blood pressure and aortic stiffness is needed to see whether increased aortic stiffness leads to more pronounced hypertension in later life.

In our retrospective MRI study we found that the combination of hypertension and abnormal arch morphology in TS patients is associated with dilation of the ascending aorta. Abnormal aortic stiffness -a theoretical common cause of abnormal arch morphology, hypertension and aortic dilation - could be the explanatory factor for this association. Unfortunately, we did not measure aortic distensibility in this retrospective study. Further analysis of the relation between aortic stiffness and aortic arch morphology, dilation and blood pressure could help to confirm or reject the hypothesis that an intrinsic aortic wall abnormality is the common cause of aortic arch deformation, hypertension and aortic dilation.

IV.3 Integrating the classical risk factors for aortic dilation in a general model

As presented in the previous section, the aetiology of aortic dilation and dissection in TS is presumably multifactorial. In this paragraph a hypothetic model for the development of aortic dilation in TS is presented including all classical risk factors and their interactions (figure 12).

The genetic defect of TS causes 4 cardiovascular problems that are related to aortic dilation and dissection (presented in a double line box): intrinsic vascular wall abnormality, hypertension, bicuspid aortic valve and coarctation. Each of these defects can contribute to the dissection process, either directly or via promotion of aortic dilation. In the figure, well accepted relations are presented in full lines, less established ones in dashed lines.

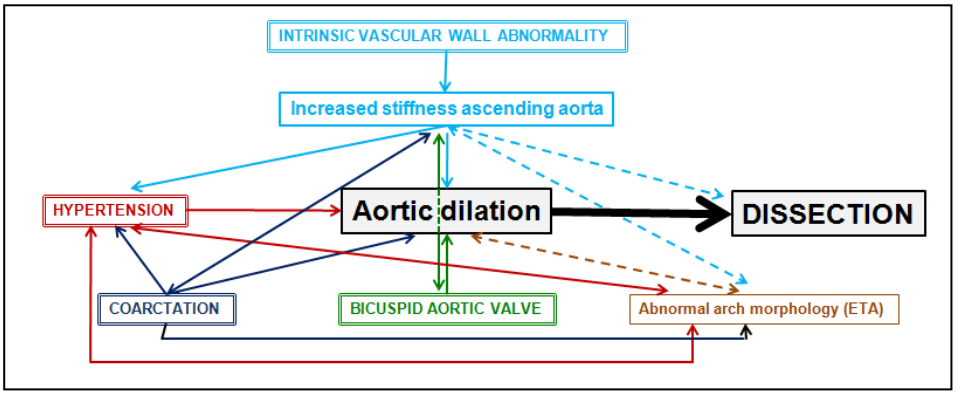

Figure 12: hypothetic model combining all different risk factors for aortic dissection. Well accepted relations are presented in full lines, less established ones in dashed lines.

The **increased stiffness** of the ascending aorta in TS adds to the risk of aortic dissection by inducing arterial hypertension and aortic dilation but it probably also has a directly aggravating effect on the risk for dissection. In a normal situation with normal elastic aortic wall properties, the wave generated by the cardiac cycle travels along the aorta towards the periphery and is reflected back from that periphery. The pressure wave at any point along the aorta, is the resultant of the antegrade and reflected wave. In case of an increased aortic stiffness, the waves travel fast and the reflected wave merges with the systolic part of the incident wave causing a high pressure in systole [122]. Hypertension elevates the aortic wall stress thus promoting aortic dilation and increasing the risk for aortic dissection [30].

Increased stiffness of the ascending aorta is theoretically also one of the causes of the abnormal arch morphology that is frequently seen in TS and that is related to aortic dilation. Increased aortic

stiffness is more pronounced in TS with a bicuspid aortic valve and is seen patients with an aortic coarctation.

Hypertension is associated with aortic dilation and is accepted as a major risk factor for aortic dissection both in the general and the TS population. Endocrine disorders, unfavourable body composition and sympathic over-activation increase the risk for developing high blood pressure and probably also add to the risk for aortic dissection. Hypertension is often seen in TS patients with coarctation or ETA.

Aortic coarctation is associated with abnormal arch morphology, with dilation of the aorta, with increased stiffness of the ascending aorta and with arterial hypertension. It still remains unclear whether aortic coarctation is a risk factor for aortic dissection if it is not associated with aortic dilation.

Bicuspid aortic valves are associated with dilation of the ascending aorta and progression towards aortic dissection. TS patients with a BAV have a more severe stiffening of the ascending aorta than those with a TAV.

It is very likely that the individual risk of each TS patient depends on the presence and the severity off all factors described in the model. It remains unclear what the relative importance is of each individual abnormality but the presence of a bicuspid aortic valve and/or aortic dilation seems to be associated with the highest risk [19, 61]. It is likely that patients who combine several risk factors are more prone to progress towards dissection. Future research should focus on risk stratification including all dissection promoting pathologies, trying to classify patients into those with low, moderate and high risk on more than aortic diameters alone. Hypothetically, patients with an ASI <25 mm/m² might benefit for preventive surgery if they combine multiple risk factors like increased aortic stiffness, abnormal arch morphology, BAV and hypertension. On the contrary, it appears that TS patients with a TAV, normal aortic arch morphology, normal aortic elasticity, normal aortic diameters and normal arterial blood pressures have a very low risk for aortic dissection . Cardiovascular follow-up in these patients could probably be limited but until now prospective data are insufficient to reduce the number of visits to the cardiologist in clinical practice.

IV.4 New insights for the cardiovascular follow-up of TS patients

Current guidelines on cardiovascular follow-up of TS patients focus on the diagnosis of the classical risk factors for aortic dissection: congenital heart defects, quantification of aortic dilation and hypertension. Repeated echocardiographic evaluation, at least every 3 to 10 year, in combination with regular cardiac MRI for an optimal visualisation of the aorta is advocated, also in patients without apparent cardiac problem [11, 27, 35, 41, 133]. Based on the data presented in this thesis, we concluded that abnormal aortic stiffness plays an additional role in the pathophysiology of aortic dilation and probably also in the progression towards dissection. It is therefore proposed to add measurements of aortic wall properties to the cardiovascular evaluation and follow-up of TS patients.

Follow-up of the ***ultrasound derived PWV*** between the carotid and the femoral artery is of limited use in TS patients. In TS, abnormal aortic stiffness is limited to the proximal aorta and the measurement of the carotid-femoral PWV fails to detect this abnormality as is shown in papers III.3.1 and III.3.2. Moreover, the distance travelled by the pulse is measured with a tape measure from the carotid to the femoral measuring site but this presumes a normal aortic arch morphology without elongation or abnormal kinking. As TS patients often have an abnormal arch morphology, tape measure is not representative for the real aortic length.

Follow-up of ***MRI derived PWV*** is possible in TS patients because the distance travelled by the pulse is measured exactly inside the vessel, taking into account abnormal elongation and kinking. Moreover, in contrast to ultrasound, MRI enables the selective measurement of the PWV of the proximal aorta. In adult TS patients, regular MRI is promoted to screen for progressive aortic dilation. Measurement of PWV could be included in the same exam. In paediatric TS patients, the use of MRI is complicated by the long duration of the image acquisition and the need for deep sedation in young children. It is therefore not the technique of first choice in this age group.

The elastic property of the ascending aorta can be easily appreciated in TS children by the use of ***ultrasound derived*** **distensibility and stiffness index**. In adults, images are often hampered by obesity and the barrel shape of the thorax making this technique of limited use in this adult patient group.

MRI derived **distensibility and stiffness index** allows good quality imaging in all patients but requires a long scanning time and sedation in young children.

Based on the previous discussion, we propose to determine aortic stiffness repetitively in TS patients.

In childhood ultrasound derived distensibility and stiffness index can be used as it easy to perform, cheap and well tolerated. In adults, it is more appropriate to use MRI derived PWV. Future longitudinal research however is necessary to compare the prognostic value of PWV and distensibility/stiffness index in the risk stratification for aortic dilation and dissection.

IV.5 Current protocol for the cardiovascular follow-up of Turner patients in Ghent University Hospital

Benefits of cardiovascular follow-up

Cost-benefit studies on the use of standardised follow-up of TS patients are lacking. Nevertheless, regular checkup including an elaborated cardiovascular examination is promoted in all international guidelines as there is no doubt about the increased cardiovascular risk in TS patients. Moreover, screening can theoretically lead to a decreased morbidity and mortality as treatment is available for several well established risk factors like hypertension, overweight, dyslipidemia and severe aortic aneurysm.

Follow-up in the Ghent University Hospital, the historical perspective

Before 2009, the cardiovascular follow-up and treatment of TS patients in the Ghent University Hospital was not standardised. At the moment of diagnosis, paediatric and adult patients were referred to the cardiologist for blood pressure measurement, ECG and echocardiography. If the initial screening showed a cardiovascular problem, further follow-up was scheduled based on the judgement of the treating physician. If the initial exam was normal, follow-up was not routinely organised.

Since 2009, the follow-up and treatment of TS patients in the Ghent University Hospital is organised in a multidisciplinary Turner Clinic where staff members join regularly to discuss patients, protocols and research projects. Clinical care is centred in the paediatric or adult endocrinology department were all TS patients are examined at least yearly. Based on an internal protocol, patients are referred on a regular basis to the different caregivers involved.

At the start of the Turner Clinic, the care for paediatric and adult TS patients was revisited and the first protocol on cardiovascular follow-up and treatment was established in 2009 [36]. In an international collaboration with the Dutch Turner Clinics, this subsequently evolved to the creation of the “Nederlands-Belgische richtlijnen voor de opvolging en behandeling van patiënten met het syndroom van Turner” that includes an elaborated chapter on the cardiovascular health of TS patients. With the advancing insights in the complex phenomenon of cardiovascular health in TS, partially based on the results of this PhD research, the protocol for cardiovascular follow-up and treatment has been refined. The most recent follow-up protocol is presented below (figure 13). The adaptations based on the new insights from this PhD research are marked in italic. The current insights in the treatment of cardiovascular disease in TS are described earlier in the introduction of this PhD thesis

Cardiovascular follow-up of paediatric Turner patients

In the Ghent University Hospital, paediatric TS patients are seen regularly at the Paediatric Cardiology Department. The frequency of the check-ups depends on the presence or absence of a CHD or aortic dilation (figure 12). All patients are evaluated by an experienced paediatric cardiologist at the time of diagnosis. If no CHD or aortic dilation (diameter of the aortic root and diameter of the ascending aorta with z-score <2) is present at that time, patients are examined approximately once every 5 years: at the start of growth hormone treatment (or at the age of 7 years), at the start of puberty induction (or at the age of 13 years in case of spontaneous menarche) and before transition to the adult cardiology department. If the patient has a CHD or a dilated ascending aorta, cardiovascular follow-up is organised on a yearly basis or more frequently if appropriate. Paediatric TS patients with a BAV without aortic dilation are seen every 2 years.

A cardiovascular check-up consists minimally of an ECG, in office blood pressure measurement at the 4 limbs and a complete ultrasound examination with measurement of the aortic diameters. Based on the results of this PhD research, we recently added the measurement of aortic wall stiffness using M-mode. A 24h blood pressure recording is routinely planned once before puberty induction and before transition, but is also performed electively in patients with in office hypertension, coarctation or important aortic dilation. Even if the ultrasound is normal, a cardiac MRI is performed before transition for the evaluation of aortic diameters, aortic arch morphology and PWV.

Cardiovascular follow-up of adult Turner patients

Adult TS patients are evaluated regularly by an adult cardiologist experienced in TS and adult CHD (figure 13). Basically, the follow-up of adult TS patients does not differ from that of children and includes an ECG, blood pressure measurement at the 4 limbs and cardiovascular imaging by ultrasound and MRI. Recently we added MRI derived PWV of the aorta to the follow-up protocol.

Patients without CHD or aortic dilation (aortic diameter <20 mm/m²) have a complete cardiovascular check-up every 3 to 5 years. Patients with a BAV are evaluated every 1 to 2 years depending on the valvular function and the association with aortic dilation. If the TS patient has a dilated aorta or a CHD, the cardiovascular assessments are more frequent with a minimum of once a year. A cardiac MRI is performed at least every 5 years, but the frequency is increased in TS patients with important aortic dilation and/or an associated CHD.

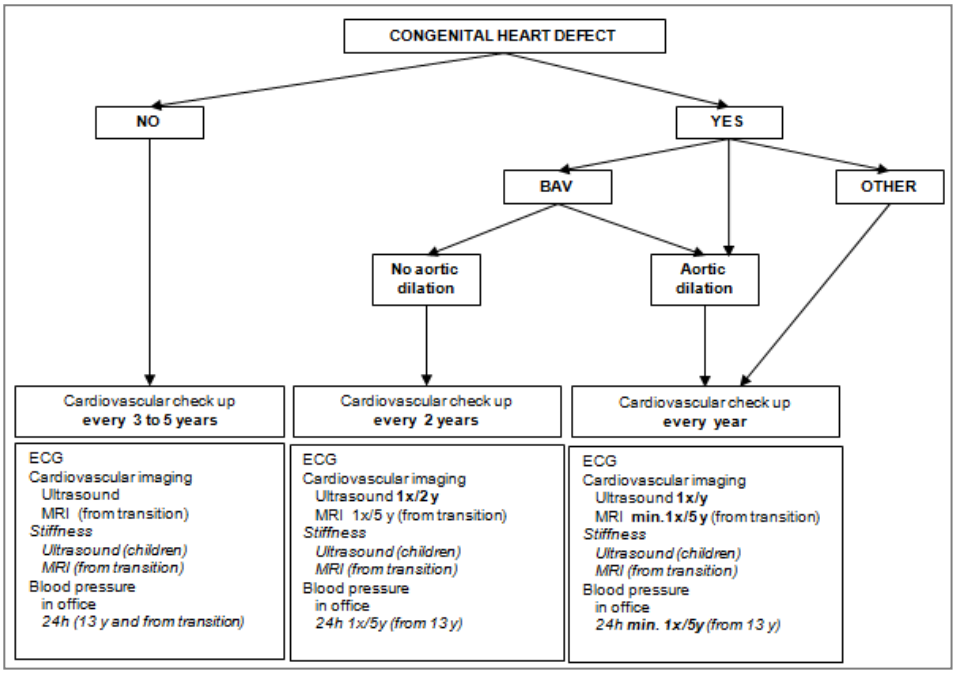

Figure 13: flowchart for the follow-up of TS patients.

Adaptations of the protocol based on the insight of the PhD research are marked in italic.

Congenital heart defect includes congenital CHD and isolated dilation of the ascending aorta (Z-score ≥2 for children and ASI ≥20 mm/m² for adults)

Cardiovascular follow-up of pregnant TS patients

An elaborated cardiovascular screening is necessary prior to conception for all TS patients who want to conceive either spontaneous or via oocyte donation. This work-up includes a complete ultrasound exam with measurement of the aortic diameters, a cardiac MRI and 24h blood pressure measurement. As pregnancy increases the risk for aortic dilation, repeated echocardiography with measurement of the aortic dimensions is performed in the first, second and third trimester and in the postpartum period. Pregnancy is discouraged in TS patients with coarctation, previous cardiac surgery, aortic dilation ≥25 mm/m² or uncontrolled hypertension [9, 17].

Cardiovascular follow-up of male patients with mosaic 45,X/46,XY

Until recently, males with 45,X/46,XY were not referred for cardiovascular screening. Based on the results of the first publication (III.1), these patients are now treated similar to females with TS (figure 13).

IV.6 Challenges and limitations of the research papers

Challenge of studying a rare disease

Turner syndrome is a rare disease occurring in approximately 1/2000 to 1/5000 live born girls. Based on these reported incidences and the number of women aged 0-60 years in Flanders (2008, Stat.be), the number of TS patients in Flanders is roughly estimated at 400 to 1040. For the paediatric population under the age of 15, the estimated number of TS varies between 100 and 250. These low numbers of TS patients inevitably lead to small study populations.

In the first paper (III.1) we selectively included TS patients with 45,X/46,XY, a rare mosaic form found in only 6% of TS patients and compared them with another rare group of males with 45,X/46,XY. At the time of the study, the Belgian Registry for Growth and Puberty problems contained 34 patients with a mosaic 45,X/46,XY (24 females and 10 males) followed in 7 university hospitals. Although we did major efforts to include them all in the study, only 11 could be enrolled (32%). Two centres were not interested to participate (respectively 2 and 6 patients), 4 patients were lost to follow-up, 10 patients refused to participate and 1 male patient deceased suddenly at the age of 24 years. Seven additional patients were included via the multidisciplinary clinic for disorders of sexual development (DSD) of the Ghent University Hospital. Recruitment via the Belgian Registry for Growth and Puberty Problems and a university DSD outpatient clinic could cause a selection bias towards short boys and/or boys born with ambiguous genitalia and does not include asymptomatic 45,X/46,XY males who might have a milder cardiovascular phenotype. Future research should include asymptomatic males with 45,X/46,XY who present at the fertility clinic but due to the privacy legislation it is very difficult to reach this specific population.

The second paper (III.2) retrospectively reviewed the MRI of 74 adult TS patients. Although the absolute number is relatively low, it accounts for 80% (74/92) of the total number of adult TS patients followed in Ghent University Hospital. A similar remark can be formulated for the fourth paper (III.3.2). International multicenter studies are needed to increase the study population but these large studies require a different management with elaborated funding and administrative staff.

In the third paper (III.3.1) all 15 prepubertal TS girls followed at the Ghent University Hospital were included. As Turner syndrome is frequently diagnosed during adolescence or even adulthood, these patients probably represent less than 50% of all prepubertal TS girls. This could possibly have caused a bias towards the more severe phenotypes.

Possible bias of studying a syndrome with a variety of genotypes and phenotypes

The genotype of TS patients is very heterogeneous and includes patients with monosomy 45,X, with isochromosome 46,X,i(X), deletion 46,X,del (X) or a variety of mosaic forms. Although CHD and aortic dilation are more prevalent in TS patients with monosomy 45,X, there is no clear correlation between the genotype and the cardiac phenotype. The genes responsible for the different cardiovascular anomalies remain currently unknown.

Apart from the first paper that only included patients with 45,X/46,XY, the other publications describe TS patients with various karyotypes. The limited number of the patients did not allow investigating the relation between karyotype and the prevalence and severity of aortic arch abnormality (III.2) or aortic wall stiffness (III.3.1 and III.3.2).The second paper (III.2) was a retrospective analysis including patients that were recently transferred to our Ghent University Hospital. In some medical files, the original genetic result was lacking making it impossible to classify the patient in a certain genotype group.

The phenotype of TS patients is highly variable; some patients remain undiagnosed until adulthood while others are not unrecognized at all. We can therefore not rule out that the TS populations we studied represent the most severe phenotypes and may therefore be biased toward a more affected group.

Difficulty of choosing an adequate control group

The third paper (III.3.1) included healthy prepubertal girls as controls for the TS group. As TS girls have a small stature, we decided to match not only for sex and age but also for height; we only included girls with a height below the 25^{th} percentile. Although none of our TS patients was obese, they tend to have a higher weight compared to their small height. In contrast, constitutional small girls tend to have low weights. As a consequence, there was a significant difference in BMI between the 2 groups. We cannot rule out a possible bias of this different constitution on aortic wall elastic properties.

In the fourth paper (III.3.2) the control group consisted of a historical cohort. On average, control patients were taller and weighed more than TS patients while their BMI was slightly lower. The mean age of the control group was 10 years older than the TS group. This considerable difference may not have been completely overcome through statistical modelling. However, finding a stiffer aorta in the TS group that is 10 years younger than the control group probably adds power to the conclusion that an abnormally stiffer aorta presents at an early age.

Possible bias of drug use in a group of patients who require chronic medical treatment

The most distinctive features of TS are small stature and premature ovarian failure. The first is treated by administering supraphysiologic doses of growth hormone, the latter by hormone replacement therapy. From an ethical point of view it is very difficult to stop one of these treatments for a longer period just for the purpose of research

In the fourth paper (III.3.1) the possible role of estrogen replacement was countered by studying prepubertal TS girls. However, we could not rule out a bias of GH, as 12 of the 15 patients were on GH treatment.

In the second (III.2) and fourth (III.3.2) publication, we retrospectively checked the medical files of the TS patients for the duration and dosage of GH and estrogen therapy. There were too many missing data to study the effect of GH or HRT on aortic arch morphology and blood pressure or aortic dilation and aortic stiffness.

Unknown contribution of other risk factors

TS patients can present a variety of pathology like diabetes, thyroid dysfunction, dyslipidemia and renal dysfunction. All these disorders can have a detrimental influence on the cardiovascular health status of TS patients.

In the third publication (III.3.1) none of the very young TS patients had comorbidity or chronic drug use apart from GH treatment. The study protocols in adult TS patients did not intend to study the impact of dyslipidemia, thyroid function, renal function, smoking habits and physical activity on aortic arch morphology, aortic dilation or aortic stiffness. These data were therefore not included in the analysis, but a possible bias cannot be ruled out.

Limitations of non-invasive clinical research

Animal models of TS are lacking and research on the cardiovascular pathology in this syndrome is limited to clinical research in human TS patients.

Studies in children predominantly use non-invasive methods that create minimal discomfort or pain. MRI imaging was therefore not considered in the third paper (III.3.1) as it is time consuming and requires the administration of IV contrast fluid. Ultrasound on the other hand is well tolerated and is performed in a relatively short time interval. Although visualisation can be difficult in adult TS patients, the quality of the images in our study population was adequate for measuring all parameters; M-mode based calculation of distensibility and stiffness was possible in all participants. Due to the limitations of the technique, it was impossible to measure parameters of stiffness of the distal aorta. Ultrasound derived PWV showed to be inadequate in TS patients.

Calculation of distensibility and stiffness index requires systolic and diastolic blood pressure values. In the clinical research protocols (III.3.1 and III.3.2), the blood pressure was measured non-invasively at the arm which might not be representative for the central pressure that is needed. Theoretically, the abnormal aortic wall properties of TS patients can lead to an increased transmission velocity of both forward and reflected waves, which causes the reflected wave to arrive earlier in the central aorta and increases the central pressure in systole. To evaluate a possible impact of blood pressure bias, distensibility and stiffness index of prepubertal TS girls were recalculated with blood pressure values that were increased with 10%. Distensibility increased and stiffness index decreased, but the difference between TS and controls remained statistically significant.

Blood pressure measurement in Turner patients

Taken into account that TS patients often have an abnormal arch morphology, measuring the blood pressure at the left arm can theoretically underestimate the real blood pressure. Moreover, in office blood pressure measurement can lead to underdiagnosis of arterial hypertension in TS as nocturnal hypertension and abnormal dipping are frequently seen in these patients. Additionally, the definition of hypertension has slightly changed over time and the definition of hypertension was not the same in the different study protocols.

In the first paper (III.1) blood pressure was measured in office at both arms. Hypertension was defined as a blood pressure above the 95th percentile in children and above 130/85 mmHg in adults. We could not conclude on the prevalence of nocturnal hypertension or abnormal nocturnal dipping in 45,X/46,XY patients.

In the second paper (III.2) blood pressure values were recorded from the medical files. In many cases, the side of the blood pressure measurement was not recorded. Ambulatory blood pressure measurement was only available in 22 cases. Patients were considered hypertensive if the in office blood pressure exceeded 140/90 mmHg, if the mean ambulatory blood pressure exceeded 130/80 mmHg or if they were treated with antihypertensive drugs. Although this stratification reflects clinical practice, it might have under- or overestimated the number of hypertensive TS patients.

In the third paper (III.3.1), we used the in office blood pressure values at the right arm for the calculation of distensibility and stiffness index to avoid interference of aortic arch morphology on the blood pressure measurements. Additionally, blood pressure was measured in office at the 4 limbs. The post hoc analysis, could not detect a significant difference (or a trend) in the blood pressure values between the left and right arm in TS patients. Twenty-four hour recording was only available in 11 girls, not enough to study the relation between mean blood pressure or abnormal nocturnal dipping and aortic wall elastic properties.

In the fourth paper (III.3.2) blood pressure was taken at the left arm during the MRI exam. Yet again, the sensitivity analyses in study III.3.1 and III.3.2 showed conclusions on distensibility and stiffness index to be robust for 10% increase or decrease in the blood pressure measurements in TS patients.

IV.7 Future research

This PhD research added information on the aetiology of aortic dilation in Turner syndrome but several highways and sidetracks still remain to be explored. Future research on the pathogenesis of aortic dilation and dissection in TS should therefore try to unravel the complex connection between abnormal aortic stiffness, aortic dilation and associated risk factors.

Additional information could be gathered by:

- comparing aortic dimension and aortic wall distensibility in non-syndromic patients with BAV and Turner patients to clarify the relative contribution of the intrinsic aortic wall property and the add-on effect of BAV in TS;
- following aortic arch morphology in time to determine whether abnormal arch morphology is present from birth or develops progressively later in life;
- measuring aortic distensibility repetitively to understand the role of aortic stiffness in the development of aortic dilation, aortic arch deformation and hypertension;
- measuring aortic distensibility and aortic diameters repetitively to clarify the relative role of both parameters in the evolution toward dilation and dissection;
- developing experimental flow models that could help to study flow alterations in stiff aortic arches and the association of abnormal flow with aortic arch deformation and head vessel dilation;
- measuring aortic stiffness parameters in males and females with 45,X/46,XY to clarify if the abnormal aortic stiffness is linked to the X chromosome haploinsufficiency;
- following males with 45,X/46,XY over time to clarify if those with a tricuspid aortic valve are also at risk for aortic dilation;
- studying the relationship between the Turner karyotype and the presence of CHD, the presence of abnormal aortic stiffness and the severity of aortic dilation;
- studying the relationship between the prenatal nuchal translucency and the presence of CHD, the presence of abnormal aortic stiffness and the severity of aortic dilation. This will probably only be possible for the paediatric population, as it will be very difficult to gather the data on nuchal translucency retrospectively;
- studying the relationship between the presence of dysmorphic features (including webbed neck) and the presence of CHD, the presence of abnormal aortic stiffness and the severity of aortic dilation;
- studying the usefulness of betablockers and losartan in the prevention of accelerated aortic growth;
- cooperating in international research on the histological nature of the aortic wall of TS patients.

IV.8 General conclusion of the PhD thesis: abnormal aortic stiffness, the missing link for aortic dilation and dissection

Aortic dissection is a major issue in Turner syndrome as it is associated with an increased risk for morbidity and mortality. Aortic dilation and CHD play a major role in the progression to this often lethal complication. Current guidelines advocate regular cardiovascular follow-up that include the evaluation of traditional risk factors like blood pressure, aortic dimensions, the presence of a bicuspid aortic valve and coarctation or ETA. They however seemed insufficient to explain all cases of aortic dissection.

Our data suggest that an intrinsic genetically determined increased aortic wall stiffness cooperates with and modulates the other well known risk factors for aortic dilation. We propose to add it as “the missing link” in the risk stratification for aortic dilation and dissection in Turner syndrome and to include it in the regular follow-up of TS patients.

CHAPTER V

SUMMARY

V.1 Summary of the PhD thesis (in English)

Turner syndrome is a chromosomal disorder occurring in 1/2000 to 1/5000 live born girls. Cardiovascular pathology is common in TS patients and includes structural (left sided) cardiovascular defects, hypertension, ECG alterations and aortic dilation and dissection. Morbidity and mortality are increased compared to the general population, aortic dissection being the most important cause of early demise. The presence of a bicuspid aortic valve, coarctation and hypertension – all highly prevalent in TS – increases the risk for aortic dilation and dissection but this often lethal complication also presents in TS patients without one of these classical risk factors. In this thesis, additional information on the pathogenesis of aortic dilation in TS was sought.

Cardiovascular pathology in TS is probably related to the genetic haploinsufficiency of the X chromosome. We found cardiac pathology that is typically associated with TS pathology (bicuspid aortic valves, aortic dilation and ECG alterations) in males with mosaic 45,X/46,XY (one of the TS genotypes). In those male 45,X/46,XY patients, cardiovascular pathology was more frequent in those who also had other TS related abnormalities. Based on this findings cardiac screening and lifetime monitoring in all males with 45,X/46,XY mosaicism according to the existing guidelines for Turner syndrome is advised.

Besides aortic dilation, elongation of the aortic arch is a common finding in TS. Our study showed that abnormal arch morphology is present in nearly 40% of TS patients and can also be seen in patients without associated coarctation or abnormal aortic valve. A significant association was found between abnormal arch morphology and hypertension in TS patients. Patients with an abnormal arch morphology more often had a dilation of the ascending aorta, suggesting that abnormal arch morphology might be an additional risk factor in the risk stratification for aortic dilation and dissection.

We found that TS patients with a bicuspid or tricuspid aortic valve have an increased stiffness of the ascending aorta from a young age on although this abnormality is more pronounced in TS patients with a BAV. In young TS girls severe dilation of the ascending aorta was only found in patients with a bicuspid aortic valve. In adult TS patients aortic dilation was found in patients with a bicuspid or a tricuspid valve although the dilation was more pronounced in those with a bicuspid valve. We concluded that Turner patients exhibit a predominant stiffening and dilation of the proximal aorta, especially if their aortic valve is bicuspid. These abnormalities are present from an early age, suggesting an intrinsic wall pathology inherent to the TS syndrome.

Our data suggest that an intrinsic genetically determined increased aortic wall stiffness cooperates with and modulates the other well known risk factors for aortic dilation. We propose to add it as “the missing link” in the risk stratification for aortic dilation and dissection in Turner syndrome and to include it in the regular follow-up of TS patients.

V.2 Samenvatting van de doctoraatsthesis (in het Nederlands)

Turner syndroom (TS) is een zeldzame genetische aandoening die optreedt in 1/2000 tot 1/5000 levend geboren meisjes. Ze wordt gekenmerkt door het verlies van een gedeelte of van heel het X-chromosoom. Vele Turner patiënten lijden aan hart- en vaatziekten waarbij structurele hartafwijkingen, hoge bloeddruk, ECG afwijkingen, dilatatie (abnormale uitzetting) en dissectie (ontstaan van scheurtjes in de vaatwand) van de aorta worden beschreven. In vergelijking met “de algemene bevolking” is de morbiditeit en mortaliteit in patiënten met TS sterk toegenomen. Vooral dissectie van de aorta, die kan optreden op zeer jonge leeftijd, speelt hierin een belangrijke rol. Bicuspide aortaklep (aortaklep met 2 ipv 3 klepblaadjes), coarctatio (vernauwing thv de grote lichaamsslagader) en hypertensie (hoge bloeddruk) komen vaak voor bij TS patiënten en zijn gekende risicofactoren voor het ontwikkelen van aortadilatatie en dissectie. Er zijn in de literatuur echter ook patiënten met aortadissectie beschreven die niet één van deze gekende klassieke risicofactoren vertoonden. In dit thesisonderzoek werd gezocht naar bijkomende informatie over het ontstaan van aortadilatatie in TS.

Vermoedelijk ligt haplo-insufficiëntie van het X-chromosoom aan de basis van de hart- en vaatziekten in TS patiënten. Mozaïek 45,X/46,XY is één van de gekende genotypes die het TS veroorzaken. Het wordt ook teruggevonden bij mannelijke patiënten met gonadale dysgenese. In de eerste publicatie wordt beschreven dat typische Turner gerelateerde hartafwijkingen zoals bicuspide aortaklep, aortadilatatie en ECG afwijkingen ook bij mannen met mozaïek 45,X/46,XY worden teruggevonden. Cardiovasculaire afwijkingen werden het vaakst gevonden bij mannelijke patiënten die ook andere typische Turner kenmerken vertonen. Op basis van deze bevinding adviseren wij om levenslange cardiovasculaire opvolging te voorzien bij alle patiënten met 45,X/46,XY, ook bij diegenen met een mannelijk fenotype.

De tweede publicatie beschrijft een retrospectieve MRI studie bij volwassen vrouwen met TS waarin wordt aangetoond dat 40% van de TS patiënten een abnormale aortaboogmorfologie vertoont. Deze wordt ook gevonden bij TS vrouwen zonder coarctatio of abnormale aortaklepmorfologie. We vonden een significante associatie tussen de aanwezigheid van een abnormaal gehaakte boog en hypertensie. Patiënten met een abnormale boogmorfologie hadden ook vaker een gedilateerde aorta ascendens. Dit suggereert dat een abnormale boogmorfologie een bijkomende risicofactor zou kunnen vormen voor het ontstaan van aorta dilatatie en mogelijk dissectie.

In een prospectieve studie vergeleken we de elasticiteit van de aorta van patiënten met TS met die van normale controles. Bij patiënten met TS vonden we een abnormaal stijvere aorta ascendens, zowel bij diegenen met een bicuspide als diegenen met een tricuspide aortaklep. Het verschil was echter meer uitgesproken bij TS patiënten met een bicuspide aortaklep. In jonge prepuberale Turner meisjes vonden we enkel een significante dilatatie van de aorta ascendens bij diegenen met een bicuspide aortaklep terwijl dilatatie van de aorta ascendens bij volwassen patiënten significant groter was bij patiënten met een bicuspide aortaklep in vergelijking met diegenen met een tricuspide aortaklep. Uit deze resultaten besloten we dat TS patiënten een abnormale stijfheid vertonen van de aorta ascendens, die nog meer uitgesproken is in de aanwezigheid van een bicuspide aortaklep.

Deze afwijking is reeds op jonge leeftijd terug te vinden wat suggereert dat het om een intrinsiek probleem gaat met slechts een beperkt effect van versnelde veroudering.

Onze data suggereren dat het Turner syndroom gekenmerkt wordt door een abnormale stijfheid van de aorta die intrinsiek genetisch is bepaald en die andere goedgekende risicofactoren van aortadilatatie moduleert en versterkt. We stellen dan ook voor om abnormale vaatstijfheid van de aorta toe te voegen als “missing link” in de risicostratificatie voor aortadilatatie en dissectie in TS en om bepaling van stijfheid en elasticiteit het te includeren in de follow-up van TS patiënten.

CHAPTER VI

APPENDIX

-

Review paper on arterial hypertension

Arterial hypertension in Turner syndrome: a review of the literature and a practical approach for diagnosis and treatment.

Katya De Groote, Laurent Demulier, Julie De Backer, Daniel De Wolf, Jean De Schepper, Guy T'Sjoen, Tine De Backer

Journal of Hypertension 2015; 33(7): 1342-1351.

REFERENCE LIST

1. Akyurek N, Atabek ME, Eklioglu BS, Alp H. Ambulatory blood pressure and subclinical cardiovascular disease in children with turner syndrome. *Pediatr Cardiol* 2014; **35**(1):57-62.

2. Alsoufi B, Cai S, Van Arsdell GS, Williams WG, Caldarone CA, Coles JG. Outcomes after surgical treatment of children with partial anomalous pulmonary venous connection. *Ann Thorac Surg* 2007; **84**(6):2020-2026.

3. Backeljauw PF, Bondy C, Chernausek SD, Cernich JT, Cole DA, Fasciano LP, et al. Proceedings from the Turner Resource Network symposium: The crossroads of health care research and health care delivery. *Am J Med Genet A* 2015; **167A**(9):1962-1971.

4. Baguet JP, Douchin S, Pierre H, Rossignol AM, Bost M, Mallion JM. Structural and functional abnormalities of large arteries in the Turner syndrome. *Heart* 2005; **91**(11):1442-1446.

5. Bannink EM, van der Palen RL, Mulder PG, de Muinck Keizer-Schrama SM. Long-Term Follow-Up of GH-Treated Girls with Turner Syndrome: BMI, Blood Pressure, Body Proportions. *Horm Res* 2009; **71**(6):336-342.

6. Bechtold SM, Dalla PR, Becker A, Meidert A, Dohlemann C, Schwarz HP. Partial anomalous pulmonary vein connection: an underestimated cardiovascular defect in Ullrich-Turner syndrome. *Eur J Pediatr* 2004; **163**(3):158-162.

7. Blethen SL. Complications of growth hormone therapy in children. *Curr Opin Pediatr* 1995; **7**(4):466-471.

8. Bondy C, Bakalov VK, Cheng C, Olivieri L, Rosing DR, Arai AE. Bicuspid aortic valve and aortic coarctation are linked to deletion of the X chromosome short arm in Turner syndrome. *J Med Genet* 2013; **50**(10):662-665.

9. Bondy C, Rosing D, Reindollar R. Cardiovascular risks of pregnancy in women with Turner syndrome. *Fertil Steril* 2009; **91**(5):e31-e32.

10. Bondy CA. Turner syndrome 2008. *Horm Res* 2009; **71**(Suppl 1):52-56.

11. Bondy CA. Care of Girls and Women with Turner Syndrome: A Guideline of the Turner Syndrome Study Group. *J Clin Endocrinol Metab* 2007; **92**(1):10-25.

12. Bondy CA, Ceniceros I, Van PL, Bakalov VK, Rosing DR. Prolonged rate-corrected QT interval and other electrocardiogram abnormalities in girls with Turner syndrome. *Pediatrics* 2006; **118**(4):e1220-e1225.

13. Bondy CA, Van PL, Bakalov VK, Ho VB. Growth hormone treatment and aortic dimensions in Turner syndrome. *J Clin Endocrinol Metab* 2006; **91**(5):1785-1788.

14. Bondy CA, Van PL, Bakalov VK, Sachdev V, Malone CA, Ho VB, et al. Prolongation of the cardiac QTc interval in Turner syndrome. *Medicine (Baltimore)* 2006; **85**(2):75-81.

15. Booher AM, Eagle KA. Diagnosis and management issues in thoracic aortic aneurysm. *Am Heart J* 2011; **162**(1):38-46.

16. Brandenburg H, Steegers EA, Gittenberger-de Groot AC. Potential involvement of vascular endothelial growth factor in pathophysiology of Turner syndrome. *Med Hypotheses* 2005; **65**(2):300-304.

17. Cabanes L, Chalas C, Christin-Maitre S, Donadille B, Felten ML, Gaxotte V, et al. Turner syndrome and pregnancy: clinical practice. Recommendations for the management of patients with Turner syndrome before and during pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2010; **152**(1):18-24.

18. Campens L, Demulier L, De Groote K., Vandekerckhove K, De Wolf D., Roman MJ, et al. Reference values for echocardiographic assessment of the diameter of the aortic root and ascending aorta spanning all age categories. *Am J Cardiol* 2014; **114**(6):914-920.

19. Carlson M, Airhart N, Lopez L, Silberbach M. Moderate aortic enlargement and bicuspid aortic valve are associated with aortic dissection in Turner syndrome: report of the international turner syndrome aortic dissection registry. *Circulation* 2012; **126**(18):2220-2226.

20. Carlson M, Silberbach M. Dissection of the aorta in Turner syndrome: two cases and review of 85 cases in the literature. *J Med Genet* 2007; **44**(12):745-749.

21. Chalard F, Ferey S, Teinturier C, Kalifa G. Aortic dilatation in Turner syndrome: the role of MRI in early recognition. *Pediatr Radiol* 2005; **35**(3):323-326.

22. Chang HJ, Clark RD, Bachman H. The phenotype of 45,X/46,XY mosaicism: an analysis of 92 prenatally diagnosed cases. *Am J Hum Genet* 1990; **46**(1):156-167.

23. Cicognani A, Mazzanti L, Tassinari D, Pellacani A, Forabosco A, Landi L, et al. Differences in carbohydrate tolerance in Turner syndrome depending on age and karyotype. *Eur J Pediatr* 1988; **148**(1):64-68.

24. Ciotti GR, Vlahos AP, Silverman NH. Morphology and function of the bicuspid aortic valve with and without coarctation of the aorta in the young. *Am J Cardiol* 2006; **98**(8):1096-1102.

25. Cittadini A, Berggren A, Longobardi S, Ehrnborg C, Napoli R, Rosen T, et al. Supraphysiological doses of GH induce rapid changes in cardiac morphology and function. *J Clin Endocrinol Metab* 2002; **87**(4):1654-1659.

26. Clark EB. Neck web and congenital heart defects: a pathogenic association in 45 X-O Turner syndrome? *Teratology* 1984; **29**(3):355-361.

27. Conway GS, Band M, Doyle J, Davies MC. How do you monitor the patient with Turner's syndrome in adulthood? *Clin Endocrinol (Oxf)* 2010; **73**(6):696-699.

28. Cools M, Pleskacova J, Stoop H, Hoebeke P, Van Laecke E, Drop SL, et al. Gonadal pathology and tumor risk in relation to clinical characteristics in patients with 45,X/46,XY mosaicism. *J Clin Endocrinol Metab* 2011; **96**(7):E1171-E1180.

29. Coutinho T, Bailey KR, Turner ST, Kullo IJ. Arterial stiffness is associated with increase in blood pressure over time in treated hypertensives. *J Am Soc Hypertens* 2014; **8**(6):414-421.

30. Cozijnsen L, Braam RL, Waalewijn RA, Schepens MA, Loeys BL, van Oosterhout MF, et al. What is new in dilatation of the ascending aorta? Review of current literature and practical advice for the cardiologist. *Circulation* 2011; **123**(8):924-928.

31. Cramer JW, Bartz PJ, Simpson PM, Zangwill SD. The Spectrum of Congenital Heart Disease and Outcomes After Surgical Repair Among Children With Turner Syndrome: A Single-Center Review. *Pediatr Cardiol* 2014; **35**(2):253-260.

32. Cripe L, Andelfinger G, Martin LJ, Shooner K, Benson DW. Bicuspid aortic valve is heritable. *J Am Coll Cardiol* 2004; **44**(1):138-143.

33. Dalla PR, Bechtold S, Kaab S, Buckl M, Urschel S, Netz H, et al. QTc interval prolongation in children with Ulrich-Turner syndrome. *Eur J Pediatr* 2006; **165**(12):831-837.

34. Dalla PR, Bechtold S, Urschel S, Netz H, Schwarz HP. QTc interval prolongation in children with Turner syndrome: the results of exercise testing and 24-h ECG. *Eur J Pediatr* 2009; **168**(1):59-64.

35. Davenport ML. Approach to the patient with Turner syndrome. *J Clin Endocrinol Metab* 2010; **95**(4):1487-1495.

36. De Groote K, De Backer J. Cardiovasculaire opvolging bij het syndroom van Turner. *Tijdschrift voor Geneeskunde* 2011; **67**:550-553.

37. De Groote K, Demulier L, De Backer J, De Wolf D, De Schepper J, T'sjoen G., et al. Arterial hypertension in Turner syndrome: a review of the literature and a practical approach for diagnosis and treatment. *J Hypertens* 2015; **33**(7):1342-1351.

38. Denes AM, Landin-Wilhelmsen K, Wettergren Y, Bryman I, Hanson C. The proportion of diploid 46,XX cells increases with time in women with Turner syndrome--a 10-year follow-up study. *Genet Test Mol Biomarkers* 2015; **19**(2):82-87.

39. Donadille B, Rousseau A, Zenaty D, Cabrol S, Courtillot C, Samara-Boustani D, et al. Cardiovascular findings and management in Turner syndrome: insights from a French cohort. *Eur J Endocrinol* 2012; **167**(4):517-522.

40. Ducarme G, Graesslin O, Alanio E, Bige V, Gaillard D, Gabriel R. [Increased nuchal translucency and cystic hygroma in the first trimester: prenatal diagnosis and neonatal outcome]. *Gynecol Obstet Fertil* 2005; **33**(10):750-754.

41. Dulac Y, Pienkowski C, Abadir S, Tauber M, Acar P. Cardiovascular abnormalities in Turner's syndrome: what prevention? *Arch Cardiovasc Dis* 2008; **101**(7-8):485-490.

42. Elsheikh M, Casadei B, Conway GS, Wass JA. Hypertension is a major risk factor for aortic root dilatation in women with Turner's syndrome. *Clin Endocrinol (Oxf)* 2001; **54**(1):69-73.

43. Elsheikh M, Conway GS. The impact of obesity on cardiovascular risk factors in Turner's syndrome. *Clin Endocrinol (Oxf)* 1998; **49**(4):447-450.

44. Erbel R, Aboyans V, Boileau C, Bossone E, Bartolomeo RD, Eggebrecht H, et al. 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). *Eur Heart J* 2014; **35**(41):2873-2926.

45. Etz CD, Zoli S, Brenner R, Roder F, Bischoff M, Bodian CA, et al. When to operate on the bicuspid valve patient with a modestly dilated ascending aorta. *Ann Thorac Surg* 2010; **90**(6):1884-1890.

46. Fernandes SM, Khairy P, Sanders SP, Colan SD. Bicuspid aortic valve morphology and interventions in the young. *J Am Coll Cardiol* 2007; **49**(22):2211-2214.

47. Finsterer J, Zartl M, Samec P. Spontaneous cerebral haemorrhage without hypertension in non-mosaic 45X Turner's syndrome. *J Clin Neurosci* 2000; **7**(4):341-343.

48. Freriks K, Timmermans J, Beerendonk CC, Verhaak CM, Netea-Maier RT, Otten BJ, et al. Standardized multidisciplinary evaluation yields significant previously undiagnosed morbidity in adult women with Turner syndrome. *J Clin Endocrinol Metab* 2011; **96**(9):E1517-E1526.

49. Fudge EB, Constantacos C, Fudge JC, Davenport M. Improving Detection of Hypertension in Girls with Turner Syndrome Using Ambulatory Blood Pressure Monitoring. *Horm Res Paediatr* 2014; **81(1)**:25-31.

50. Fuentes K, Silveira DC, Papamitsakis NI. Spontaneous carotid artery dissection in a patient with Turner syndrome. *Cerebrovasc Dis* 2007; **24**(6):543-544.

51. Giordano R, Forno D, Lanfranco F, Manieri C, Ghizzoni L, Ghigo E. Metabolic and cardiovascular outcomes in a group of adult patients with Turner's syndrome under hormonal replacement therapy. *Eur J Endocrinol* 2011; **164**(5):819-826.

52. Gittenberger-de Groot AC, Bartelings MM, Poelmann RE, Haak MC, Jongbloed MR. Embryology of the heart and its impact on understanding fetal and neonatal heart disease. *Semin Fetal Neonatal Med* 2013; **18**(5):237-244.

53. Goldstein SR, Kerenyi T, Scher J, Papp C. Correlation between karyotype and ultrasound findings in patients with failed early pregnancy. *Ultrasound Obstet Gynecol* 1996; **8**(5):314-317.

54. Gomez D, Al Haj ZA, Borges LF, Philippe M, Gutierrez PS, Jondeau G, et al. Syndromic and non-syndromic aneurysms of the human ascending aorta share activation of the Smad2 pathway. *J Pathol* 2009; **218**(1):131-142.

55. Gotzsche CO, Krag-Olsen B, Nielsen J, Sorensen KE, Kristensen BO. Prevalence of cardiovascular malformations and association with karyotypes in Turner's syndrome. *Arch Dis Child* 1994; **71**(5):433-436.

56. Gravholt CH. Epidemiological, endocrine and metabolic features in Turner syndrome. *Eur J Endocrinol* 2004; **151**(6):657-687.

57. Gravholt CH. Epidemiological, endocrine and metabolic features in Turner syndrome. *Arq Bras Endocrinol Metabol* 2005; **49**(1):145-156.

58. Gravholt CH, Hansen KW, Erlandsen M, Ebbehoj E, Christiansen JS. Nocturnal hypertension and impaired sympathovagal tone in Turner syndrome. *J Hypertens* 2006; **24**(2):353-360.

59. Gravholt CH, Hjerrild BE, Mosekilde L, Hansen TK, Rasmussen LM, Frystyk J, et al. Body composition is distinctly altered in Turner syndrome: relations to glucose metabolism, circulating adipokines, and endothelial adhesion molecules. *Eur J Endocrinol* 2006; **155**(4):583-592.

60. Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. *J Clin Epidemiol* 1998; **51**(2):147-158.

61. Gravholt CH, Landin-Wilhelmsen K, Stochholm K, Hjerrild BE, Ledet T, Djurhuus CB, et al. Clinical and epidemiological description of aortic dissection in Turner's syndrome. *Cardiol Young* 2006; **16**(5):430-436.

62. Gravholt CH, Mortensen KH, Andersen NH, Ibsen L, Ingerslev J, Hjerrild BE. Coagulation and fibrinolytic disturbances are related to carotid intima thickness and arterial blood pressure in Turner syndrome. *Clin Endocrinol (Oxf)* 2012; (76):649-665.

63. Gravholt CH, Naeraa RW, Nyholm B, Gerdes LU, Christiansen E, Schmitz O, et al. Glucose Metabolism, Lipid Metabolism, and Cardiovascular Risk Factors in Adult Turner's Syndrome. *Diabetes Care* 1998; **21**(7):1062-1070.

64. Groenink M, den Hartog AW, Franken R, Radonic T, de W, V, Timmermans J, et al. Losartan reduces aortic dilatation rate in adults with Marfan syndrome: a randomized controlled trial. *Eur Heart J* 2013; **34**(45):3491-3500.

65. Grotenhuis HB, Westenberg JJ, Steendijk P, van der Geest RJ, Ottenkamp J, Bax JJ, et al. Validation and reproducibility of aortic pulse wave velocity as assessed with velocity-encoded MRI. *J Magn Reson Imaging* 2009; **30**(3):521-526.

66. Hagan PG, Nienaber CA, Isselbacher EM, Bruckman D, Karavite DJ, Russman PL, et al. The International Registry of Acute Aortic Dissection (IRAD): new insights into an old disease. *JAMA* 2000; **283**(7):897-903.

67. Hagman A, Kallen K, Bryman I, Landin-Wilhelmsen K, Barrenas ML, Wennerholm UB. Morbidity and mortality after childbirth in women with Turner karyotype. *Hum Reprod* 2013; **28**(7):1961-1973.

68. Hauser M, Kuhn A, Petzuch K, Wolf P, Vogt M. Elastic properties of the ascending aorta in healthy children and adolescents. Age-related reference values for aortic wall stiffness and distensibility obtained on M-mode echocardiography. *Circ J* 2013; **77**(12):3007-3014.

69. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr* 1978; **93**(1):62-66.

70. Hiratzka LF, Creager MA, Isselbacher EM, Svensson LG, Nishimura RA, Bonow RO, et al. Surgery for Aortic Dilatation in Patients With Bicuspid Aortic Valves: A Statement of Clarification From the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2010; (121):e266-e369.

71. Hjerrild BE, Mortensen KH, Sorensen KE, Pedersen EM, Andersen NH, Lundorf E, et al. Thoracic aortopathy in Turner syndrome and the influence of bicuspid aortic valves and blood pressure: a CMR study. *J Cardiovasc Magn Reson* 2010; **12**(1):12.

72. Ho VB, Bakalov VK, Cooley M, Van PL, Hood MN, Burklow TR, et al. Major vascular anomalies in Turner syndrome: prevalence and magnetic resonance angiographic features. *Circulation* 2004; **110**(12):1694-1700.

73. Hook EB, Warburton D. The distribution of chromosomal genotypes associated with Turner's syndrome: livebirth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. *Hum Genet* 1983; **64**(1):24-27.

74. Hook EB, Warburton D. Turner syndrome revisited: review of new data supports the hypothesis that all viable 45,X cases are cryptic mosaics with a rescue cell line, implying an origin by mitotic loss. *Hum Genet* 2014; **133**(4):417-424.

75. Hyett J, Perdu M, Sharland G, Snijders R, Nicolaides KH. Using fetal nuchal translucency to screen for major congenital cardiac defects at 10-14 weeks of gestation: population

based cohort study
96. *Bmj* 1999; **318**(7176):81-85.

76. Irioka T, Mizusawa H. Ischemic stroke in a young adult with Turner syndrome. *Neurol Sci* 2011; **32**(2):317-319.

77. Iyer NP, Tucker DF, Roberts SH, Moselhi M, Morgan M, Matthes JW. Outcome of fetuses with Turner syndrome: a 10-year congenital anomaly register based study. *J Matern Fetal Neonatal Med* 2012; **25**(1):68-73.

78. Jagannath AD, Rastogi U, Spooner AE, Lin AE, Agnihotri AK. Aortic dissection and moyamoya disease in Turner syndrome. *Am J Med Genet A* 2010; **152A**(8):2085-2089.

79. Karnis MF. Catastrophic consequences of assisted reproduction: the case of Turner syndrome. *Semin Reprod Med* 2012; **30**(2):116-122.

80. Kawel-Boehm N, Maceira A, Valsangiacomo-Buechel ER, Vogel-Claussen J, Turkbey EB, Williams R, et al. Normal values for cardiovascular magnetic resonance in adults and children. *J Cardiovasc Magn Reson* 2015; **17**:29.

81. Kim HK, Gottliebson W, Hor K, Backeljauw P, Gutmark-Little I, Salisbury SR, et al. Cardiovascular anomalies in Turner syndrome: spectrum, prevalence, and cardiac MRI findings in a pediatric and young adult population. *AJR Am J Roentgenol* 2011; **196**(2):454-460.

82. Kneppo C, Eichhorn JG, Ley S, Schenk JP, Gorenflo M, Bettendorf M. Assessment of aortic morphology and compliance in children and adolescents with Ullrich-Turner syndrome (UTS) using magnetic resonance imaging (MRI). *J Pediatr Endocrinol Metab* 2014; **27**(9-10):915-922.

83. Komori H, Matsuishi T, Abe T, Nagata Y, Ohtaki E, Kojima K, et al. Turner syndrome and occlusion of the internal carotid artery. *J Child Neurol* 1993; **8**(4):412-415.

84. Kosho T, Muroya K, Nagai T, Fujimoto M, Yokoya S, Sakamoto H, et al. Skeletal features and growth patterns in 14 patients with haploinsufficiency of SHOX: implications for the development of Turner syndrome. *J Clin Endocrinol Metab* 1999; **84**(12):4613-4621.

85. Kuhn A, Baumgartner D, Baumgartner C, Horer J, Schreiber C, Hess J, et al. Impaired elastic properties of the ascending aorta persist within the first 3 years after neonatal coarctation repair. *Pediatr Cardiol* 2009; **30**(1):46-51.

86. Landin-Wilhelmsen K, Bryman I, Wilhelmsen L. Cardiac malformations and hypertension, but not metabolic risk factors, are common in Turner syndrome. *J Clin Endocrinol Metab* 2001; **86**(9):4166-4170.

87. Langrish JP, Mills NL, Bath LE, Warner P, Webb DJ, Kelnar CJ, et al. Cardiovascular effects of physiological and standard sex steroid replacement regimens in premature ovarian failure. *Hypertension* 2009; **53**(5):805-811.

88. Larson EW, Edwards WD. Risk factors for aortic dissection: a necropsy study of 161 cases. *Am J Cardiol* 1984; **53**(6):849-855.

89. Laurent S, Cockcroft J, Van Bortel L., Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; **27**(21):2588-2605.

90. Lawson SA, Urbina EM, Gutmark-Little I, Khoury PR, Gao Z, Backeljauw PF. Vasculopathy in the young Turner syndrome population. *J Clin Endocrinol Metab* 2014; **99**(10):E2039-E2045.

91. Layton KF, Kallmes DF, Cloft HJ, Lindell EP, Cox VS. Bovine aortic arch variant in humans: clarification of a common misnomer. *AJNR Am J Neuroradiol* 2006; **27**(7):1541-1542.

92. Lin AE, Lippe BM, Geffner ME, Gomes A, Lois JF, Barton CW, et al. Aortic dilation, dissection, and rupture in patients with Turner syndrome. *J Pediatr* 1986; **109**(5):820-826.

93. Lopez L, Arheart KL, Colan SD, Stein NS, Lopez-Mitnik G, Lin AE, et al. Turner syndrome is an independent risk factor for aortic dilation in the young. *Pediatrics* 2008; **121**(6):e1622-e1627.

94. Loscalzo ML, Van PL, Ho VB, Bakalov VK, Rosing DR, Malone CA, et al. Association between fetal lymphedema and congenital cardiovascular defects in Turner syndrome. *Pediatrics* 2005; **115**(3):732-735.

95. Madriago E, Nguyen T, McFerson M, Larson EV, Airhart N, Moller JH, et al. Frequency and Outcomes of Cardiac Operations and Catheter Interventions in Turner Syndrome. *Am J Cardiol* 2012; **110**(4):580-585.

96. Maeda T, Ohno M, Matsunobu A, Yoshihara K, Yabe N. A cytogenetic survey of 14,835 consecutive liveborns. *Jinrui Idengaku Zasshi* 1991; **36**(1):117-129.

97. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013; **34**:1925-1938.

98. Matura LA, Ho VB, Rosing DR, Bondy CA. Aortic dilatation and dissection in Turner syndrome. *Circulation* 2007; **116**(15):1663-1670.

99. Matura LA, Sachdev V, Bakalov VK, Rosing DR, Bondy CA. Growth hormone treatment and left ventricular dimensions in Turner syndrome. *J Pediatr* 2007; **150**(6):587-591.

100. Mavinkurve M, O'Gorman CS. Cardiometabolic and vascular risks in young and adolescent girls with Turner syndrome. *BBA Clin* 2015; **3**:304-309.

101. Mazzanti L, Cacciari E. Congenital heart disease in patients with Turner's syndrome. Italian Study Group for Turner Syndrome (ISGTS). *J Pediatr* 1998; **133**(5):688-692.

102. Meszaros I, Morocz J, Szlavi J, Schmidt J, Tornoci L, Nagy L, et al. Epidemiology and clinicopathology of aortic dissection. *Chest* 2000; **117**(5):1271-1278.

103. Michelena HI, Corte AD, Prakash SK, Milewicz DM, Evangelista A, Enriquez-Sarano M. Bicuspid aortic valve aortopathy in adults: Incidence, etiology, and clinical significance. *Int J Cardiol* 2015; **201**:400-407.

104. Moaref A, Khavanin M, Shekarforoush S. Aortic distensibility in bicuspid aortic valve patients with normal aortic diameter. *Ther Adv Cardiovasc Dis* 2014; **8**(4):128-132.

105. Moberg K, De Nobele S., Devos D, Goetghebeur E, Segers P, Trachet B, et al. The Ghent Marfan Trial--a randomized, double-blind placebo controlled trial with losartan in Marfan patients treated with beta-blockers. *Int J Cardiol* 2012; **157**(3):354-358.

106. Mortensen KH, Andersen NH, Gravholt CH. Cardiovascular Phenotype in Turner Syndrome--Integrating Cardiology, Genetics, and Endocrinology. *Endocr Rev* 2012; **33**:677-714.

107. Mortensen KH, Erlandsen M, Andersen NH, Gravholt CH. Prediction of aortic dilation in Turner syndrome - enhancing the use of serial cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2013; **15**(1):47-58.

108. Mortensen KH, Hansen KW, Erlandsen M, Christiansen JS, Gravholt CH. Ambulatory arterial stiffness index in Turner syndrome: the impact of sex hormone replacement therapy. *Horm Res* 2009; **72**(3):184-189.

109. Mortensen KH, Hjerrild BE, Andersen NH, Sorensen KE, Horlyck A, Pedersen EM, et al. Abnormalities of the major intrathoracic arteries in Turner syndrome as revealed by magnetic resonance imaging. *Cardiol Young* 2010; **20**(2):1-10.

110. Mortensen KH, Hjerrild BE, Stochholm K, Andersen NH, Sorensen KE, Lundorf E, et al. Dilation of the ascending aorta in Turner syndrome - a prospective cardiovascular magnetic resonance study. *J Cardiovasc Magn Reson* 2011; **13**:24.

111. Muscat P, Lidov M, Nahar T, Tuhrim S, Weinberger J. Vertebral artery dissection in Turner's syndrome: diagnosis by magnetic resonance imaging. *J Neuroimaging* 2001; **11**(1):50-54.

112. Myers PO, Fasel JH, Kalangos A, Gailloud P. Arteria lusoria: developmental anatomy, clinical, radiological and surgical aspects. *Ann Cardiol Angeiol (Paris)* 2010; **59**(3):147-154.

113. Napolitano C, Novelli V, Francis MD, Priori SG. Genetic modulators of the phenotype in the long QT syndrome: state of the art and clinical impact. *Curr Opin Genet Dev* 2015; **33:17-24.**:17-24.

114. Nathwani NC, Unwin R, Brook CG, Hindmarsh PC. Blood pressure and Turner syndrome. *Clin Endocrinol (Oxf)* 2000; **52**(3):363-370.

115. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004; **114**(2 Suppl 4th Report):555-576.

116. Nielsen J, Wohlert M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Hum Genet* 1991; **87**(1):81-83.

117. Nienaber CA, Eagle KA. Aortic dissection: new frontiers in diagnosis and management: Part I: from etiology to diagnostic strategies. *Circulation* 2003; **108**(5):628-635.

118. Nistri S, Grande-Allen J, Noale M, Basso C, Siviero P, Maggi S, et al. Aortic elasticity and size in bicuspid aortic valve syndrome. *Eur Heart J* 2008; **29**(4):472-479.

119. Nistri S, Sorbo MD, Marin M, Palisi M, Scognamiglio R, Thiene G. Aortic root dilatation in young men with normally functioning bicuspid aortic valves. *Heart* 1999; **82**(1):19-22.

120. O'Gorman CS, Syme C, Lang J, Bradley TJ, Wells GD, Hamilton JK. An evaluation of early cardiometabolic risk factors in children and adolescents with Turner syndrome. *Clin Endocrinol (Oxf)* 2013; **78**(6):907-913.

121. O'Rourke M. Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. *Hypertension* 1990; **15**(4):339-347.

122. O'rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens* 2002; **15**(5):426-444.

123. Okuno S, Nishi N, Sakaki T. Putaminal hemorrhage in a case of Turner's syndrome with hyperaldosteronemia. *No Shinkei Geka* 2005; **33**(2):171-176.

124. Olivieri LJ, Baba RY, Arai AE, Bandettini WP, Rosing DR, Bakalov V, et al. Spectrum of aortic valve abnormalities associated with aortic dilation across age groups in Turner syndrome. *Circ Cardiovasc Imaging* 2013; **6**(6):1018-1023.

125. Ostberg JE, Brookes JA, McCarthy C, Halcox J, Conway GS. A comparison of echocardiography and magnetic resonance imaging in cardiovascular screening of adults with Turner syndrome. *J Clin Endocrinol Metab* 2004; **89**(12):5966-5971.

126. Ostberg JE, Donald AE, Halcox JP, Storry C, McCarthy C, Conway GS. Vasculopathy in Turner syndrome: arterial dilatation and intimal thickening without endothelial dysfunction. *J Clin Endocrinol Metab* 2005; **90**(9):5161-5166.

127. Ostberg JE, Storry C, Donald AE, Attar MJ, Halcox JP, Conway GS. A dose-response study of hormone replacement in young hypogonadal women: effects on intima media thickness and metabolism. *Clin Endocrinol (Oxf)* 2007; **66**(4):557-564.

128. Ostberg JE, Thomas EL, Hamilton G, Attar MJ, Bell JD, Conway GS. Excess visceral and hepatic adipose tissue in Turner syndrome determined by magnetic resonance imaging: estrogen deficiency associated with hepatic adipose content. *J Clin Endocrinol Metab* 2005; **90**(5):2631-2635.

129. Ou P, Bonnet D, Auriacombe L, Pedroni E, Balleux F, Sidi D, et al. Late systemic hypertension and aortic arch geometry after successful repair of coarctation of the aorta. *Eur Heart J* 2004; **25**(20):1853-1859.

130. Ou P, Celermajer DS, Raisky O, Jolivet O, Buyens F, Herment A, et al. Angular (Gothic) aortic arch leads to enhanced systolic wave reflection, central aortic stiffness, and increased left ventricular mass late after aortic coarctation repair: evaluation with magnetic resonance flow mapping. *J Thorac Cardiovasc Surg* 2008; **135**(1):62-68.

131. Ou P, Mousseaux E, Celermajer DS, Pedroni E, Vouhe P, Sidi D, et al. Aortic arch shape deformation after coarctation surgery: effect on blood pressure response. *J Thorac Cardiovasc Surg* 2006; **132**(5):1105-1111.

132. Oulego-Erroz I, onso-Quintela P, Mora-Matilla M, Gautreaux MS, Lapena-Lopez de AS. Ascending aorta elasticity in children with isolated bicuspid aortic valve. *Int J Cardiol* 2013; **168**(2):1143-1146.

133. Pinsker JE. Clinical review: Turner syndrome: updating the paradigm of clinical care. *J Clin Endocrinol Metab* 2012; **97**(6):E994-1003.

134. Poprawski K, Michalski M, Lawniczak M, Lacka K. Cardiovascular abnormalities in patients with Turner syndrome according to karyotype: own experience and literature review. *Pol Arch Med Wewn* 2009; **119**(7-8):453-460.

135. Prandstraller D, Mazzanti L, Giardini A, Lovato L, Tamburrino F, Scarano E, et al. Correlations of phenotype and genotype in relation to morphologic remodelling of the aortic root in patients with Turner's syndrome. *Cardiol Young* 2009; **19**(3):264-271.

136. Prandstraller D, Mazzanti L, Picchio FM, Magnani C, Bergamaschi R, Perri A, et al. Turner's syndrome: cardiologic profile according to the different chromosomal patterns and long-term clinical follow-Up of 136 nonpreselected patients. *Pediatr Cardiol* 1999; **20**(2):108-112.

137. Quezada E, Lapidus J, Shaughnessy R, Chen Z, Silberbach M. Aortic dimensions in Turner syndrome. *Am J Med Genet A* 2015; **167(A)**(11):2527-2532.

138. Radetti G, Crepaz R, Milanesi O, Paganini C, Cesaro A, Rigon F, et al. Cardiac performance in Turner's syndrome patients on growth hormone therapy. *Horm Res* 2001; **55**(5):240-244.

139. Ross JL, Feuillan P, Long LM, Kowal K, Kushner H, Cutler GB, Jr. Lipid abnormalities in Turner syndrome. *J Pediatr* 1995; **126**(2):242-245.

140. Sachdev V, Matura LA, Sidenko S, Ho VB, Arai AE, Rosing DR, et al. Aortic valve disease in Turner syndrome. *J Am Coll Cardiol* 2008; **51**(19):1904-1909.

141. Schoemaker MJ, Swerdlow AJ, Higgins CD, Wright AF, Jacobs PA. Mortality in women with turner syndrome in Great Britain: a national cohort study. *J Clin Endocrinol Metab* 2008; **93**(12):4735-4742.

142. Schwartz PJ, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Bosi G, et al. Prevalence of the congenital long-QT syndrome. *Circulation* 2009; **120**(18):1761-1767.

143. Sharma J, Friedman D, Dave-Sharma S, Harbison M. Aortic distensibility and dilation in Turner's syndrome. *Cardiol Young* 2009; **19**(6):568-572.

144. Sheikh AS, Ali K, Mazhar S. Acute aortic syndrome. *Circulation* 2013; **128**(10):1122-1127.

145. Sluysmans T, Colan SD. Theoretical and empirical derivation of cardiovascular allometric relationships in children. *J Appl Physiol* 2005; **99**(2):445-457.

146. Snellen HA, van Ingen HC, Hoefsmit EC. Patterns of anomalous pulmonary venous drainage. *Circulation* 1968; **38**(1):45-63.

147. Surerus E, Huggon IC, Allan LD. Turner's syndrome in fetal life. *Ultrasound Obstet Gynecol* 2003; **22**(3):264-267.

148. Sybert VP. Cardiovascular malformations and complications in Turner syndrome. *Pediatrics* 1998; **101**(1):E11.

149. Sybert VP, McCauley E. Turner's syndrome. *N Engl J Med* 2004; **351**(12):1227-1238.

150. Tak T, Crouch E, Drake GB. Persistent left superior vena cava: incidence, significance and clinical correlates. *Int J Cardiol* 2002; **82**(1):91-93.

151. Telvi L, Lebbar A, Del Pino O, Barbet JP, Chaussain JL. 45,X/46,XY mosaicism: report of 27 cases. *Pediatrics* 1999; **104**(2 Pt 1):304-308.

152. Terasaki T, Hashimoto Y, Miyashita F, Kozaki Y, Naganuma M, Uchino M, et al. A case of Turner syndrome complicated with brain infarction. *Rinsho Shinkeigaku* 2002; **42**(2):145-148.

153. Trolle C, Hjerrild B, Cleemann L, Mortensen KH, Gravholt CH. Sex hormone replacement in Turner syndrome. *Endocrine* 2012; **41**(2):200-219.

154. Turner HH. A Syndrome of infantilism, congenital webbed neck, and cubitus valgus. *Endocrinology* 1938; **23**:566-574.

155. Turtle EJ, Sule AA, Bath LE, Denvir M, Gebbie A, Mirsadraee S, et al. Assessing and addressing cardiovascular risk in adults with Turner syndrome. *Clin Endocrinol (Oxf)* 2013; **78**(5):639-645.

156. Turtle EJ, Sule AA, Webb DJ, Bath LE. Aortic dissection in children and adolescents with Turner syndrome: risk factors and management recommendations. *Arch Dis Child* 2015; **100**(7):662-666.

157. van den Berg J, Bannink EM, Wielopolski PA, Hop WC, van Osch-Gevers L, Pattynama PM, et al. Cardiac status after childhood growth hormone treatment of Turner syndrome. *J Clin Endocrinol Metab* 2008; **93**(7):2553-2558.

158. van den Berg J, Bannink E, Wielopolski P, Pattynama PM, de Muinck Keizer-Schrama SM, Helbing W. Aortic distensibility and dimensions and the effects of growth hormone treatment in the turner syndrome. *Am J Cardiol* 2006; **97**(11):1644-1649.

159. van Egmond H., Orye E, Praet M, Coppens M, De vloo-Blancquaert A. Hypoplastic left heart syndrome and 45X karyotype. *Br Heart J* 1988; **60**(1):69-71.

160. Virdis R, Cantu MC, Ghizzoni L, Ammenti A, Nori G, Volta C, et al. Blood pressure behaviour and control in Turner syndrome. *Clin Exp Hypertens A* 1986; **8**(4-5):787-791.

161. Voges I, Jerosch-Herold M, Hedderich J, Pardun E, Hart C, Gabbert DD, et al. Normal values of aortic dimensions, distensibility, and pulse wave velocity in children and young adults: a cross-sectional study. *J Cardiovasc Magn Reson* 2012; **14**:77-14.

162. Vogt M, Kuhn A, Baumgartner D, Baumgartner C, Busch R, Kostolny M, et al. Impaired elastic properties of the ascending aorta in newborns before and early after successful coarctation repair: proof of a systemic vascular disease of the prestenotic arteries? *Circulation* 2005; **111**(24):3269-3273.

163. Westwood M, Tajbakhsh SH, Siddals KW, Whatmore AJ, Clayton PE. Reduced pericellular sensitivity to IGF-I in fibroblasts from girls with Turner syndrome: a mechanism to impair clinical responses to GH. *Pediatr Res* 2011; **70**(1):25-30.

164. Wu MH, Chen HC, Kao FY, Huang SK. Risk of Systemic Hypertension and Cerebrovascular Accident in Patients With Aortic Coarctation Aged <60 Years (from a National Database Study). *Am J Cardiol* 2015; **116**(5):779-784.

165. Wuhl E, Witte K, Soergel M, Mehls O, Schaefer F. Distribution of 24-h ambulatory blood pressure in children: normalized reference values and role of body dimensions. *J Hypertens* 2002; **20**(10):1995-2007.

166. Yener N, Oktar GL, Erer D, Yardimci MM, Yener A. Bicuspid aortic valve. *Ann Thorac Cardiovasc Surg* 2002; **8**(5):264-267.

167. Zanjani KS, Thanopoulos BD, Peirone A, Alday L, Giannakoulas G. Usefulness of stenting in aortic coarctation in patients with the Turner syndrome. *Am J Cardiol* 2010; **106**(9):1327-1331.

168. Zhou J, Arepalli S, Cheng CM, Bakalov VK, Bondy CA. Perturbation of the transforming growth factor beta system in Turner syndrome. *Beijing Da Xue Xue Bao* 2012; **44**(5):720-724.

169. Zuckerman-Levin N, Zinder O, Greenberg A, Levin M, Jacob G, Hochberg Z. Physiological and catecholamine response to sympathetic stimulation in Turner syndrome. *Clin Endocrinol (Oxf)* 2006; **64**(4):410-415.

CURRICULUM VITAE

Personal details

First name:	Katya Els Charlotte
Place of birth:	Duffel
Date of birth:	February 6, 1971
Marital state:	married (20/09/1997) to Koen Van Herck
Children:	Morgane (°29/08/2000) and Jules (°24/08/2003)

Education – Degrees

2004-2005	Post-graduate training Fetal Cardiology at the Brompton Hospital, London, UK
2002-2005	Post-graduate training Paediatric Cardiology at Ghent University Hospital, Ghent, Belgium (AEPC certificate May 2005)
1998-2002	Post-graduate training Paediatrics at the University of Antwerp (UA), Antwerp, Belgium (Diploma October 2002)
1996-1998	Clinical Research - University of Antwerp, dept. Of Paediatrics and Neonatology, Antwerp, Belgium
1989-1996	Graduated as Medical Doctor summa cum laude at the University of Antwerp (UIA), Antwerp, Belgium (Diploma June 1996)
1984-1989	High School Heilige Familie Instituut, Berchem, Belgium (June 1989) Major: Latin, Greek and languages

Current Job position

2006-	Senior Consultant - Ghent University Hospital, Paediatric and Fetal Cardiology
2012-	Visiting Consultant - ZNA campus Middelheim, Antwerp, Fetal Cardiology
2006-	Visiting Consultant - Alma Ziekenhuis, Eeklo, Paediatric Cardiology
2006-	Visiting Consultant - Antwerp University Hospital, Paediatric and Fetal Cardiology

Other functions and memberships

2009-	Belgian National Foundation for Research in Pediatric Cardiology (representative of Parents' Organisations) - Member
2009-	Association for European Paediatric Cardiology (AEPC), working group "Psychosocial care in congenital heart disease from fetus to adult" - President
2005-	Association for European Paediatric Cardiology (AEPC) - Member
2005-	Belgian Association of Paediatrics (BVK) - Member
2005-	Kinderhartfonds vzw - Secretary

Prizes, Awards

June 2002	Pfizer Educational Grant, University of Antwerp (UIA), Antwerp, Belgium
15/03/2002	Nestlé-price for the poster "Retrospectieve analyse van cardiochirurgische interventies bij adolescenten in het UZ Gent". K De Groote, D De Wolf, D

	Matthys, H Verhaaren, B Suys, K François. 30th Annual Meeting of the Belgian Society for paediatrics (BVK), Terhulpen, Belgium
19/03/1999	Nestlé-price for the poster "Hemodynamic changes in different patterns of response to inhaled nitric oxide in newborns with persistent pulmonary hypertension." K. De Groote, B. Suys, B. Van Overmeire. 27th Annual Meeting of the Belgian Society for paediatrics (BVK), Terhulpen, Belgium
13/12/1997	ABOTT-price for the oral présentation: "La ventilation à haute fréquence est favorable pour la fonction diastolique du nouveau-né." K De Groote, B. Van Overmeire. Journées Francophones de Recherche en Néonatologie, Nantes, France
June 1996	2de Specia-price for medical student curriculum, University of Antwerp (UIA), Antwerp, Belgium

Publications in peer reveiwed journals

De Groote K, Devos D, Van Herck K, De Wolf D, Van der Straaten S, Rietzschel E, Raes A, Vandekerckhove K, Panzer J, De Wilde H, De Schepper J. Increased aortic stiffness in prepubertal girls with Turner syndrome. J Cardiol 2016 (in press).

François K, Vandekerckhove K, De Groote K, Panzer J, De Wolf D, De Wilde H, Bové T. Current outcomes of the bi-directional cavopulmonary anastomosis in single ventricle patients: analysis of risk factors for morbidity and mortality, and suitability for Fontan completion. Cardiol Young 2016; 26(2): 288-297.

De Groote K, Demulier L, De Backer J, De Wolf D, De Schepper J, De Backer T. Arterial hypertension in Turner syndrome, a review of literature and a practical approach for diagnosis and treatment. Journal of Hypertension 2015; 33(7): 1342-1351.

Campens L, Demulier L, De Groote K, Vandekerckhove K, De Wolf D, Roman MJ, Devereux RB, De Paepe A, De Backer J. Reference values for echocardiographic assessment of the diameter of the aortic root and ascending aorta spanning all age categories. Am J Cardiol 2014; 114 (6): 914-920.

Bove T, Vandekerckhove K, Devos D, Panzer J, De Groote K, De Wilde H, De Wolf D, De Backer J, Demulier L, François K. Functional analysis of the anatomical right ventricular components: should assessment of right ventricular function after repair of tetralogy of Falot be refined? Eur J Cardiothorac Surg 2014; 45(2): e6-12.

Francois,K, Creytens D, De Groote K, Panzer J, Vandekerckhove K, De Wolf D, Bove T. Analysis of the aortic root in patients with tetralogy of Fallot undergoing early repair: form follows function. J Thor Card Surg 2014;148(4):1555-1559.

De Groote K, Devos D, Van Herck K, Demulier L, Buysse W, De Schepper J, De Wolf D. Abnormal aortic arch morphology in Turner syndrome patients is a risk factor for hypertension. Heart Vessels 2014; 30(5)à: 618-625.

De Groote K, Cools M, De Schepper J, Craen M, François I, Devos D, Carbonez K, Eyskens B, De Wolf D. Cardiovascular pathology in males and females with 45,X/46,XY mosaicism. PLoS ONE 2013;8(2): e54977.

Bové T, François K, Van De Kerckhove K, Panzer J, De Groote K, De Wolf D, Van Nooten G. Assessment of a right-ventricular infundibulum-sparing approach in transatrial-transpulmonary repair of tetralogy of Fallot. European Jounal of Cardio-Thoracic Surgery 2011; 41(1): 126-133.

François K, Bové T, Panzer J, De Groote K, Vandekerckhove K, De Wilde H, De Wolf D. Univentricular heart and Fontan staging: analysis of factors impacting on body growth. European Journal of Cardio-Thoracic Surgery 2012;41(6):e139-e145.

Claerhout I, Buijsrogge M, Delbeke P, Walraedt S, De Schepper S, De Moerloose B, De Groote K, Decock C. The use of propranolol in the treatment of periocular infantile haemangiomas: a review. British Journal of Ophthalmology 2011;95(9):1199-1202.

De Wilde H, Zaqout M, De Groote K, De Wolf D. Cardiogenic shock after judo (case presentation). Acta Paediatrica 2010;99(6):799-800.

De Wilde H, Zaqout M, De Groote K, De Wolf D. Cardiogenic shock after judo (discussion and diagnosis). Acta Paediatrica 2010;99(6):949-951

François K, Zaqout M, Bové T, Vandekerckhove K, De Groote K, Panzer J, De Wilde H, De Wolf D. The fate of the aortic root after early repair of tetralogy of Fallot. European Jounal of Cardio-Thoracic Surgery 2010;37(6):1254-1258.

Bové T, De Groote K, Panzer J, Vandekerckhove K, Suys B, De Wolf D, Van Nooten G. Pleural effusions, water balance mediators and the influence of lisinopril after completion Fontan procedures. European Jounal of Cardio-Thoracic Surgery 2009;36(1):57-62.

Bové T, De Meulder F, Vandenplas G, De Groote K, Panzer J, Suys B, DeWolf D, François K. Midterm assessment of the reconstructed arteries after the arterial switch operation. Annals of Thoracic Surgery 2008;85(3):823-830.

De Wolf D, Vanderbruggen K, Verbist A, Suys B, Verhaaren H, François K, Bove T, Panzer J, Decaluwe W, De Groote K, Matthys D. Percutaneous interventions for congenital aortic stenosis (review). Acta Cardiologica 2006;61(2):204-205.

Suys B, De Groote K, Decaluwe W, Panzer J, Francois K, Bové T, Matthys D, Verhaaren H, De Wolf D. Congenital left heart outflow abnormalities in the newborn (review). Acta Cardiologica 2006;61(2):210-211.

Bove T, François K, De Groote K, Suys B, De Wolf D, Van Nooten G. Closure of artrial septal defects: is there still a place for surgery? Acta Chirurgica Belgica 2005;105:497-503.

Francois K, Tamim M, Bove T, De Groote K, De Wolf D, Matthys D, Suys B, Verhaaren H, Van Nooten G. Is morbidity influenced by staging in the fontan palliation? A single center review.

Pediatric Cardiology 2005;26(4):350-355.

De Groote K, Verhaaren H, Suys B, De Wolf D. Percutanous closure by a vascular plug of a fistula between the superior caval vein and the left atrium. Pediatric Cardiology 2005;26(5):675-676.

Bove T, François K, De Groote K, Suys B, De Wolf D, Verhaaren H, Matthys D, Moerman A, Poelaert J, Vanhaesebroeck P, Van Nooten G. Outcome analysis of major cardiac operations in low birth weight neonates. Annals of Thoracic Surgery 2004;78:181-187.

Van Overmeire B, Allegaert K, Casaer A, Debauche C, Decaluwé W, Jespers A, Weyler J, Harrewijn I, Langhendries JP, and the Multicentre Ibuprofen Prophylaxis Study (MIPS) Investigators. Prophylactic ibuprofen in premature infants: a multicentre,randomised, double-blind, placebo-controlled trial. Lancet 2004; 364: 1945-1949.

De Groote K, Suys B, Deleeck A, De Wolf D, Matthys D, Van Overmeire B. How accurately can QT interval be measured in newborn infants? European Journal of Pediatrics 2003;162:875-879.

Van Hal G, De Groote K, Van Damme P, Pepermans K, Van Cauwenberghe K, Meheus A. Pros and cons of an illicit drug users' registration system by means of judicial data in Flanders (Belgium). Public Health 2001;115(1):70-77

Van Overmeire B, Smets K, Lecoutere D, Van De Broek H, Weyler J, De Groote K, Van Langhendries JP. A comparison of Ibuprofen and Indomethacine for closure of patent ductus arteriosus. New England Journal of Medicine 2000;343:674-681.

Franco-Belgium Collaborative NO Trial Early compared with delayed inhaled nitric oxide in moderately hypoxaemic neonates with respiratory failure: a randomised controlled trial. The Franco-Belgium Collaborative NO Trial Group. Lancet 1999;354(9184):1066-1

Van Damme K, Cramm M, Thoelen S, De Groote K, Meheus A. Inactivated Hepatitis A vaccine: Reactogenicity, immunogenicity and long-term antibody persistence. Journal of Medical Virology 1994;44:446-451.

Other publications (proceedings, non peer-reviewed journals, scientific reports)

De Groote K. Het slechtnieuwsgesprek: impact op de patiënten en de zorgverlener. Tijdschr voor Geneeskunde 2014; 70(21): 1236-1239.

De Groote K. Cardiovasculaire afwijkingen bij het syndroom van Turner en Noonan. Percentiel 2011;16(3):83-85.

De Groote K, De Backer J. Cardiovasculaire opvolging bij het syndroom van Turner. Tijdschrift voor geneeskunde 2011;67(11):550-553

De Groote K, Verhaaren H. Foetale echocardiografie anno 2005. Percentiel 2005;10(2).

François K, Bove T, De Groote K, De Wolf D, et al.. De chirurgische behandeling van de tetralogie van Fallot. Tijdschrift voor geneeskunde 2004; 60(21):1523-1529.

De Wolf D, Suys B, François K, Bove T, De Groote K, Verhaaren H, Taeymans Y, Matthys. Therapeutische hartkatheterisatie voor aangeboren hartafwijkingen. Bloedvaten-hart-longen 2003;8:241-245.

De Wolf D, Suys B, François K, Bove T, De Groote K, H Verhaaren, Taeymans Y, Mattys D. Therapeutische hartkatheterisatie voor aangeboren hartafwijkingen. Percentiel 2003;8:107-111.

De Wolf D, Suys B, François K, De Groote K, Verhaaren H, Taeymans Y, Mattys D. Evolutie van de indicaties voor hartkatheterisatie bij kinderen. Tijdschrift voor Geneeskunde 2002;58(20):1341-1349.

Matthys K, Vanhercke D, Van Aken S, De Groote K, Coomans I, Verdonck P. Non-invasive assessment of hemodynamics in adolescents with arterial tonometry and Doppler ultrasound during a conventional stress test. Computers in Cardiology 2002;29:517-520

De Groote K, Deleeck A, Suys B, Van Overmeire B. How accurate can we measure QTc interval in the newborn infant? Tijdschrift voor kindergeneeskunde 1999;suppl. 1:39.

De Groote K, Van Overmeire B. Inhalatie van stikstofmonoxide (iNO) als behandeling van pulmonale hypertensie van de pasgeborene - een overzicht. Tijdschrift voor Geneeskunde 1998;54(12):817-824.

De Groote K, Van Damme P, Deprettere A, Michielsen P. Prevention of vertical transmission of hepatitis B virus infection, is there a standard policy in Flanders (Belgium)? Acta Gastroenterologica Belgica 1997;60(4):255-258

Van Damme P, De Groote K, Deprettere A, Michielsen P. Hepatitis B in België: epidemiologie en vaccinatiebeleid. Tijdschrift voor Geneeskunde 1997;53:1135-1145.

De Groote K, Van Hal G, Van Damme P, Van Cauwenberghe K. Registratie en retrospectief onderzoek van druggebruikers in 12 parketten in Vlaanderen en Brussel, 1996. Welzijnsgids, noden en verslaving 1997;23:17-40.

De Groote K, Van Damme P, Deprettere A. Hepatitis B - neonati van HBsAg-positieve moeders. VAXinfo 1996;16:5-7.

De Groote K, Van Hal G, Van Damme P, Van Cauwenberghe K. Zesde drugsregistratie via de parketten van Vlaanderen en Brussel, 1996. In: (Bianca Booms; Jean Paul Wydoodt, eds.) Alcohol, illegale drugs en medicatie - VAD 1996;pp. 58-62.

De Groote K. Verticale transmissie van hepatitis B in Vlaanderen. Eindwerk voor het behalen van het diploma van Arts (Graduate Thesis - June 1996). University of Antwerp, Antwerp, Belgium 1996;29 pp.

Van Damme P, Cramm M, Thoelen S, De Groote K, Vranckx R, Meheus A. Horizontal transmission of Hepatitis B virus. Proceedings Book of the International Symposium on Viral Hepatitis and Liver Disease, Tokyo, 10-14/05/1993. 1993;

Oral presentations as invited speeker

De Groote K. 50th Advantages and disadvantages of modern communication in the communication with patients. Annual meeting of the Association for European Paediatric and Congenital Cardiology (AEPC). in Rome, Italy. 3/06/2016.

De Groote K. Geavanceerde prenatale diagnostiek bij congenitale hartafwijkingen. Symposium Neonatologie of the Bruges, AZ Sint Jan. Bruges, Belgium. 22/01/2011.

De Groote K Life choices in adolescents with congenital heart disease. 44th Annual meeting of the Association for European Paediatric and Congenital Cardiology (AEPC). Annual meeting of the AEPC, Innsbruck, Austria. 26/05/2010.

De Groote K. Thrombo-embolic events post-Fontan. 38th Annual meeting of the Belgische Vereniging voor Kindergeneeskunde. Brussels, Belgium, 19/03/2010.

De Groote K. Paediatric cardiology beyond the heart: pathology of the aorta. Best in paediatrics of Novartis. Chantilly, France. 26/02/2010-27/02/2010.

De Groote K, De Backer J. Cardiovasculaire pathologie in Turner syndroom. Symposium naar aanleiding van de voorstelling van Turnerkliniek of the Turner Clinic Ghent University Hospital. Ghent, Belgium. 10/09/2009.

De Groote K Rechterkamer embryologie en foetale problemen. 2de Vlaams interuniversitair Symposium Congenitale Cardiology of the Belgische Vereniging voor Kindercardiologie. Leuven, Belgium. 7/06/2008.

De Groote K. When the news is bad: what about the feelings of the caregivers. Biannual meeting of the AEPC psychosoial working group from fetus to adult. Bruges, Belgium. 27/02/2008-29/02/2008.

Oral presentations at (inter)national conferences

De Groote K. Cardiovascular pathology in males and femals with 45,X/46,XY mosaicism. 4th International DSD Symposium of the I-DSD Registry. Glasgow, UK. 7/06/2013-9/06/2013.

De Groote K, De Wolf D, Vandekerckhove K, Panzer J, De Wilde H, Sluysmans T, Gewillig M, De Schepper J. Males with 45,X/46,XY have similar cardiovascular problems as females with Turner syndrome. 40th Annual meeting of of the Belgische Vereniging voor Kindergeneeskunde. Brussel, Belgium. 23/03/2013-24/03/2013.

De Groote K, Panzer J, Suys B, Verhaaren H, Matthys D, François K, Bove T, De Wolf D. The personalised patient passport improves communication with patients and staff. Biannual meeting of the AEPC psychosoial working group from fetus to adult. Belfast, Ireland. 5/03/2006 -7/03/2006

De Groote K, Suys B, Verhaaren H, Matthys D, François K, De Wolf D. Complication after Ross procedure for congenital aortic valvar stenosis. 6th EuroEcho meeting of the European Association of Cardiovascular Imaging (EACVI). Munich, Germany. 4/12/2002-7/12/2002.

De Groote K, Van Overmeire B. The impact of high frequency oscillatory ventilation versus

conventional ventilation on the heart function of the preterm infant during the first days of life. 2nd European Conference on high frequency ventilation. Ovifat,Belgium. 20/10/1998.

De Groote K, Van Overmeire B. La ventilation à haute fréquence est favorable pour la fonction diastolique du nouveau-né. Journées Francophones de Recherche en Néonatologie. Nantes, France. 12/12/1997-14/12/1997.

De Groote K, Van Overmeire B. HFOV is gunstiger voor de diastolische hartfunctie van de premature pasgeborene. Negentiende Congres Kindergeneeskunde of the NVK. Veldhoven, The Netherlands. 5/11/1997.

De Groote K, Deleeck A, Suys B, Van Overmeire B. How accurate can we measure QTc interval in the new-born infant. 21e Congres Kindergeneeskunde of the NVK. Veldhoven, The Netherlands. 5/11/1999.

DANKWOORD

De titel van deze PhD thesis bevat niet toevallig het woord “queste”. Het verwoordt exact wat dit onderzoeksproject voor mij betekend heeft: een queeste, een zoektocht in al zijn letterlijke en figuurlijke betekenissen.
Het is een zoektocht geweest naar wetenschappelijke kennis in de hoop daarmee een bijdrage te leveren aan een betere zorg voor onze patiënten. Een streven ook naar persoonlijk erkenning in de academische wereld en in het eigen ziekenhuis. Tijdens deze zoektocht naar waarheid en kennis is er één persoon die ik ongevraagd steeds weer opnieuw tegenkwam: mezelf. Soms was het een verrassend aangename ontmoeting waar kracht uit werd geput, andere confrontaties waren minder luisterrijk. Een persoonlijkheidstest en een karaktertraining is het zeker geweest…

Het was een lange tocht, die bijna 8 jaar heeft geduurd. Net zoals bij elke queeste was ook mijn traject onvoorspelbaar en grillig. Ik trotseerde hoge bergen en diepe donkere dalen. Er zijn zijpaden geweest waarop ik onverrichter zake moest omkeren en die veel kostbare tijd en energie hebben gekost. Hier en daar waren er verraderlijke putten waar de tocht vertraagde; er was veel creativiteit en volharding nodig om deze obstakels te overwinnen. Gelukkig waren er ook mooie groene vlaktes waar de tocht lekker opschoot en waar mooie resultaten werden geboekt.

Een queeste zou geen queeste zijn als ze niet gepaard ging met wonderbaarlijke ontmoetingen. Ik ben op mijn tocht ontelbare mensen tegengekomen die kortdurend mijn pad hebben gekruist. Anderen hebben grote stukken van de weg met me meegelopen en enkelen hebben zelfs het ganse traject samen met mij afgelegd. Alhoewel ik niet iedereen met naam kan noemen, ben ik al die mensen oneindig dankbaar.

Toch wil ik enkele personen specifiek bedanken. Mijn promotor, prof. dr. Daniël De Wolf en mijn tweede promotor prof.dr. Jean De Schepper hebben de aanzet gegeven voor dit grote avontuur. Ze hebben me hun vertrouwen geschonken om zelf mijn pad te banen en de queeste te voltooien op de manier die ikzelf goed achtte. Bedankt voor deze mogelijkheid, Daniël en Jean. Ik ben blij dat ik het einde van dit avontuur zonder al te veel kleerscheuren heb gehaald. Dank ook, Daniël, voor het vertrouwen dat u al jaren in me stelt. U hebt ons kindercardiologische team uitgebouwd tot een stevige kern waarin ik op mijn eigen manier mijn eigen plaats mag innemen. Bedankt voor dat vertrouwen!
Ook mijn oprechte dank aan de leden van de examenjury voor hun eerlijke mening en opbouwende kritiek bij het lezen van “mijn verhaal”: prof François, prof. Callewaert, prof. Deneyer, prof. Gheeraert, prof. Matthys, prof. Suys en prof. T’Sjoen. Met de hulp van jullie suggesties is het eindresultaat geworden wat het nu is: een thesis waar ik heimelijk toch een beetje trots op ben. Ook prof Vandewalle, voorzitter van de examcommissie, ben ik grote dank verschuldigd voor zijn niet aflatende aanmoedigingen en tips bij praktische PhD-vragen. Van harte dank daarvoor, Johan!
Een hele speciale en hele oprechte “dank u wel” wil zeggen aan prof. Dirk Matthys. U gaf me bijna 15 jaar geleden de mogelijkheid om vanuit Antwerpen naar Gent te komen en om me te specialiseren in de mooiste subdiscipline die er bestaat: de kindercardiologie. Met uw hulp ben ik ondertussen ook een vast lid van de grote en mooie familie die “pediatrie” heet. U bent ook diegene die me 2 jaar geleden aanmaande om er wat vaart achter te zetten. U wilde me graag zien promoveren voor uw emeritaat. Het is me net gelukt… Dank ook aan U en Dora voor de gastvrijheid

die u me verleende aan het begin van mijn carrière als supervisor. Er zijn niet veel mensen die kunnen zeggen dat ze bij hun grote baas mochten logeren tijdens hun wachtdienst.

De aandachtige lezer heeft vermoedelijk opgemerkt dat er nog enkele andere namen herhaaldelijk opduiken in dit verhaal.
Dr. Dan De Vos, zonder jouw gedrevenheid en medewerking was deze PhD-thesis niet mogelijk geweest. Alle MRI beelden die ik gebruikte zijn van jouw hand. Bedankt voor de vele mooie illustraties die je me bezorgde en voor de interessante discussies die we voerden bij de interpretatie van de gegevens.
Dr. Laurent Demulier, “Turner-dokter” op de volwassen cardiologie”. Bedankt voor je kritische opmerkingen die de vele overlegmomenten tot een grote meerwaarde maakten. Met jouw empathisch vermogen zijn onze volwassen vrouwen met Turner syndroom in de beste handen. Dank ook aan prof. dr. Julie De Backer en prof. dr. Tine De Backer, twee vrouwen die het gemaakt hebben in de onderzoekswereld en waar ik veel bewondering voor heb. Ondanks jullie zeer drukke bezigheden kon ik steeds een beroep op jullie expertise.
Prof. dr. Martine Cools, u wijdde me in in de mysterieuze wereld van de DSD. Uw inzichten waren onontbeerlijk in de interpretatie van de eerste studieresultaten.
Prof. dr. Rita Craen, uw enthousiasme overtuigde me om me te verdiepen in de cardiovasculaire problemen van Turner patiënten. U gaf me ook de suggestie om ook eens te kijken naar mannen met 45,X/46,XY. Het was het allereerste startschot voor het verdere PhD-onderzoek.
Een woord van dank ook aan alle andere leden van de Turner Kliniek: er is in Gent, mede door jullie gedrevenheid, een uniek multidisciplinair team uitgebouwd. Vanuit heel België vinden patiënten de weg naar ons centrum en de populatie Turner patiënten die door ons wordt gevolgd is enorm toegenomen. Enkel op deze manier wordt klinisch onderzoek mogelijk. Dank dus, voor al jullie goede zorgen en extra inspanningen voor “onze” Turner patiënten. Hopelijk ziet ook onze directie de mogelijkheden die deze kliniek ons biedt en is ze bereid om haar financiële duit in het zakje te doen.

Klinisch onderzoek is enkel mogelijk met de onbaatzuchtige medewerking van patiënten en hun familie. Mijn oprechte dank aan allen die bereid waren om voor mijn onderzoek naar het UZ Gent te komen, zeer speciaal ook alle “controle meisjes en hun ouders” die voor mij een uurtje van hun vrije tijd opofferden. Dank aan alle collegae kinderendocrinologen die hun patiënten motiveerden om deel te nemen aan de studieprotocollen: prof. dr. Jean De Schepper, prof. dr. Rita Craen, prof. dr. Martine Cools, dr. Kathleen De Waele, dr. Sara Van Aken, dr. Saskia Van der Straaten voor het UZ Gent en dr. Inge François voor de KU Leuven.
Een bijzondere woord van dank ook aan de Stichting ter Bevordering van het Onderzoek in Kindercardiologie dat Belgisch multicenter onderzoek niet enkel aanmoedigt maar ook financieel ondersteunt. Dank aan prof dr. Marc Gewillig (KU Leuven) en prof. dr. Thierry Sluysmans (UCL) voor de inclusie van hun patiënten in de eerste studie.

En dan zijn er de vele supporters langs de weg. Zonder jullie aanmoedigingen, steun en praktische hulp was de eindmeet vermoedelijk nooit bereikt.

Eerst en vooral “mijn mannen”, zoals ik ze graag noem: dr. Kristof Vandekerckhove, dr. Seppie Panzer, dr. Hans De Wilde en dr. Mahmoud Zaqout. Jullie waren van zeer nabij getuige van mijn soms moeizame tocht. Ik kon met mijn frustraties steeds bij jullie terecht en in de laatste fase maakten jullie het mogelijk om me terug te trekken in mijn cocon om mijn wedervaren op een rustige manier uit te schrijven. Dikke, dikke merci! Ik hoop voor jullie binnenkort hetzelfde te kunnen doen.
Chris Leyman, onze vaste kindercardio-verpleegkundige. In mijn beginperiode was je vaak mijn rots in de cardiologiebranding en het aantal ECG’s en bloeddrukken die je voor mijn studies nam zijn ondertussen niet meer te tellen. Bedankt, ik zal je heel erg missen, volgend jaar…
En dan de vrouwen van de dienst: Ilse Coomans en Christelle Maes, vanaf het prille begin waren jullie “mijn zusters in het kindercardiologische mannen-bastiljon”. Steeds bereid tot een babbeltje, steeds bereid tot hulp! Mijn dank voor jullie steun op alle vlakken is niet in woorden uit te drukken. Bedankt ook aan alle andere secretaresses die de laatste jaren onze dienst kwamen versterken en die een onmisbare hulp zijn in onze dagelijkse werking.
En dan zijn er de ontelbare andere mensen die het dagelijkse werk in het UZ Gent aangenaam maken en die me aanmoedigden op momenten dat het pad onbegaanbaar leek. Ik kom op erg veel verschillende diensten en het zijn er dus veel te veel om persoonlijk op te noemen: mijn collegae kinderartsen, cardiologen, chirurgen, artsen en vroedvrouwen op de prenatale diagnostiek, psychologen, ontelbare verpleegkundigen en logistieke medewerkers, sociale assistenten, secretaresses, … Aan iedereen: bedankt voor wie jullie zijn en wat jullie dagelijks presteren!

En dan uiteindelijk het thuisfront, zij die het meest geleden hebben onder mijn lange mentale afwezigheid. Mama en papa, bedankt voor de fijne en warme thuis waarin ik mocht opgroeien. Bedankt voor het vertrouwen dat ik kreeg en alle mogelijkheden die jullie me geboden hebben. Bedankt ook mama, voor het mooie ontwerp dat de cover van dit PhD-werk tot een persoonlijk kunstwerk maakt. Dank ook aan mijn schoonouders, broer, schoonzussen en schoonbroers voor hun morele steun tijdens de lange tocht.

Morgane en Jules, mijn twee grootste schatten. Het is een lange tocht geweest, ook voor jullie. Bij de start van het verhaal Jules was nog een schattige kleuter en Morgane een vrolijk klein meisje in het tweede leerjaar. Onderweg zijn jullie uitgegroeid tot 2 grote knappe tieners. Jullie hebben de laatste 2 jaren niet zo veel aan jullie mama gehad. Het was ofwel werken in het ziekenhuis, ofwel werken thuis. De verloren tijd kan ik niet meer terugbrengen, maar ik beloof wel dat ik er vanaf nu vaker “echt zal zijn” voor jullie.

En dan, last but not least: Koen. Bij het dankwoord aan het einde van een verdediging wordt de echtgenoot traditioneel als laatste bedankt, meestal voor zijn inbreng in het huishouden en de zorg voor de kinderen. Dat ga ik nu niet doen … Jouw rol in dit werk was zoveel meer dan dat. Jij bent in dit verhaal mijn Gandalf: mijn persoonlijke huisprofessor, aanwezig van begin tot einde en nu en dan opduikend met een bijna magische oplossing. Je was bij elke fase van het onderzoek betrokken: bij het begin van een project als we samen de methodologie bespraken, bij de verwerking als de statistiek weer eens (te) moeilijk was, bij het nalezen van de artikels… Jouw expertise als onderzoeker heb ik steeds mogen (mis)bruiken. Ook in de eindfase was jouw inbreng onbetaalbaar bij de layout van het drukwerk, commentaar op de dia’s, kiezen van een outfit… En nu

is dit hoofdstuk van ons verhaal ten einde. Eindelijk! Ik zou je willen beloven dat er vanaf nu meer tijd vrijkomt voor ons twee, maar dat zal niet alleen aan mij liggen.
Bedankt, Koen. Zonder jou was dit niet verhaal nooit geschreven.