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**TWIN-TWIN TRANSFUSION  
SYNDROME (TTTS) –  
outcomes with special  
reference to cardiovascular  
function**

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To my Family



# ABSTRACT

## *Background*

Fetal environment has become a subject for increasing interest when studying health and disease in adults. Monozygous (MZ) twins, especially gestations complicated with twin-twin transfusion syndrome (TTTS), offer a unique opportunity to study adverse developmental programming of the cardiovascular system. TTTS affects about 10 % of pregnancies with a common placenta, because of unbalanced blood flow across deep arteriovenous connecting vessels. The divergent hemodynamic loads of the donor and the recipient fetus often result in myocardial hypertrophy of the recipient heart.

The aims of this thesis were to evaluate intrauterine environmental contributions to vascular functions in twins with discordant birth weight (Paper I), to study long term effects of TTTS on cardiac structure and function (Papers II and IV) and to determine infant survival and neonatal outcome after fetoscopic laser coagulation therapy of TTTS in Sweden (Paper III).

## *Methods and Results*

An observational study of 31 twin-pairs, mean age eight years, with discordant weight at birth, showed that systolic blood pressure (SBP) was higher and endothelial function lower in the smaller twin. In MZ twins with a history of TTTS (n= 9 pairs), there was no significant difference in SBP, but donor twins had narrower carotid arteries than recipient twins and carotid strain was higher (Paper I).

Echocardiography of eleven TTTS twin-pairs, mean age 9.6 years, prenatally treated with amnioreductions, showed no difference in cardiac structure, but recipients had significantly lower diastolic ventricular filling compared with donors (Paper II). When examining a laser treated cohort of 19 TTTS twin-pairs, mean age 4.5 years, and 19 age-matched singleton controls, we found signs of a minor decrease in early diastolic ventricular filling in recipients compared with donors, but no differences in heart function or structure compared with controls (Paper IV).

From a hospital-based register of the first Swedish cohort of laser treated TTTS pregnancies (n = 71), we found that overall survival from treatment to one year of age was 46 %, and that in 61 % of gestations, at least one twin survived infancy. Mean gestational age at birth was 30 weeks and mechanical ventilation was needed in 46 % of liveborn twins (Paper III).

## *Conclusions*

Exposure to fetal growth retardation may contribute to higher blood pressure, arterial narrowing and endothelial dysfunction in childhood (Paper I). Fetal and infant survival after fetoscopic laser coagulation of TTTS is still limited. If very preterm delivery is necessary, the neonatal team has to prepare for taking care of two high-risk neonates most likely requiring respiratory support (Paper III). Despite different and severe fetal cardiac loading conditions, our long-term follow-up studies of twins surviving TTTS showed an overall cardiac structure and function within normal range. The signs of reduced diastolic function found in the group treated with amnioreductions (Paper II) were less pronounced in the laser treated cohort (Paper IV).

These observations indicate that the cardiac morbidity caused by TTTS resolves in childhood. This has important implications as clinical decision making in TTTS frequently involves choosing between accepting increased fetal cardiac morbidity in the recipient twin and delivering two very preterm babies.

## LIST OF PUBLICATIONS

- I. Halvorsen CP, Andolf E, Hu J, Pilo C, Winbladh B, Norman M.  
Discordant twin growth in utero and differences in blood pressure and endothelial function at 8 years of age.  
*Journal of Internal Medicine* 2006 Feb;259(2):155-63.
- II. Halvorsen CP, Bilock SL, Pilo C, Sonesson SE, Norman M.  
Childhood cardiac function after twin-to-twin transfusion syndrome - a 10-year follow up.  
*Acta Paediatrica* 2009 Sep;98(9):1468-74.
- III. Halvorsen CP, Ek S, Dellgren A, Grunewald C, Kublickas M, Westgren M, Norman M.  
Survival and neonatal outcome after fetoscopic guided laser occlusion (FLOC) of twin-to-twin transfusion syndrome (TTTS) in Sweden.  
*Journal of Perinatal Medicine* 2012; 40(5): 533–538.
- IV. Halvorsen CP, Mohlkert LA, Norman M, Sonesson SE.  
Childhood cardiac outcome of twin-twin transfusion syndrome after intrauterine laser treatment: an echocardiographic study of ventricular function.  
*Submitted.*

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## LIST OF ABBREVIATIONS

AA	Abdominal aorta
AAA	Arterioarterial anastomosis
ACh	Acetylcholine
AGA	Appropriate-for-gestational age
AoV	Aortic valve
AV	atrioventricular
AVA	Arteriovenous anastomosis
A wave	Late (atrial) peak diastolic filling wave measured with Doppler flow
a'	Late (atrial) peak diastolic myocardial movement measured with DTI
BHC	Birth head circumference
BL	Birth length
BP	Blood pressure
BPD	Bronchopulmonary dysplasia
BSA	Body surface area
BW	Birth weight
CCA	Common carotid artery
CVD	Cardiovascular disease
DA	Diamniotic
DBP	Diastolic blood pressure
DC	Dichorionic
DOHaD	Developmental origins of health and disease
DTI	Doppler tissue imaging
DVP	Deepest vertical pocket
DZ	Dizygous
E wave	Early peak diastolic filling wave measured with Doppler flow
EDV	End diastolic volume
ESV	End systolic volume
ET	Ejection time
e'	Early peak diastolic myocardial movement measured with DTI
IMTc	Intima-media thickness of carotid artery
IVCT	Isovolumetric contraction time
IVH	Intraventricular hemorrhage
IVRT	Isovolumetric relaxation time
IVS	Interventricular septum
FLOC	Fetoscopic laser occlusive coagulation
GA	Gestational age
LV long SF	Longitudinal shortening fraction of left ventricle
LV maj	Left ventricular longitudinal end-diastolic diameter
LVED	Left ventricular end-diastolic diameter
LVOT	Left ventricular outflow tract
LVPW	Left ventricular posterior wall

MA	Monoamniotic
MAPSE	Mitral annular plane systolic excursion
MC	Monochorionic
MPI	Myocardial performance index
MV	Mitral valve
MZ	Monozygous
PDA	Patent ductus arteriosus
PPHN	Persistent pulmonary hypertension of the newborn
PU	Perfusion unit
PV	Pulmonary valve
RDS	Respiratory distress syndrome
ROP	Retinopathy of the premature
RVAW	Right ventricular anterior wall
RV long SF	Longitudinal shortening fraction of right ventricle
RV maj	Right ventricular longitudinal end-diastolic diameter
RVOT	Right ventricular outflow tract
RVOTO	Right ventricular outflow tract obstruction
SBP	Systolic blood pressure
SF	Shortening fraction
SGA	Small-for-gestational age
STE	Speckle tracking echocardiography
SV	Stroke volume
TAPS	Twin-anemia-polycythemia sequence
TAPSE	Tricuspid annular plane systolic excursion
TTTS	Twin-twin transfusion syndrome
TV	Tricuspid valve
Vmax	Peak velocity
VTI	Velocity time integral
VVA	Venovenous anastomosis
VVI	Velocity vector imaging

# 1 INTRODUCTION

Cardiovascular disease (CVD) is estimated to account for one third of the total mortality in the world. The World Health Organisation (WHO) recently developed a CVD action plan where objectives included interventions to reduce the burden of major CVD risk factors such as smoking, unhealthy diet, harmful use of alcohol and physical inactivity. However, risk factors for CVD originating in the perinatal period are less known to the public. Due to improvements of fetal and neonatal care, the group of neonates surviving a complicated perinatal period is increasing, and the gap in knowledge on adverse fetal environmental effects on later health and disease has emerged as an important research field.

Monozygous (MZ) twin gestations complicated with twin-twin transfusion syndrome (TTTS) or discordant fetal growth offer unique opportunities to study different fetal environmental effects on later development. TTTS affects about 10 % of monozygous (MC) pregnancies, with an overall incidence of less than 0.1 % [1].

In the 1990's, a cohort of twin pregnancies was prospectively collected at the maternal and obstetrical unit of Södersjukhuset in Stockholm. The TTTS pregnancies, a special clinical challenge, were at that time offered treatment with amnioreductions and indomethacin in order to reduce the risk for preterm delivery. Since there was a lack of data on TTTS long term outcome I was, as a neonatologist with a special interest in pediatric cardiology, asked to do a pediatric follow-up of this cohort. As the recommended fetal treatment gradually changed from amnioreductions to laser coagulation of placental anastomoses, there was a need for follow-up of this new fetal therapy as well.

## **2 BACKGROUND**

### **2.1 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE**

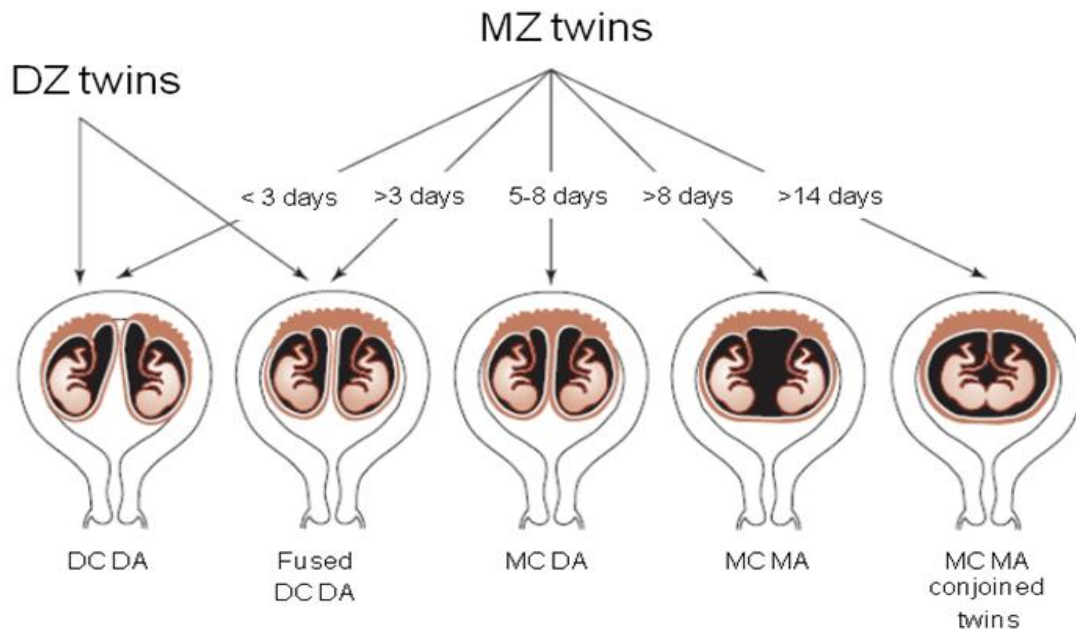
After decades of discussions about either genetic or environmental factors' influence on the development of the human phenotype, most researchers agree on the importance of both. The DOHaD (developmental origins of health and disease) concept focuses on how the environment during early human development interacts with the genome to influence health in later life. To be able to understand potential health consequences of fetal exposures to adverse intrauterine environment, many researchers have studied offspring of gestations with perinatal complications, e.g. growth disturbances, preterm birth and maternal disease during pregnancy. Scandinavian researchers followed by David Barker were among the first to present associations between low birth weight and increased risk for hypertension, ischemic heart disease and diabetes in adulthood [2, 3]. To disentangle environmental and genetic contributions to early development, multiple pregnancies – sharing genetic and early familial conditions – have been used as a model for research purposes.

### **2.2 MULTIPLE PREGNANCY**

The rate of multiple pregnancies varies around the world, representing about 1-3% of all pregnancies. In Sweden, the reported multiple birth rate has been 1.5 % during the past decade. Of these, triplets and quadruplets represent < 2 % [4].

#### **2.2.1 Zygoty- chorionicity**

Zygoty of a twin gestation depends on if the fetuses come from one or two fertilized eggs; one egg = monozygous (MZ), two eggs = dizygous (DZ). The DZ gestation is usually dichorionic (DC) – i.e. there are two placentas, although these can sometimes be fused and on fetal scans appear to be monochorionic (MC). The development of chorionicity in a MZ gestation is dependent of the time for cell division. An early division of the ovum (< 60 hours from conception) results in a DC pregnancy with different amniotic cavities, i.e. diamniotic (DA). When division occurs after 3-8 days, the pregnancy will become MC DA, whereas a late cell division (> 8 days) will generate MC twins in a common amniotic cavity, i.e. monoamniotic (MA). Very rarely, the fertilized cell divides after more than 14 days, and then the twins become conjoined or “siamese” [5], Figure 1.

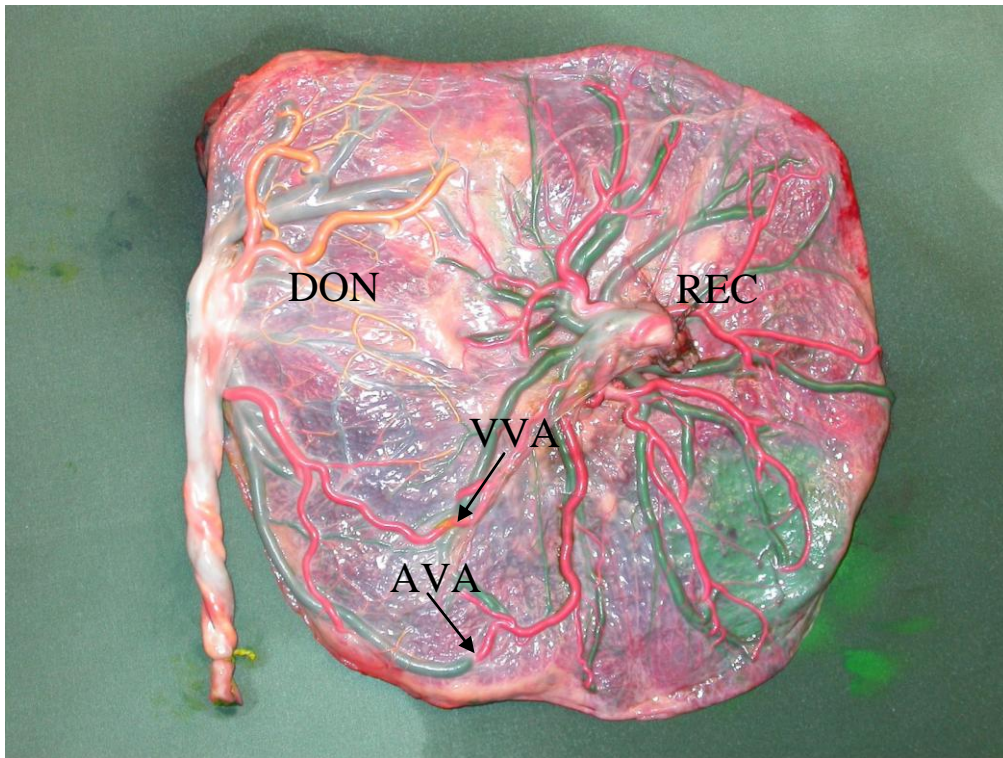


**Figure 1.** Chorionicity in relation to time from fertilization to cell division. Modified and published with permission [6].

When a twin pregnancy is discovered, determination of chorionicity is of major importance as MC placentation is considered a malformation and combined with a higher risk for complications than the DC. With ultrasound scanning, chorionicity is easiest determined in the first trimester, by searching for a lambda (DC) or a T-sign (MC) at the membrane-placenta interface [7]. MC occurs in 20 % of all twin pregnancies and in 70 % of MZ pregnancies [1]. Growth discordances, congenital malformations, acute and chronic transfusions are known complications of MC gestations [1].

### 2.3 TWIN-TWIN TRANSFUSION SYNDROME (TTTS)

Twin-twin transfusion syndrome (TTTS) is usually evident in the first or second trimester as a pronounced discordance of amniotic fluid volume [8]. TTTS is one of the most severe fetal complications carrying an incidence of about 10 % of all MC pregnancies and carrying a mortality of 80 % if untreated [8, 9]. In a normal MC gestation, there are communicating vessels (anastomoses) in the common placenta, linking circulations of the twin fetuses. Depending on the number and types of present anastomoses, the exchange of blood may be balanced or unbalanced. TTTS occurs when the net transfusion from one twin (the donor) exceeds the flow from the other twin (the recipient). The transfusions through deep arteriovenous anastomoses (AVA) to the recipient twin, are then insufficiently compensated by the flow through superficial venovenous (VVA) and arterioarterial (AAA) anastomoses [10], Figure 2. The AVA number has been suggested to be of inferior importance compared with the size of anastomoses [11]. TTTS usually presents during the first trimester. The earlier the presentation, the poorer the prognosis [12]. To evaluate the severity and eventual progress of TTTS, staging according to Quintero is well established, [13], Table 1.



**Figure 2.** TTTS placenta with insertions of donor (DON) and recipient (REC) umbilical cords and color dye injected arteriovenous (AVA) and venovenous (VVA) anastomoses. Note the larger vessels of the recipient placenta territory.

Table 1. Quintero staging system of TTTS based on ultrasonographic findings [13].

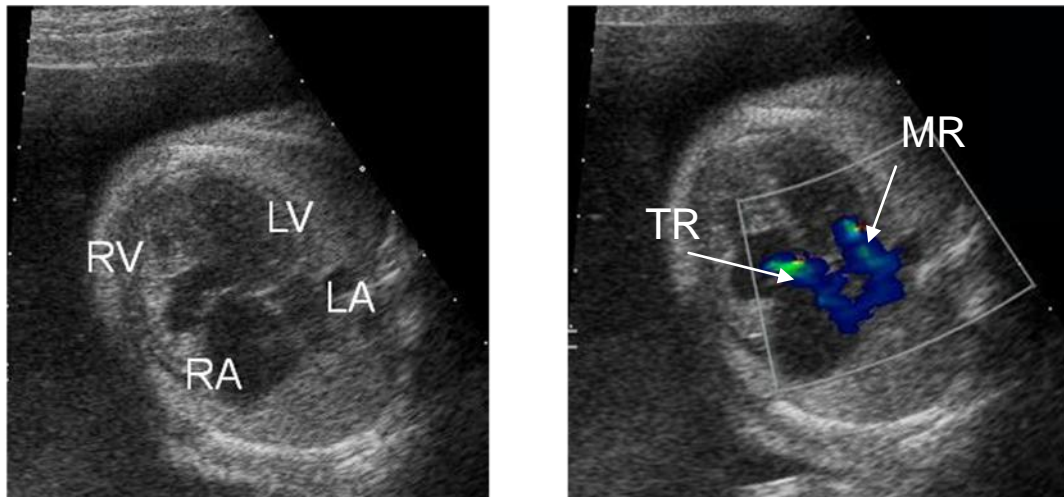
Quintero stages	Sonographic or Doppler findings
<b>I</b>	Polyhydramnios, $DVP \geq 8$ cm in recipient's sac and oligohydramnios, $DVP \leq 2$ cm in donor's sac with visible bladder in donor
<b>II</b>	As in I but no visible bladder in donor
<b>III</b>	As in II and abnormal Doppler flow of umbilical artery*, umbilical vein** or ductus venosus ***
<b>IV</b>	As in III and hydrops of either twin
<b>V</b>	Fetal demise of one or both twins

DVP= deepest vertical pocket. \*Absent or reversed end-diastolic velocity. \*\*Pulsatile flow. \*\*\*Reversed flow.

### 2.3.1 Clinical manifestations

As a result of hypovolemia, the donor is exposed to renal hypoperfusion, leading to oliguria and oligohydramnios. In its attempt to compensate for the volume depletion and hypotension, the donor responds with an increased production of renin [14]. This release results in an upregulation of angiotensin-II synthesis and an intrarenal vasoconstriction in the donor with an accentuation of the oligohydramnios. Meanwhile in the recipient, the hypervolemia results in atrial distension mediating a release of atrial natriuretic peptide, decreasing tubular reabsorption and thus worsening the

polyhydramnios. Paradoxically, the upregulated renin production of the donor transferred through the AVA results in fetal hypertension of the recipient despite its own downregulation [14-16]. As a consequence of the hypertensive disorder, the recipient gradually develops myocardial hypertrophy primarily of the right ventricle, followed by atrioventricular regurgitations and a decreased diastolic ventricular filling [17], Figure 3. Renin-angiotensin (RAS) activators have been found to be produced in the kidney of the donor and in the placental territory of the recipient (Ref Galea P, Placenta 2008).



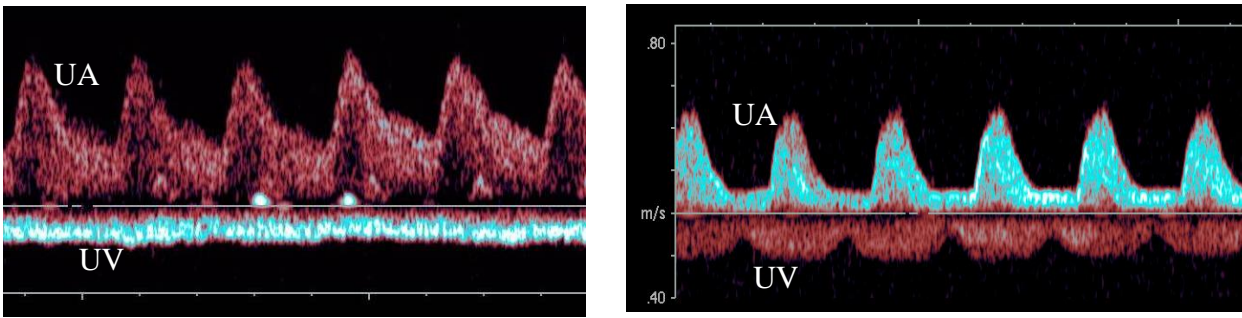
**Figure 3.** Heart of a recipient fetus with right ventricular (RV) hypertrophy and tricuspid and mitral valve regurgitations (TR, MR).

Endothelin-1, another potent vasoconstrictor, has been found to occur in twice as high serum concentrations in recipients compared with their donors, and with the highest levels observed in recipients with severe fetal hydrops [18]. All vasoactive mediators transported across the anastomoses may contribute to the development of hypertension and cardiomyopathy in the recipient fetus [19]. In other studies of TTTS gestations, elevated levels of brain natriuretic peptide and cardiac Troponin-T have been reported in recipients, suggesting a myocardial damage and remodeling with possible long term effects on cardiac function [20, 21]. However, for early diagnosis of TTTS in the first trimester, there has been no data supporting high levels of these substances.

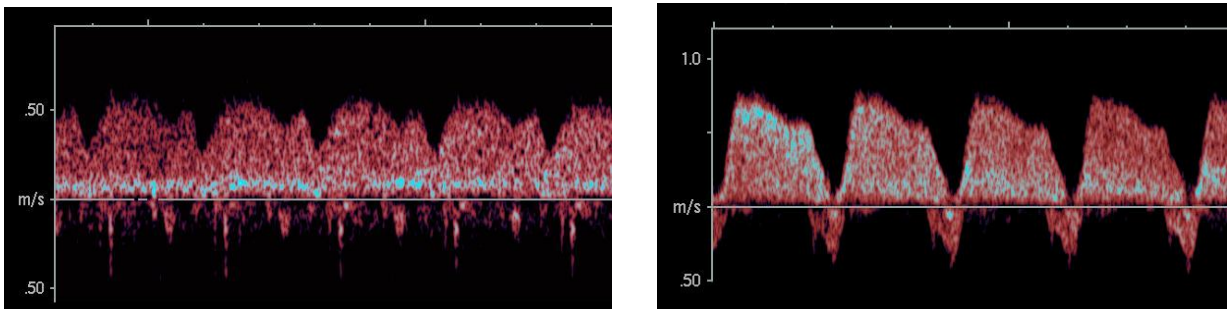
### 2.3.2 Diagnostics

#### *Fetal ultrasonography*

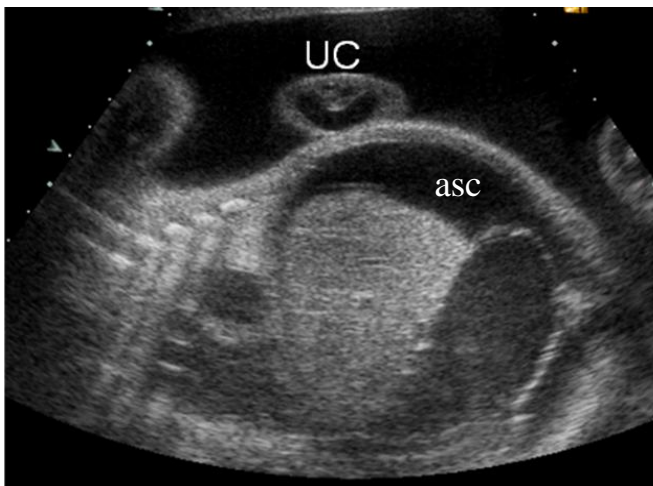
As the chronic unbalanced volume shift makes the donor hypovolemic, oliguric, hypotensive and subsequently growth retarded, the recipient becomes hypervolemic, polyuric and hypertensive. With an aggravating oliguria, the urinary bladder of the donor will be invisible. The hemodynamic alterations of the twin fetuses are reflected in Doppler flow abnormalities of umbilical vessels. The end-diastolic flow of the umbilical artery of the donor becomes absent or reversed whereas a pulsatile Doppler signal of the umbilical vein in addition with a reversed flow in ductus venosus can be seen in the recipient, Figures 4 and 5. In advanced stages of TTTS, the fetuses might develop ascites or hydrops as an indication of severe cardiovascular compromise, Figure 6.



**Figure 4.** Pulse wave Doppler in umbilical vessels of a normal fetus (left) and a recipient fetus with systemic hypertension (right). Umbilical artery (UA) and vein (UV).



**Figure 5.** Pulse wave Doppler in ductus venosus of a normal fetus (left) and of a recipient fetus with systemic hypertension (right).



**Figure 6.** A recipient fetus with ascites (asc). UC= umbilical cord.

For specific grading of cardiovascular severity, the CHOP scoring system has been suggested by Rychik et al [17]. The prognostic value of the scoring system has been questioned and considered too time consuming to be useful in a clinical setting [22]. As there is no non-invasive method to measure fetal systemic blood pressure, the ultrasonographer may identify indirect signs. Fetal hypertension is first observed as ventricular hypertrophy with atrioventricular regurgitation followed by a compromised



diastolic filling resulting in a prolongation of isovolumetric relaxation time (IVRT) and a monophasic atrioventricular inflow profile with short duration. Late signs of fetal hypertension includes systolic ventricular dysfunction [17, 21, 23, 24].

Quantifiable changes in left and right ventricular wall thickness of recipients as well as an increased myocardial performance index (MPI) indicating impaired global ventricular function has been demonstrated in early stages of TTTS [25, 26]. The right ventricle of the recipient is earlier and more severely affected than the left, and can occasionally also develop right ventricular outflow obstruction (RVOTO) and pulmonary valve stenosis due to impaired flow owing to hypertrophy [27-30]. In contrast, the hypovolemic donor twin shows little cardiac pathology on fetal echocardiography but does manifest a slightly hyperdynamic heart and central hemodynamics similar to that seen in other kinds of placental dysfunction.

While conventional Doppler assesses the velocity of blood flow by measuring high-frequency, low-amplitude signals from blood cells, Doppler tissue imaging (DTI) uses Doppler shifts of the myocardium to quantify the higher amplitude and lower velocity signals of tissue motion (displacement and velocity) [31]. However, the angle dependency of the DTI technique limits the usefulness. Since speckle tracking echocardiography, also named velocity vector imaging (VVI), is angle independent, it may be used as a supplement for assessment of systolic function [32]. Lately, several centers have reported VVI assessments of TTTS fetuses [33-35]. Recipient fetuses demonstrated early impairment of ventricular strain, whereas donors exhibited higher strain values indicating a hyperdynamic myocardium [34, 36, 37]. Ventricular strain measured with speckle tracking has so far not been studied in TTTS children after birth.

Despite a few reports of prognostic variables in TTTS, such as the presence of AAA [38, 39], at present there is no known fetal or maternal variable that identifies patients at risk for progression. Different European centers have established guidelines for surveillance and treatment of TTTS, but such guidelines are still lacking in Sweden.

Chronic TTTS differs from other forms of twin transfusions by the discordance of amniotic fluid between twins, Table 1. In the twin-anemia-polycythemia-sequence (TAPS), few and very small AVA at the chorionic plate results in a large inter-twin hemoglobin difference with one anemic and one polycythemic twin fetus. TAPS should be suspected if there are Doppler findings of abnormal and divergent peak systolic velocities of middle cerebral arteries in MC twins but no oligo/polyhydramnios [40, 41]. Due to the inter twin transfusions, there is a difference in reticulocyte count at birth. A third form, the acute twin-twin transfusion, occurs right before or at delivery and results in one anemic and one polycythemic twin, without discordant reticulocyte count.

### **2.3.3 Fetal intervention and treatment**

To reduce the risk for premature rupture of membranes and preterm labor due to the polyhydramnios distending the uterus, different treatment strategies have been developed. Selective feticide by umbilical cord occlusion (trying to save at least one

twin), septostomy, amnioreductions alone or in combination with indomethacin, have all been used to prevent adverse outcome [42-44]. Serial amnioreductions was most frequently used until the late 1980s, when De Lia pioneered the use of selective fetoscopic laser occlusive coagulation (FLOC) [45]. FLOC is executed under local anesthetics with an ultrasound guided fetoscope inserted into the amnion cavity of the recipient, whereas the vessels crossing the vascular equator are identified and coagulated by pulsed laser of 50-60 W effect. The procedure is terminated with amniodrainage [46, 47]. Complications reported with FLOC are e.g. placental abruption, rupture of membranes and fetal demise.

A meta-analysis of laser treatment of TTTS reported an 80 % chance of delivering at least one liveborn twin [48]. With a high incidence of neonatal deaths, primarily due to very preterm birth, reporting the rate of overall survival passed the neonatal period is recommended and more adequate than reporting liveborn rates. Currently, an overall survival rate of 50-70 % can be expected [49]. So far, there are only two randomized controlled trials (RCT) comparing serial amnioreductions with FLOC and both were terminated earlier than planned due to interim analyses showing significantly higher survival rate after FLOC. The overall survival after FLOC at 6 months of age was 56 % in the Eurofetus study [43] and 45 % at 30 days in the NICHD trial [50]. With current level I evidence for FLOC treatment vs. amnioreduction for TTTS  $\geq$  stage II, laser treatment of stage I is still questioned as there may be a chance of either spontaneous regress or a non-progress.

Centers of fetal medicine treating TTTS are still challenged to improve rates of fetal survival, but there are also reasons for the perinatal team to consider best ways to increase neonatal survival in donors and recipients. Outcome on survival, neonatal disease and neurodevelopment after establishing a new therapeutic method is important, and Swedish data have been missing.

#### **2.3.4 Blood pressure and vascular function after TTTS**

Based on fetal hypertension (recipient) and upregulation of renin synthesis (donor), BP monitoring has been suggested in TTTS neonates. Neonatal BP has in smaller series been reported to be higher in recipients than in donors [51-54]. Only a few studies have focused on long term vascular consequences of TTTS. Pulse wave velocity, reflecting vascular stiffness, was found to be increased in the brachial artery of donors compared with recipients, a finding suggested to be an effect of fetal arterial remodeling and a risk factor for later hypertension [55, 56]. Furthermore, a meta-analysis of pulse wave velocity studies has confirmed that it is predictive for total cardiovascular events [57].

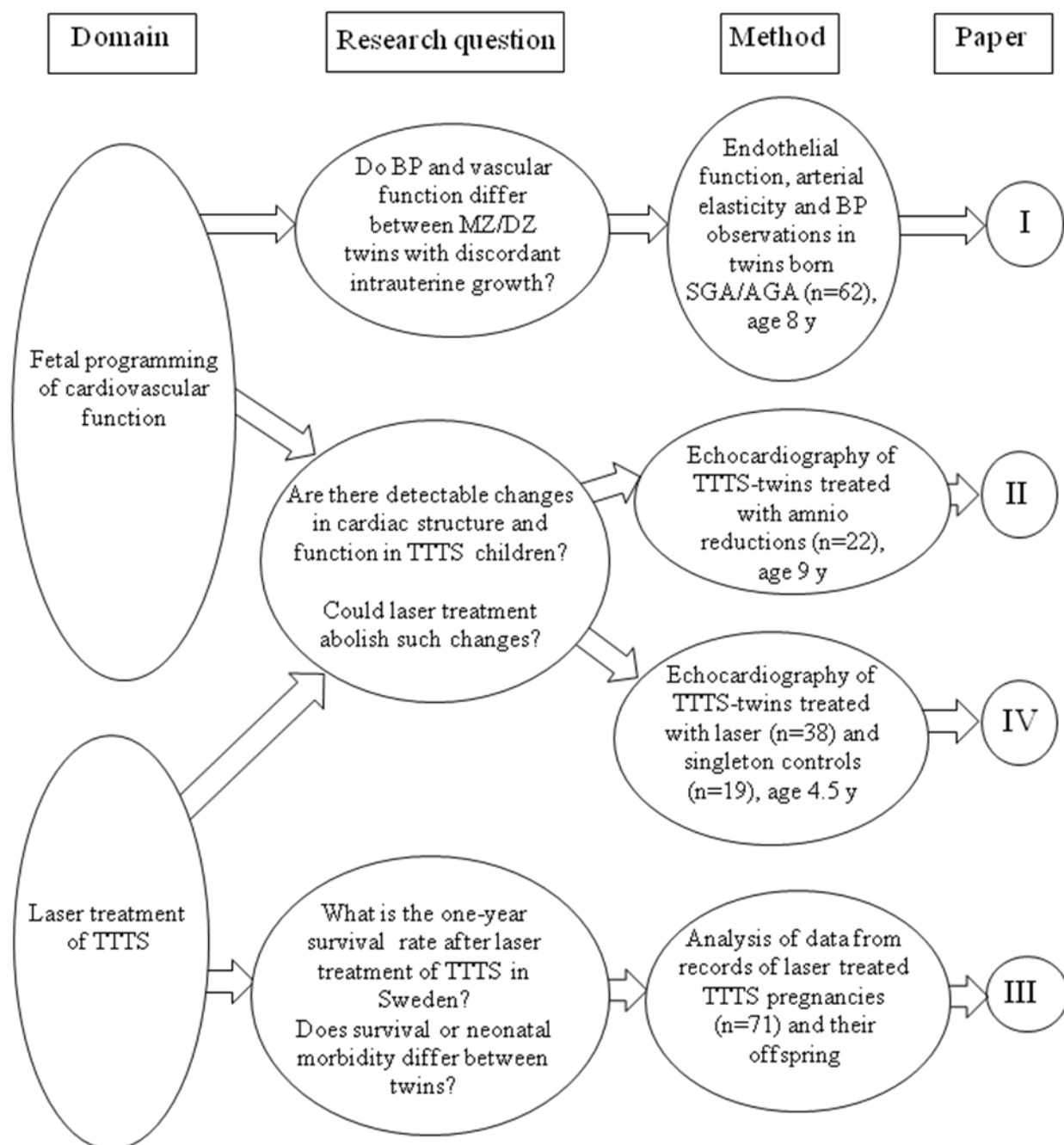
### 3 AIMS

The overall aim of this thesis was to study outcome in twin pregnancies complicated with TTTS or fetal growth retardation, in order to provide pregnant women and obstetricians with up-to-date information for antenatal counseling, to improve perinatal care and to define the need for long-term cardiovascular follow-up.

The specific objectives were:

- To evaluate whether discordant fetal growth contributes to later differences in blood pressure and vascular functions in twins (Paper I).
- To perform a ten year follow-up of cardiac structure and function in twins treated with amnioreductions due to TTTS (Paper II).
- To determine infant survival and neonatal outcome after fetoscopic laser coagulation therapy of TTTS in Sweden (Paper III).
- To study cardiac structure and function in childhood after TTTS treated with fetoscopic laser coagulation (Paper IV).

OVERVIEW



## 4 MATERIAL AND METHODS

### 4.1 SETTINGS AND STUDY COHORTS

All twins included in Papers I- II were born within the Stockholm region and assessments were conducted in clinical settings at Karolinska University Hospital in Solna, Danderyd or Huddinge. All cardiac examinations of Paper IV were conducted in the pediatric cardiology unit of Sachs' Children and Youth Hospital. Data of the laser treated women and their offspring of Paper III, were collected in a hospital-based register at the Centre for Fetal Medicine at Karolinska in Huddinge.

Table 2. Number of twin subjects (fetuses or children) studied in Papers I-IV.

Paper	I	II	III	IV
<b>MZ –with TTTS</b>				
<i>no laser treatment</i>	18	22*		
<i>laser treatment</i>			142	38
<b>MZ – no TTTS</b>	26			
<b>DZ</b>	18			
<b>Singleton controls</b>				19
<b>Total</b>	62	22*	142	57

\* 18/22 subjects same as in Paper I

#### 4.1.1 Paper I

This observational study of intrauterine growth retardation and later blood pressure and vascular function, consisted of twins recruited from two different birth cohorts of the Stockholm region. From log-books of all births ( $n > 60,000$ ) at Karolinska and Danderyd in Stockholm during 1991–1997 we found 35 live born same-sexed twin pairs in whom one was appropriate for gestational age (AGA; birth weight = mean  $\pm$  2 SD) and the other small for gestational age (SGA; birth weight more than 2 SD below the mean) according to Swedish reference data [58]. Very preterm delivery (gestational age:  $< 32$  weeks) was excluded. Four families were lost to follow-up, the remaining 31 families were invited and 22 accepted to participate in the study. Another twin cohort, consisting of all consecutive and prospectively diagnosed TTTS pairs in which both twins survived and were born in Stockholm during 1994–1997, was also included ( $n = 9$  pairs). The TTTS pregnancies were diagnosed at Södersjukhuset in Stockholm, where six of the women underwent treatment with amnioreductions alone or in combination with indomethacin. Altogether 62 twins (34 girls, 28 boys) were studied at an age of 7.8 (4.6–11.8) years and divided into three groups: (i) monozygous without TTTS (MZ without TTTS), (ii) monozygous with TTTS (MZ with TTTS) and (iii) same-sexed

dizygous (DZ) twins. Chorionicity was established on the basis of histology of the separating membrane. In cases of dichorionic placentas (n =12) or unknown chorionicity (n =2), the determination of monozygosity relied on parental reporting of mistaken identity.

#### **4.1.2 Paper II**

The subjects of this study were recruited from the same TTTS cohort from Södersjukhuset as assessed in Paper I, but with the inclusion of another two pairs for a total of eleven twin-pairs, with mean age 9.6 (7.2-11.8) years. Besides treatment with one or serial amnioreductions, some were also treated with indomethacin. Because of the risk for closure of the ductus arteriosus before birth, all fetuses treated with indomethacin were monitored with echocardiography every 1 to 2 weeks. During gestation, three recipient fetuses gradually developed cardiomyopathy illustrated by cardiomegaly, regurgitant atrioventricular valves, ventricular hypertrophy and reduced systolic function. Abnormal findings in the right ventricle preceded and were more severe than those observed in the left ventricle. In two of these recipient fetuses, there were also signs of right ventricular outflow tract obstruction (RVOTO). Distribution of Quintero stages in the group was: two of stage I, eight of stage II, and one of stage IV.

#### **4.1.3 Paper III**

Data was prospectively collected from all pregnancies treated with fetoscopic laser occlusive coagulation between Oct 2001 and July 2010, at the Center for Fetal Medicine at Karolinska University Hospital in Huddinge. There were 123 pregnant women referred to the center for fetal medicine at Karolinska University Hospital in Huddinge for consideration of fetoscopic laser coagulation treatment due to suspected TTTS. Among the referrals there were two triplet pregnancies. In 14 of the referrals, TTTS diagnosis could not be confirmed. All confirmed TTTS pregnancies were graded using the Quintero staging system [13]. Laser treatment was considered indicated if Quintero stage was II-IV and gestational duration was  $\leq 26$  weeks, n=71; stage II = 34, III = 31 and IV = 6. In addition, two women with Quintero stage I were treated due to pronounced maternal discomfort from excessive polyhydramnios. Overall, 73 pregnant women with confirmed TTTS were treated with FLOC. Twenty-six treated patients were referred from hospitals within the region (37 %) and 42 (59 %) from other regions of Sweden. Three women (4 %) came from other Nordic countries (Norway, n=2, Finland, n=1). During the study period, TTTS-patients living in the most southern part of Sweden, and in need of FLOC, were referred to Denmark or Germany.

The median gestational age at FLOC was 21 (range 17-26) weeks. Three women had been treated with amnioreductions before FLOC and eight underwent amnioreductions after FLOC. Due to an insufficient result of the first FLOC, two women were treated twice. There was no maternal mortality related to the intervention.

#### **4.1.4 Paper IV**

This observational cohort study of cardiovascular function of TTTS twin pairs surviving fetoscopic laser occlusion was conducted from Dec 2011 until Jan 2013. Children were recruited from the laser treated cohort studied in Paper III. Only twins with survival of both twins were included, to be able to make within-pair comparisons.

Of twenty-one surviving twin-pairs, one family did not respond to mail and another declined to participate. Nineteen twin-pairs were examined by the same experienced technician at the pediatric cardiology unit at Sachs' Children and Youth Hospital. In addition, a gender- and age matched control group of nineteen healthy singletons was examined.

## 4.2 CARDIOVASCULAR MEASUREMENTS

### 4.2.1 Blood pressure determinations (Papers I, II and IV)

BP was recorded after 30-45 min of rest. An automated oscillometric sphygmomanometer was used with an appropriately sized cuff around the right upper arm. The mean value of three consecutive determinations was regarded as the subject's BP. The coefficient of variation (CV) was calculated to 9.5 % for systolic (SBP) and 9.9 % for diastolic blood pressure (DBP), i.e. the same as in other pediatric studies [59, 60].

### 4.2.2 Arterial dimensions and stiffness (Paper I)

To obtain data on the mechanical properties of the abdominal aorta (AA) and the left common carotid artery (CCA) we used ultrasonography. A computer-generated pair of electronic echo-trackers (Diamove™, Teltec AB, Lund, Sweden) was used to measure the end-diastolic diameter (Dd; mm), pulse amplitude of the diameter ( $\Delta D$ ; mm) and relative strain (ratio:  $\Delta D/Dd$ , %), Figure 7. These diameter data and those of simultaneously measured BPs were computed to yield the stiffness index ( $\beta$ ) using the equation:  $\beta = \ln(SBP/DBP) \times Dd / \Delta D$  [61]. The mean value of three recordings, each consisting of about 6–10 consecutive heart cycles, was taken as the subject's reading. More on this technique has been described by Hu et al [62].

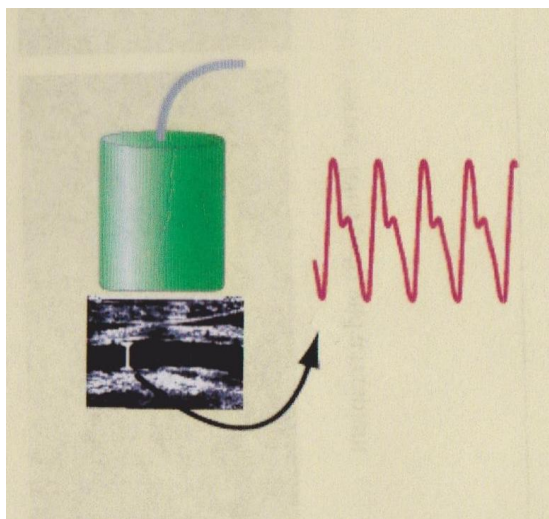


Figure 7. Ultrasonic echo-tracking system measuring arterial end-diastolic diameter and pulse amplitude.

### 4.2.3 Endothelial function (Paper I)

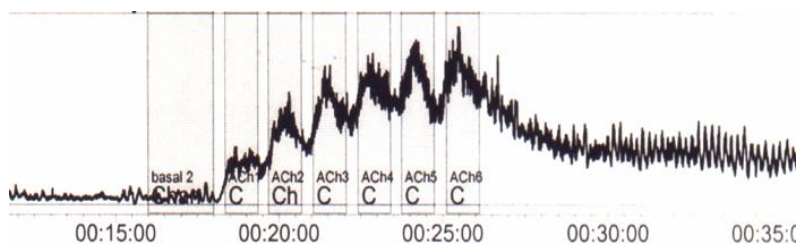
Measurements of changes in perfusion during vascular drug provocations of the skin have been used to determine endothelial function. In this study we used a laser Doppler instrument and a micropharmacology system (Perimed AB, Kista, Sweden). The laser Doppler signal is proportional to the number and velocity of moving blood cells through the illuminated superficial skin microvessels and expressed in perfusion units (PU) of output voltage (1 PU = 10 mV).

#### *Endothelium-dependent vasodilation:*

To study endothelium-dependent vasodilation, perfusion was recorded after transfer of acetylcholine (ACh) across the skin by iontophoresis (anodal current of 0.1 mA for 20 s repeated six times at 60 s intervals), Figure 8.

#### *Endothelium-independent vasodilation:*

To study endothelium-independent vasodilation, the effect of an exogenous nitric oxide donor (1% nitroglycerine) was tested on the contralateral hand. More details concerning these methods have been described by Martin et al [63].



**Figure 8.** Skin perfusion response to transdermal administration of acetylcholine with iontophoresis.

### 4.2.4 Echocardiography (Papers II and IV)

All echocardiographic assessments of Paper II were made with an Acuson Sequoia ultrasound system (Siemens Corp., Mountain View, CA, USA).

In Paper IV, echocardiographic examinations were performed using an Acuson SC2000 (Siemens Medical Solutions, Mountain View, CA, USA), by the same experienced technician at the Paediatric Cardiology Unit, Sachs' Children and Youth Hospital. Analyses were made off-line on Syngo Dynamics WS and SC2000 WP (Siemens Medical Solutions, Mountain View, CA, USA). All assessments were performed according to the standards of the European and American Societies of Echocardiography [35, 64, 65].

#### *2D and M-mode measurements (Papers II and IV)*

M-mode images from a parasternal long axis view were used to measure interventricular septum (IVS), left ventricular posterior wall (LVPW) and right ventricular anterior wall (RVAW) thickness in the end of diastole. Left ventricular end-diastolic and systolic diameters (LVED, LVES) were obtained from the same images and used to calculate shortening fraction (SF). Two dimensional (2D) images were



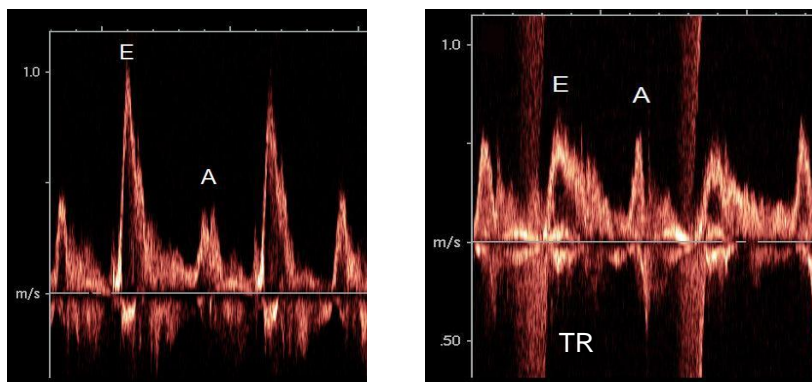
documented as 3 cine-loops, each covering two heart cycles, with a frame rate of 70-100 frames/sec. A parasternal short axis view was used for assessing 2D diameter of right ventricular outflow tract (RVOT), and a parasternal long axis view for measuring diameter of left ventricular outflow tract (LVOT).

For additional assessments (Paper IV), we used 2D records from an apical four-chamber view to measure left and right ventricular longitudinal dimensions (LVmaj, RVmaj) and the M-mode technique was used to estimate the mitral and tricuspid annular plane systolic excursion of the left (MAPSE) and right (TAPSE) ventricular free walls. TAPSE/RVmaj and MAPSE/LVmaj ratios were calculated to obtain the longitudinal shortening fractions of each ventricle (RV long SF, LV long SF). Each measurement was made at least three times and averaged. For conversion to z-score, we used reference values from Daubeney (Paper II) [66] and Pettersen (Paper IV) [67]. Z-score is a statistical measurement of a score's relationship to the mean in a group of scores. Accordingly, the mean was defined from these references.

*Blood flow and tissue Doppler (Papers II and IV):*

Pulsed wave (PW) Doppler flow velocity recordings were obtained from LVOT and RVOT for calculations of their velocity time integrals (RVOT VTI, LVOT VTI) as semi-quantitative estimates of stroke volumes. Recordings from pulmonary (PV) and aortic valves (AoV) were used to obtain peak velocity (Vmax) as an estimate of outflow patency. Continuous wave Doppler was used to measure the peak velocity of a frequently present clinically insignificant tricuspid regurgitation jet, as an indirect assessment of right ventricular systolic pressure.

Diastolic function was investigated using mitral (MV) and tricuspid valve (TV) Doppler flow velocity recordings, where early (E) and late atrial (A) filling peak velocities were measured and E/A ratios calculated, Figure 9.



**Figure 9.** Doppler assessed early (E) and late, atrial (A) right ventricular inflow in 8 year old former donor (left) and recipient (right). Note the tricuspid regurgitation (TR).

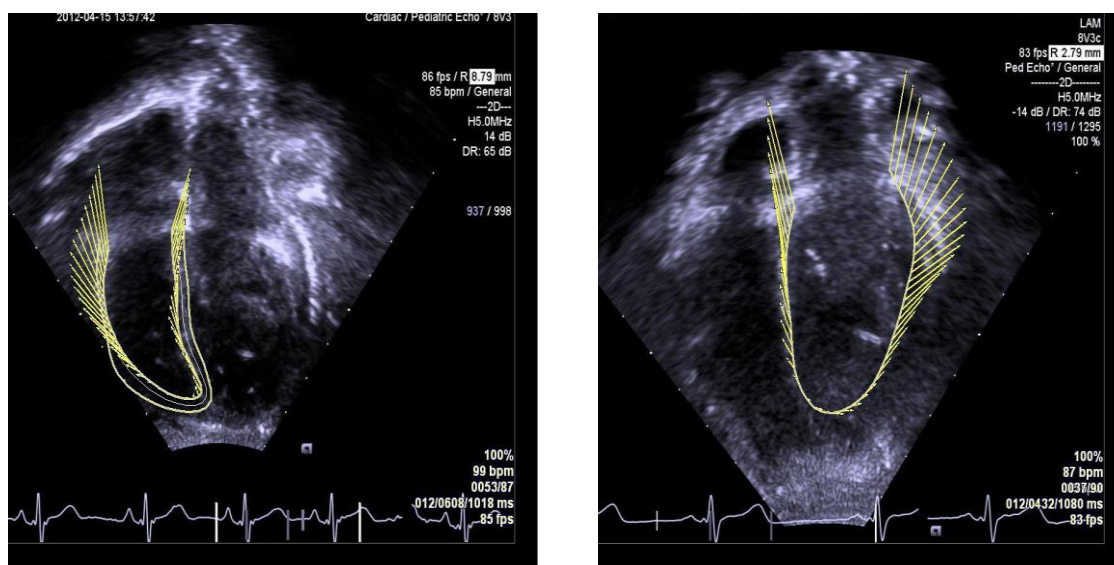
Values were compared with reference data from Eidem et al [68]. The myocardial performance index (MPI), assessing combined systolic and diastolic function of the left ventricle, was obtained by extracting the isovolumetric contraction (IVCT), isovolumetric relaxation (IVRT) and ejection (ET) time intervals from recordings of mitral valve inflow and left ventricular outflow profiles, and thereafter calculated using the formula;  $MPI = (IVCT+IVRT)/ET$ . Pulsed wave Doppler tissue imaging (DTI) was

used to record myocardial velocities in the basal free walls of the left and right ventricle. From these recordings, peak e' and a' velocities and time intervals were measured and e'/a' ratio and mpi were calculated with the same approach as for flow Doppler recordings. These records were furthermore used to measure the isovolumetric relaxation and contraction times (ivrt, ivct).

*Speckle tracking echocardiography (velocity vector imaging=VVI) (Paper IV):*

VVI can be used to study cardiac mechanics and quantify global and regional cardiac function through assessments of longitudinal and circumferential deformation where strain (%) describes myocardial deformation and is positive or negative depending on lengthening or shortening and strain rate (sec<sup>-1</sup>) is the rate of change in strain [35]. An apical four-chamber view was used to record 2D cine-loops with a frame rate of at least 70-90 frames/sec for later off-line analysis using SC2000 WP, VVI version 3.0 (Siemens Medical Solutions, Mountain View, CA, USA).

To study longitudinal deformation of the ventricles, the endocardial borders were manually traced from the free wall edge of the atrioventricular valve annulus, extending to the apex, and continuing back to the septal edge of the atrioventricular valve annulus, for left and right ventricle separately, Figure 10. From data presented by the software, we selected the global average from all segments of the ventricular walls during peak systole as estimates of ventricular deformation; namely longitudinal peak systolic global strain and strain rate. The intraobserver variability when calculating global strain was 11 % for the right ventricle and 14 % for the left ventricle.



**Figure 10.** Speckle tracking of endocardial borders to obtain longitudinal systolic strain of right and left ventricles.

*3D volumes: (Paper IV)*

Single beat 3D echocardiography was used to obtain end-diastolic and systolic volumes of right (RV EDV, RV ESV) and left ventricles (LV EDV, LV ESV), using a 4Z1c transducer from an apical four-chamber view. For each ventricle, the stroke volume (SV) was calculated by subtracting ESV from EDV.

## 4.3 MATERNAL AND NEONATAL DATA COLLECTION (PAPER

### III)

Data on all MC pregnancies referred to the center for fetal medicine at Karolinska University Hospital at Huddinge, was prospectively collected in a hospital-based register. For this purpose, records from maternal, fetal, neonatal and pediatric departments of local clinics were required. Survival up to one year of age was selected as the primary outcome. Secondary outcomes included the following neonatal data and diagnoses; Apgar score < 7 at 5 minutes, birth weight and z-score for birth weight (BW z-score), birth length, birth head circumference, respiratory distress syndrome (RDS), need for any mechanical ventilation, treatment for patent ductus arteriosus (PDA), culture-proven septicemia, a diagnosis of structural cardiac disease, renal failure, severe brain injury (defined as intraventricular hemorrhage  $\geq$  grade 3 and/or cystic periventricular leukomalacia), pulmonary hypertension, bronchopulmonary dysplasia (BPD, defined as need of supplemental oxygen at 36 weeks of postmenstrual age) and severe retinopathy of prematurity (ROP  $\geq$  grade 3).

## 4.4 STATISTICAL ANALYSES

### 4.4.1 Paper I:

To study differences between groups, chi-square and ANOVA were used. Mean within-pair differences (95 % confidence interval) were calculated (= value in the lighter twin at birth - value in the heavier twin at birth) and two-sided paired t-test was used for co-twin comparisons of continuous variables. Regression analyses were used to evaluate contributions to within-pair differences from the following risk factors or confounders: group (MZ-no TTTS, MZ-TTTS and DZ), preeclampsia, use of antenatal steroids, gender, current age, weight and height.

### 4.4.2 Paper II:

Wilcoxon test for matched pairs was used to compare the recipient with the donor within each pair of twins, and the t-test for single means was used to test if the standardized measurements of the valve areas (z-scores) deviated from reference values.

### 4.4.3 Paper III:

To study differences between groups, Chi-square test was used for categorical variables or proportions, and Student's t-test or Mann-Whitney U-test was used for continuous variables. Comparing donor and recipient twins, chi-square, paired t-test or Wilcoxon's signed ranks test was used according to Mc Nemar [69]. Linear regression was used to test for associations between maternal and obstetric characteristics and infant survival.

#### **4.4.4 Paper IV:**

For comparisons between donors, recipients and controls, we used matched paired t-tests. Variables with observed differences between groups were also analyzed using linear regression to evaluate the possible contribution of gestational age at birth or BSA, when appropriate.

For all four studies, a p-value < 0.05 was considered significant.

## **4.5 ETHICAL APPROVALS**

All families of the cardiovascular observation studies provided written informed consent (Papers I, II and IV). The women treated with fetoscopic laser coagulation were contacted by regular mail a few weeks after treatment, and asked for a written informed consent for data acquisition of medical records including data on offspring (Paper III). All study protocols were approved by Regional Ethics Review Board in Stockholm with identification numbers: 00-277, 412-00, 2010/2010-31/4.

## 5 RESULTS

### 5.1 BLOOD PRESSURE

#### 5.1.1 Paper I

The overall systolic blood pressure (SBP) was 101 (1.3) and diastolic BP (DBP) 59 (0.7) mmHg. After taking age, gender and height into account, 8/62 twin subjects had a SBP above the 90th centile, according to normative data from North American children [60]. Among those with high SBP, four were MZ-TTTS subjects (two donors and two recipients), three were MZ twins without TTTS and small at birth and one was a DZ twin with normal birth weight. The gestational age, current age- and gender distribution were similar in those with and those without a high SBP. The SBP and DBP in children with a history of maternal preeclampsia or antenatal steroid therapy did not differ significantly from those without such a history (p-values 0.67-0.83). Within all pairs there was a trend towards higher SBP (mean +2.6 mmHg, p =0.08) and pulse pressure (mean +3.0 mmHg, p=0.09) in the twin with lower birth weight, compared with the heavier twin. The differences were more marked among MZ twins without TTTS; SBP was +5.2 (CI +0.1 – +10, p=0.04) higher in the lighter than the heavier MZ twin sibling. Within-pair SBP differences did not show any significant relation to the degree of birth weight discordancy (regression coefficient: +4.8 mmHg/kg lower birth weight in the lighter twin, p = 0.39). Moreover, we found no significant within-pair differences in DBP or heart rate, Table 2. Multivariate regression analyses indicated that contributors to the within-pair difference in SBP were pregnancy history of preeclampsia, antenatal steroid treatment and monozygosity. Age, gender, current weight and height differences did not significantly affect SBP discordance.

Table 2. Within-pair blood pressure differences in 8-year-old twins with discordant birth weights.

	MZ- no TTTS n=13	MZ-TTTS n=9	DZ n=9
SBP, mmHg	<b>+5.2(+0.1;+10)</b>	-0.1(-3.2;+3.1)	+1.6(-6.3;+9.4)
DBP, mmHg	+1.4(-2.0;+4.8)	-1.4(-7.4;+4.5)	+1.1(-3.5;+5.7)
Heart rate, bpm	-3.3(-7.7;+1.0)	-1.3(-6.1;+3.5)	+4.3(-1.2;+9.8)

MZ- no TTTS = monozygous twins without TTTS, MZ-TTTS = monozygous twins with TTTS, DZ = dizygous twins, SBP=systolic blood pressure, DBP=diastolic blood pressure, bpm = beats per minute. Difference: [value in the lighter – value in the heavier twin at birth]/ value in the heavier twin at birth x 100. Mean (lower; upper limits for 95 % confidence interval) values, bold indicates p < 0.05.

### 5.1.2 Paper II

The mean (SD) of SBP; DBP was 100 ( $\pm 8.0$ ); 56.6 ( $\pm 6.6$ ) in the donor group and 101 ( $\pm 10.9$ ); 58.4 ( $\pm 8.5$ ) in the recipient group, the difference between the groups was not significant.

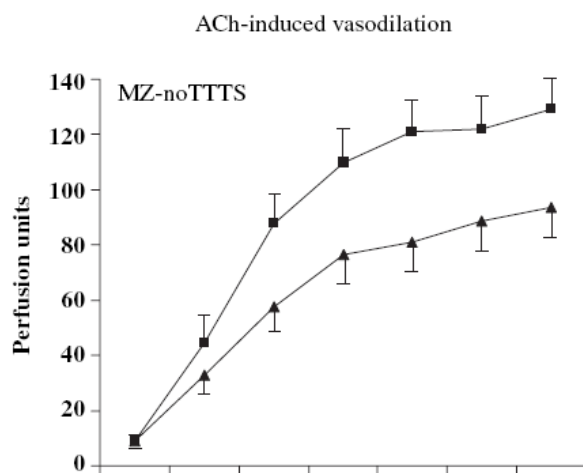
### 5.1.3 Paper IV

The mean (SD) of SBP; DBP was 91.1 ( $\pm 6.7$ ); 56.8 ( $\pm 4.6$ ) in the donor group, 90.0 ( $\pm 12.3$ ); 55.4 ( $\pm 8.4$ ) in recipient group and 56.5 ( $\pm 6.9$ ) in control group, the differences between the groups were not significant.

## 5.2 ENDOTHELIAL FUNCTION

### 5.2.1 Paper I

The overall mean basal skin perfusion was 11 (1.5) perfusion units (PU) and the maximum increase in perfusion induced by the endothelium-dependent vasodilator ACh was 110 (5.7) PU. Basal skin perfusion did not differ between the lighter and heavier twin. In the MZ group, the lighter twin had a 40 PU lower maximum response to ACh than the heavier twin ( $p < 0.05$ ), Figure 11. In the MZ-TTTS and DZ groups, the within-pair differences in responses to ACh were not significant. Preeclampsia reduced the within-pair difference in maximum skin perfusion response to ACh (6.2 PU, n.s.). In contrast, in pregnancies with discordant twin growth but without preeclampsia the ACh skin perfusion response was 34 PU lower in the twin with lower birth weight ( $p < 0.05$ ). The mean basal skin perfusion before testing endothelium-independent vasodilation was 11 (1.0) PU. The maximum increase in skin perfusion after topical application of nitroglycerin was 35 (2.4) PU. The mean difference in response to nitroglycerin between lighter and heavier twins was statistically significant among girls (- 13 PU,  $p < 0.05$ ), but not among boys (- 4.5, n.s).



**Figure 11.** Intra-pair comparisons in vasodilation response to acetylcholine between heavier (■) and lighter (▲) monozygous twin.

## **5.3 ARTERIAL DIMENSIONS AND ELASTICITY**

### **5.3.1 Paper I**

Among MZ and DZ twins, no intra-pair differences were detected in CCA dimensions, changes in pulsatile diameter or stiffness. In the MZ-TTTS group, the donor twin had a CCA diameter that was -0.3 (95 % CI: -0.6 - 0) mm or 5 % narrower than that of the recipient twin. The donor also showed +3 (95 % CI: 1-5) % higher carotid strain than the recipient. The AA strain was higher in the recipient than in the donor, however there was no significant difference in stiffness index or end-diastolic diameter between twins of any group. The results did not differ significantly from reference data for healthy school-aged singletons with normal birth weights [63]. By calculating the arterial stiffness index, we adjusted for differences in strain due to those in BP.

## **5.4 ECHOCARDIOGRAPHIC MEASUREMENTS**

### **5.4.1 Cardiac dimensions**

Paper II:

We found no significant differences between donors and recipients regarding diameters of ventricular cavities, walls and outflows, coronary arteries or AV-valves. Three donors and three recipients had tricuspid valve areas below -2 SD according to reference data reported by Daubeney et al [66]. Among those recipients, two had myocardial hypertrophy during fetal life.

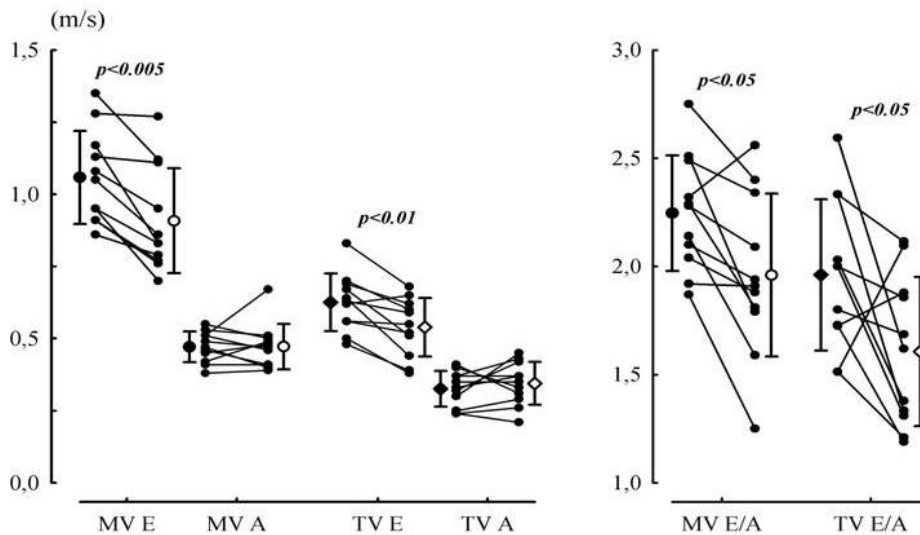
Paper IV:

LVED and thickness of septum and free ventricular walls (IVS, LVPW, RVAW) did not differ between groups after adjusting for body surface area (BSA) [67]. The unadjusted longitudinal RVmaj and LVmaj in recipients were similar to those in donors but smaller than in controls. Similar observations were made for measurements of the ventricular outflow tracts (RVOT, LVOT). With the exception of one former donor with a mild pulmonary valve stenosis diagnosed at birth, no cardiac malformations were diagnosed. Unadjusted left ventricular EDV and SV of both ventricles measured with 3D were reduced in donors compared with recipients and controls.

### **5.4.2 Systolic and diastolic function**

Paper II:

Doppler flow variables as well as M-mode derived SF did not demonstrate any differences in systolic function between twins. Recipients had lower peak velocities of early diastolic filling (E-wave) of left and right ventricle and a reduced E/A ratio compared with donors and measured with conventional Doppler, Figure 12. Similar findings were observed with DTI technique.



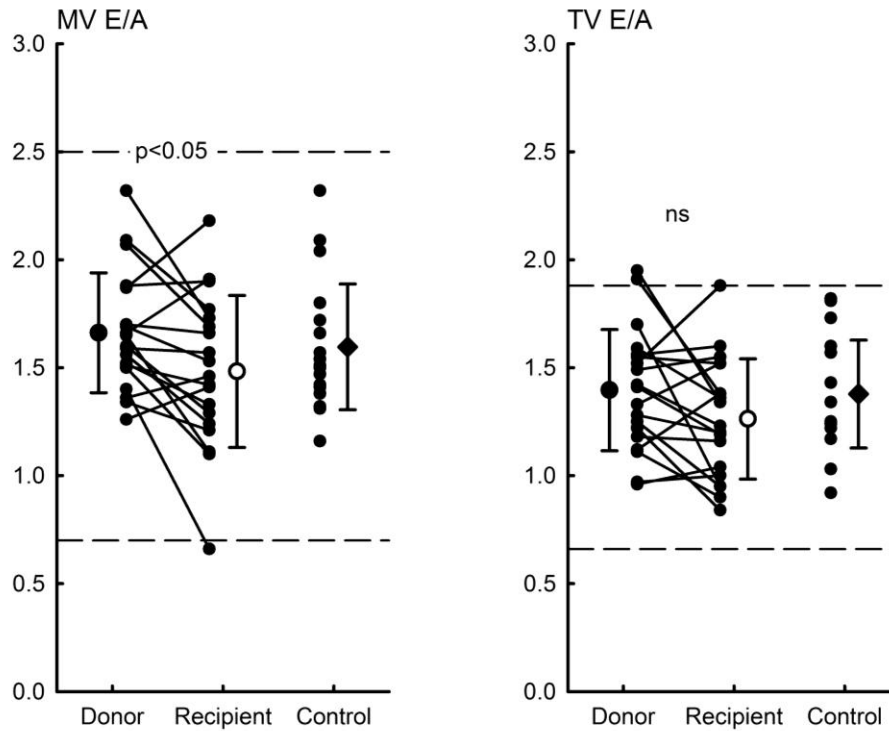
**Figure 12.** Diastolic early (E) and late (A) filling blood flow velocities through the tricuspid (TV) and mitral (MV) valves (to the left) as well as their E/A ratios (to the right). Lines connect the observations made in the donor (left) with the recipient (right) within each pair of twins. Symbols with error bars represent the mean  $\pm$  standard deviation. Filled symbols with error bars denote the former donors and unfilled symbols with error bars denote the former recipients.

#### Paper IV:

The only observed differences between donors and recipients were related to diastolic ventricular filling. Recipients had a lower E/A ratio in the mitral valve compared with donors, whereas in comparison with controls there was no statistical difference. There were no significant differences in E/A ratio of the tricuspid valve between groups, but recipients had a tissue velocity-derived  $e'/a'$  ratio that was lower than the donors', but not significantly different from the controls', Figure 13. Linear regression analysis did not show any contribution of gestational age at birth to these differences.

Longitudinal global peak systolic strain of left ventricle did not differ between donors and recipients, but donors had a slightly lower left ventricular strain than controls. There was no difference in strain rate between groups, Table 3.





**Figure 13.** Ratio of diastolic early (E) and late (A) filling blood flow velocities in the mitral (MV) and tricuspid (TV) valves. Solid lines connect observations made within each pair of twins. Symbols with error bars represent the mean  $\pm$  1 standard deviation. Broken lines are upper and lower 95 % reference limits constructed from data published by Eidem [68] (Paper IV).

Table 3. Longitudinal myocardial deformation measured with velocity vector imaging (VVI) of global strain and strain rate in donor, recipient and control children. Values are presented as mean (1 SD).

	donors, n=19	recipients, n=19	don vs. rec p- value	controls, n=19	don vs. contr p- value	rec vs. contr p- value
<b>LV peak systolic global strain, %</b>	-17.6(4.0)	-19.5(4.9)	0.18	-20.8(3.5)	0.03	0.39
<b>LV systolic global strain rate, s<sup>-1</sup></b>	-1.3(0.3)	-1.4(0.4)	0.30	-1.4(0.3)	0.24	0.93
<b>RV peak systolic global strain, %</b>	-21.8(5.2)	-20.3(6.6)	0.36	-24.6(4.0)	0.12	0.05
<b>RV systolic global strain rate, s<sup>-1</sup></b>	-1.9(0.4)	-1.8(0.7)	0.57	-1.9(0.5)	0.82	0.57

## 5.5 OUTCOMES AFTER LASER TREATMENT

### 5.5.1 Maternal and obstetric characteristics

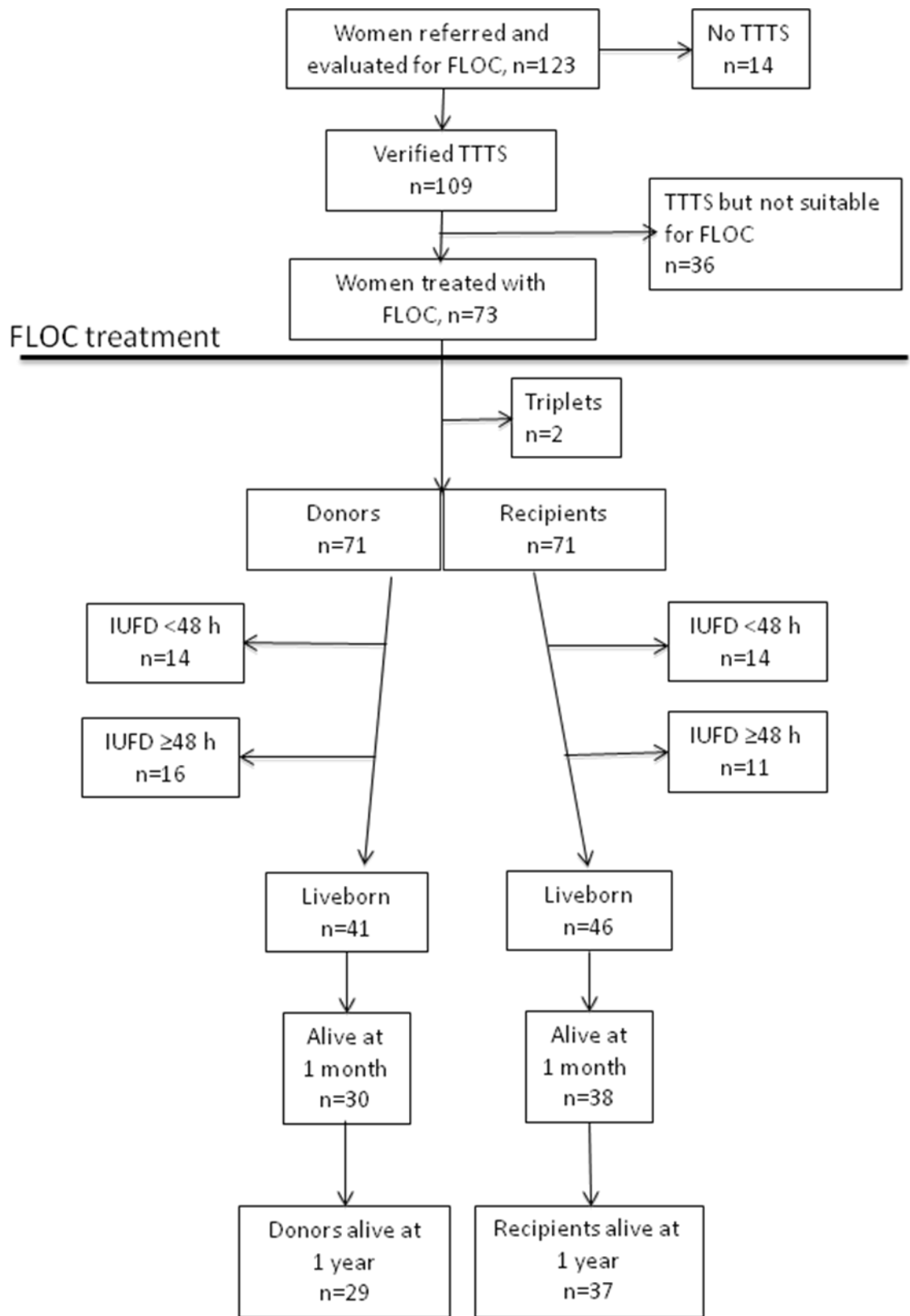
In the studied laser treated cohort, preterm birth was twice as common and extremely preterm birth ten times as common as among Swedish twin births in general, Table 4.

Table 4. Maternal and obstetric characteristics in laser treated TTTS pregnancies compared with all Swedish multiple pregnancies. Data from Swedish Medical Birth Register 2009 [70]. Values are medians or proportions.

	Laser treated cohort n=71	All Swedish duplex n=1,544
Age, years	31.3	32.0
Smoking, %	7.0	6.4
Assisted fertilization, %	16	22
Nulliparous, %	38	44
BMI, kg/m <sup>2</sup>	23	24
Caesarean section, %	66	54
Preterm (GA < 37 w), %	89	44
Very preterm born (GA < 32 w), %	53	10
Extremely preterm (GA < 28 w), %	40	3

### 5.5.2 Survival after laser treatment

In 53/71 (75 %) laser treated TTTS cases, at least one twin was liveborn and in 43/71 (60 %) at least one twin survived infancy. Fetal survival did not differ between donors (41/71 [58 %]) and recipients (46/71 [65 %], p=0.36). Among liveborn infants, survival was 29/41 (71%) in donors and 37/46 (80%) in recipients (p=0.10), Figure 14. Only one live born infant delivered before 25 gestational weeks (n=8) was alive at one year of age, Table 5. If delivery occurred after 28 gestational weeks, 88 % of donors and 96 % of recipients were still alive after one year. In pregnancies ending with two liveborn infants, both survived to 1 year in 21/34 (62 %) of pregnancies. The proportion of liveborn infants did not differ between Quintero stages I-II (61 %) and III-IV (61 %). In addition, infant survival did not correlate to gestational age at FLOC.



**Figure 14.** Fetal and infant survival in TTTS donors and recipients after treatment with fetoscopic laser occlusive coagulation therapy (FLOC).

Table 5. Infant survival in 86 liveborn TTTS twins in relation to gestational age at birth (weeks<sup>+days</sup>). Values are total numbers (donors/recipients).

	Extremely preterm					Very preterm	Moderately preterm	Term
	23-23 <sup>+6</sup>	24-24 <sup>+6</sup>	25-25 <sup>+6</sup>	26-26 <sup>+6</sup>	27-27 <sup>+6</sup>			
Gestational age at birth, w	23-23 <sup>+6</sup>	24-24 <sup>+6</sup>	25-25 <sup>+6</sup>	26-26 <sup>+6</sup>	27-27 <sup>+6</sup>	28-31 <sup>+6</sup>	32-36 <sup>+6</sup>	>37
Liveborn	3(1/2)	5(2/3)	8(4/4)	13(6/7)	8(3/5)	11(6/5)	32(15/17)	7(4/3)
Early neonatal death								
<24 h	0/1	2/2	2/1	1/1		1/1		
24 h-7d	1/0	0/1	1/1			1/0		
Late neonatal death								
8-27 d			1/0	1/0				
Post neonatal death								
28 d -1 year					0/1		1/0	
Alive at 1 year	1(0/1)	0(0/0)	2(0/2)	10(4/6)	7(3/4)	8(4/4)	31(14/17)	7(4/3)

### 5.5.3 Neonatal anthropometry and disease

As expected, donors had a significantly lower weight, length and head circumference at birth than their recipient twins ( $p < 0.01$  for all comparisons). Although neonatal morbidity was found to be high, we did not find any significant differences in major neonatal morbidities between donors and recipients, Table 6.

The rate of treatment with mechanical ventilation during neonatal stay was 46 %. The need of mechanical ventilation in relation to gestational age is described in Table 7. Persistent pulmonary hypertension of the newborn (PPHN) was reported in 11 % of recipients, but in none of the donors. Fetal myocardial hypertrophy was detected with ultrasound in twenty recipients, mostly presented as atrioventricular valve regurgitation and/or biventricular hypertrophy. Two of the donor fetuses were described as having signs of cardiac dysfunction, one of those also showing a valvular pulmonary stenosis. In the recipient group, there were two reported cases of obstructions of right ventricular outflow tract. At neonatal follow-up, one of these was diagnosed with a mild valvular pulmonary stenosis and the other with bilateral stenosis of the pulmonary branches.

Table 6. Neonatal morbidity and anthropometry in liveborn monochorionic twins treated with fetoscopic laser occlusive coagulation for twin-to-twin transfusion syndrome (TTTS). Donors and recipients reported separately. Values are numbers (%) or median (range). Z-score for BW according to Niklasson et al [71].

	Donors, n=41	Recipients, n=46
GA <sup>1</sup> at birth, weeks	30.8 (23.8-38.1)	29.0 (23.0-40.6)
BW <sup>2</sup> , g	1,230 (266-3,591)	1,380 (400-3945)
BW z-score	-2.18 (-5.32-0.81)	-0.90 (-3.19-7.25)
RDS <sup>3</sup>	23 (56 %)	24 (52 %)
Mechanical ventilation	15 (36 %)	25 (54 %)
Culture proven septicemia	6 (15 %)	12 (26 %)
IVH $\geq$ grade 3/cPVL <sup>4</sup>	6 (15 %)	5 (11 %)
PDA <sup>5</sup> treated	4 (10 %)	5 (11 %)
PPHN <sup>6</sup>	0	5 (11 %)
Renal failure	5 (12 %)	4 (8.7 %)
BPD <sup>7</sup> at 36 w	4 (9.8 %)	5 (11 %)
ROP <sup>8</sup> $\geq$ grade 3	2 (4.9 %)	4 (8.7 %)

<sup>1</sup>GA=Gestational age; <sup>2</sup>BW=birth weight; <sup>3</sup>RDS=respiratory distress syndrome; <sup>4</sup>IVH/cPVL=intraventricular hemorrhage or cystic periventricular leucomalacia; <sup>5</sup>PDA=patent ductus arteriosus; <sup>6</sup>PPHN= persistent pulmonary hypertension of the newborn; <sup>7</sup>BPD=bronchopulmonary dysplasia; <sup>8</sup>ROP=retinopathy of prematurity

Table 7. Numbers of liveborn TTTS twins treated with mechanical ventilation (MV) during the neonatal period.

Gestational age at birth, weeks <sup>+days</sup>	Donors		Recipients	
	MV	No MV	MV	No MV
< 28 <sup>+0</sup>	11	2	18	1
28-31 <sup>+6</sup>	2	4	3	2
32-36 <sup>+6</sup>	2	12	4	13
$\geq$ 37 <sup>+0</sup>	0	4	0	3

## 6 DISCUSSION

### 6.1 FINDINGS AND IMPLICATIONS

The main findings of the thesis were that:

1. Severe fetal growth retardation contributes to higher blood pressure, arterial narrowing and endothelial dysfunction in monozygous twins in childhood.
2. Survival after fetoscopic laser treatment is poor if delivery occurs extremely preterm. No donor born before 26 gestational weeks survived infancy.
3. Neonatal mortality and morbidity after laser treatment does not differ significantly between donors and recipients, except for temporary pulmonary hypertension and need for mechanical ventilation, which is more common among recipients.
4. The risk for neonatal respiratory disease needing mechanical ventilation is increased in preterm TTTS twins compared with singletons of corresponding gestational age.
5. Recipients showed a reduction in diastolic function at follow-up at 9 years, compared with donor twins if treated with amnioreductions. There were similar differences between TTTS twins in the laser treated group, although of minor significance.
6. Despite severe cardiac dysfunction demonstrated in many recipient fetuses, almost all variables used to study cardiac structure or function were within normal range at a mean age of 4.5 years.
7. Although laser treatment of TTTS does not seem to completely abolish the risk for fetal remodeling of the myocardium, there were no clinically significant differences in cardiac function in comparison with healthy singletons.

Despite many recipients exhibiting significant cardiac dysfunction during fetal life, survivors of TTTS showed only minor within-pair differences in diastolic cardiac function at follow-up, and these differences were even smaller in the laser treated cohort. Cardiac function in TTTS twins compared well with gender- and age-matched controls. Our data agrees with previous studies, suggesting that changes seen in fetal hearts of many recipients are reversible [28, 72, 73]. As our findings are based on a limited number of TTTS twins, TTTS survivors are few, and echocardiography is a harmless method, we suggest that a cardiac screening including an exercise test in late childhood should be considered for this group.

Laser treatment has been reported to reduce the intrauterine growth discordance between donor and recipient from treatment to birth [74]. In concordance with another long-term study of laser treated TTTS infants [75], donors in the laser treated cohort of Paper IV were significantly smaller at follow-up than their recipient co-twins and age-matched controls, despite some catch-up after birth. In the cohort treated with amnioreductions (Paper II), the intra-pair difference in growth was not significant at

school-age, which may be explained by a less severe TTTS, with the vast majority classified as Quintero stages I-II.

Impaired fetal growth has been suggested to result in a deficient synthesis of elastin in the walls of the aorta and other large arteries, leading to permanent changes in the mechanical properties of these vessels [76]. Loss of natural elasticity has been proposed to contribute to an increased BP at older age [77]. In Paper I, we reported narrower carotid arteries with higher strain in donors compared with recipients. Being born SGA seems to be a risk factor for increased carotid stiffness at school-age [63]. In the MZ group without TTTS, the lighter twin demonstrated a higher strain of abdominal aorta, but no differences in dimensions or in stiffness index. These differences may be explained by donors being exposed not only to intrauterine growth retardation but also to hypotension and prematurity. Prematurity has been suggested to be a risk factor for aortic narrowing [78], but not for carotid stiffness [79]. A possible long-term importance of our findings in TTTS infants compared with non TTTS

Systemic impairment of endothelial function has been found in newborns, children and young adults with low birth weight [63, 80]. The finding of impaired response of vasodilation to acetylcholine in the smaller MZ twin without TTTS as compared with the twin with a greater birth weight, could not be repeated for the MZ twins with TTTS. This may be due to shorter gestation of TTTS twins (four pairs were delivered before 32 weeks), as preterm delivery has been suggested to attenuate developmental programming of the endothelium [81].

We also found that MZ twins without TTTS born SGA demonstrated higher BP than their siblings born AGA (Paper I). In contrast, there was no difference in SBP between TTTS twins, and two donors and two recipients had values above the 90<sup>th</sup> percentile. Although recipients were born AGA, they had been exposed to abnormal circulatory load during fetal life, and recipients have showed an increased BP in the neonatal period compared with donors [53, 54]. Furthermore, children of preeclamptic mothers have developed increased BP and BMI in childhood or young adulthood according to a recent review article [82]. In our study group, maternal preeclampsia was found to be a contributor to within-pair BP differences in SBP, suggesting that preeclampsia somehow adds to the circulatory stress during pregnancy and may not be only of genetic origin.

None of the laser treated twins assessed in Paper IV showed any ventricular hypertrophy at follow-up. Interestingly, we found the dimensions of the right and left ventricular outflow tracts and longitudinal diameters to be smaller in both donors and recipients compared with age- and gender-matched controls. Discordance in body size could in this regard only be an explanation for the donor group, as recipients' body sizes did not deviate significantly from those of controls. Furthermore, we did not observe any differences in LVOT/RVOT ratios, and all outflow velocities were within normal range and similar between groups. Recently, Lewandowski et al reported larger mass, shorter longitudinal diameter and impaired systolic and diastolic strain of left ventricle in adults born preterm, measured with magnetic resonance tomography [83]. Cardiac remodeling has also been studied in lamb models, showing a switch from fetal hyperplasia to postnatal hypertrophy of cardiomyocytes at birth occurring regardless of

gestational age [84, 85]. Immature cardiomyocytes in the preterm may exhibit early remodeling based on exposure of an early increase in after load at birth [86]. Albeit statistically significant, the actual differences in ventricular size were very small and our findings of lack of differences in ventricular wall thickness or outflow obstruction are probably of greater clinical importance.

We have reported a lower rate of neonatal survival in the laser treated TTTS cohort when comparing with extremely preterm born neonates of the population-based EPRESS (Extremely Preterm infants in Sweden) study [87]. Monochorionic twins are known to demonstrate a worse perinatal outcome than singletons [88]. We speculate that a newborn with the combination of TTTS and an extremely preterm birth may demonstrate a vulnerability and morbidity comparable to a neonate born at least one week earlier. Accordingly, to improve the neonatal care and survival, information of a TTTS history, irrespective of treatment, has to be given to the neonatal team. Overall survival or neonatal morbidity did not differ significantly between donors and recipients except as regards the need for treatment with mechanical ventilation during neonatal stay and temporary pulmonary hypertension, which was more common among recipients. When considering the high rates of lung disease, e.g. RDS and pulmonary hypertension, the neonatal team has to prepare for treatment of these complications in *two* neonates.

Although preterm male neonates are known to be more vulnerable to neonatal disease than females [89], we found no correlation between gender and survival or neonatal disease in our TTTS twins.

In our studies we did not identify any infant diagnosed with a duct-dependent right ventricular outflow obstruction. However, based on the risk of right ventricular hypertrophy with a possible development of outflow obstruction in the recipient fetus, the neonatal staff needs to be prepared for an early echocardiographic assessment and possible PGE1-infusion at birth.

### **6.1.1 Methodological considerations**

There are some limitations of these studies as regards methods. One is the dependence of reliable pediatric reference data of cardiac measurements when calculating the z-scores of the study groups. Normative data are included in most software of echocardiographic equipment and we primarily used data from Daubeney, Eidem and Pettersen [66-68]. In Paper II, three donors and three recipients had tricuspid valve areas below the normal range (z-score < -2.0) according to Daubeney. When comparing these values with data presented by Pettersen, we found all tricuspid valve areas to be within normal range. To manage this, we introduced an age- and gender-matched control group in our following study (Paper IV). Echocardiographic measurements do not relate simply to measurement of the size of heart structures. Normal values of functional data, e.g. conventional Doppler and tissue Doppler data, also change across the pediatric age range, and are influenced by patient age [68, 90] and heart rate [90]. In our echocardiographic studies, we tried to keep the child calm and confident, watching a video in the lap of a parent, to get assessments and heart rates at rest. All children of Paper I and IV demonstrated normal heart rate for age according to reference data[91].



Usage of speckle tracking echocardiography is highly dependent on the 2D image quality and frame-rate. In our attempts to visualize the borders of left and right ventricles in the same clip from a longitudinal apical four-chamber view, we sometimes experienced software limitations when tracing the right ventricular free wall. VVI-analyses in a few children were therefore made on different clips for assessments of right and left ventricle respectively. Furthermore, data on longitudinal strain has been reported to show a higher reproducibility than data on radial strain [92], and we therefore chose to report only longitudinal values on strain and strain rate.

Multiple comparisons performed in the echocardiographic studies increased the likelihood of statistically significant differences due to random rather than systematic variation. However, our finding of a difference in ventricular relaxation within pairs was robust: it was found using two independent methods (Doppler flow velocimetry and tissue imaging) with reassuringly low p-values (Paper II) and similar results were reproduced in the laser treated group (Paper IV). Moreover, the findings of Papers II and IV were not confounded or modified by preeclampsia (as in Paper I) or by maternal smoking in pregnancy [78].

### **6.1.2 Strengths and limitations**

The major strengths of this thesis are the long-term follow-up of cardiac and vascular structure and function (Papers I, II and IV) and the high participation rates. The prospectively collected cohorts of the studies may have contributed to the high participation rate. Even though there are several studies of TTTS fetuses describing cardiac findings, we have not been able to identify any other study with a mean age past 21 months [73]. All cardiac assessments were performed by a skilled echotechnician, blinded to TTTS status.

Limitations to consider with all our studies are the small sample sizes with insufficient power to detect small within-pair differences. The relatively limited number of children in the different studies is based on the fact that pronounced TTTS is a rare complication of MC pregnancies. Moreover, despite the last decades' increased consciousness of TTTS among ultrasonographers, we believe that some TTTS gestations might have been undiscovered during the study time and therefore lost for possible treatment (Paper III) and follow-up (Paper I-IV).

#### *Misclassifications:*

Only a limited number of placentas of the pregnancies studied in Paper III were sent for histopathology. For that reason, we could not determine the success rate of the laser coagulation therapy. All units delivering laser treated TTTS pregnancies need to be better informed of the importance of sending placentas for analyses to gain further experience.

In studies III and IV, we identified donor and recipient status from reports of fetal sonography and delivery units. In those few cases where data on phenotype was missing in delivery and neonatal records, we chose to define the smallest twin as the donor. The accuracy of defining donor and recipient status during fetal monitoring and

after birth and whether antenatal ultrasound could predict birth order in twin pregnancy has been questioned [93]. Caesarean section is associated with an even greater inaccuracy of prediction. Moreover, reversal of the donor-recipient phenotype has been suggested to occur in about 5 % of TTTS pregnancies and although some of the reported cases have been associated with aneuploidy or genetic disorders, the pathophysiology is still unclear and could possibly also occur after laser treatment [94].

#### *Single survivors:*

Our objective was to study environmental influence on cardiac function by comparing twins with identical genotypes (Papers II and IV). In these studies, we excluded twin pairs with only one survivor and the results can therefore not be generalized to single TTTS survivors. However, since fetal demise of one twin will discontinue the inter-twin transfusions, we could hypothesize that aside from a probable immediate pressure fall including a risk for cerebral damage, the surviving twin will experience a normalizing placental circulation. Accordingly, we suggest that single survivors may not exhibit a worse cardiac outcome than twins of dual survival. In Paper III, the outcome of all twins was reported and in the planned neurodevelopmental follow-up of this cohort, twins of dual as well as of single survival will be included.

#### *Neurodevelopmental outcome after TTTS:*

When evaluating possible adverse effects of fetal interventions, neurodevelopmental follow-up is recommended. There are several studies on long-term neurodevelopmental outcome in early childhood. The rate of neurological late complications after TTTS has been reported to be 6 % for severe and 7 % for mild deficits in a study by Graef [95], whereas others have stated an incidence of 7 % for cerebral palsy and 17 % for all neurological late complications [96]. Wagner et al reported on a better neurodevelopmental long-term outcome even in milder TTTS stages (grade I) treated with lasercoagulation compared with conservative treatment. However, there was no statistical difference in perinatal survival between groups [97]. In a meta-analysis of TTTS outcome [98], the pooled analysis confirmed that the risk of cerebral injury was not related to twin type (donors 9 %, recipients 10 %,  $p=0.66$ ). The same author reported no possibility to analyze whether survival rates and neurological morbidity were related to gestational age at diagnosis and stage of disease. We have not studied neurologic outcome in the laser treated cohort, although this is planned for the future, but we found the rate of neonatal cerebral injury diagnosed with ultrasonography to be 14 % among liveborn donors and 6.5 % among recipients (Paper III). In the laser treated group of dual survivors assessed with echocardiography (Paper IV), parents reported two children (both donors) with a diagnosis of cerebral palsy.

## **6.2 ETHICAL CONSIDERATIONS**

There are several issues to consider before deciding about delivering TTTS twins preterm. First, fetoscopic laser treatment can be performed until 26 weeks of gestation, and if this has not been done earlier and there are no contraindications, it may be preferred. Second, depending on the degree of abnormalities of Doppler flows in umbilical artery, the risk for fetal demise may exceed the risk for an early neonatal

death if born extremely preterm. In Paper III, we reported that no donor and only two recipients survived delivery before gestational week 26<sup>+0</sup>. This ought to be taken into consideration before a delivery at that time is accomplished on fetal indication because of the donor. Third, an extremely preterm delivery due to a recipient fetus demonstrating cardiac dysfunction, could result in a higher risk for death or more severe neonatal complications than if waiting a few weeks. As long as ventricular hypertrophy and dysfunction only involves the right side, the myocardial alterations seem to be reversible. Despite almost half of the recipients demonstrating fetal myocardial hypertrophy, there was only one of all children studied in Papers II and IV who developed a pulmonary stenosis requiring surgery.

The median gestational age at laser therapy was 21 (range 17-26) weeks. We found that outcome was not related to gestational age at treatment. Experience and skills of the team performing laser treatment are probably the most important factors for fetal survival [99, 100]. Laser therapy may not be scattered over too many centers, and the suggestion has been made to centralize it to a few centers of excellence, where treatment of at least twenty TTTS pregnancies per year could be expected [44]. In Sweden, that number has been achieved at Karolinska University Hospital during the last couple of years. But we could still expect an improvement in outcome due to a learning curve.

### **6.3 FUTURE PERSPECTIVES AND CHALLENGES**

The etiology of TTTS is still unknown and cannot so far be prevented. Although it is a rare condition, with a better awareness about characteristic fetal signs of the syndrome among midwives and obstetricians, more TTTS gestations may be eligible for evaluation and treatment in specialized centers for fetal interventions. The findings that fetal ventricular hypertrophy of the recipient seems to be reversible, have to be considered before decision of delivery, especially if the alternative is delivering two extremely preterm babies. On the other hand, the hemodynamics of the donor are frequently impaired and severe Doppler changes of umbilical vessels are more often the reason for an early caesarean section. The decisions of trading one growth retarded donor and a pressure loaded recipient for two preterm twins is difficult. With our current results, we suggest a desisting from delivery before 28 gestational weeks, if possible.

From the results of Paper III we learnt that TTTS preterms are more exposed to severe neonatal complications than other twins or singletons of the same gestational age. This is important information to the perinatal teams preparing to take care of these babies. The high rate of treatment with mechanical ventilation may suggest that TTTS pregnancies < 32 gestational weeks are to be delivered in a tertiary neonatal intensive care unit.

The within-pair difference in diastolic function, in favor of the donor, may be a reason for echocardiographic controls not only in childhood, but also later in life as teenagers or adults. To investigate early signs of impaired cardiac function, recipients might also perform an exercise test. All children had normal BP, at the time of the study, but since

growth restriction, prematurity and myocardial dysfunction are risk factors for hypertension, donors as well as recipients could benefit from BP controls in later life. Severe fetal growth retardation contributes to higher blood pressure, arterial narrowing and endothelial dysfunction in monozygous twins in childhood.

Increases in the thickness of the intima and media of the carotid artery, as measured noninvasively by ultrasonography, are known to be directly associated with an increased risk of myocardial infarction and stroke in older adults [101]. In Paper I, we reported that donors had a significantly narrower common carotid artery than their recipient twins. To our knowledge, there is no data on carotid intima-media thickness (IMTc) in TTTS children. We have ultrasound data of IMTc of the TTTS children and controls examined for Paper IV and intend to investigate if donors exhibit an increased thickness compared with the other groups.

The high risk for preterm birth after treatment for TTTS is a major problem. Premature rupture of membranes and bleedings were the most reported complications in the study group of Paper III. There is no evidence for cervical cerclage, tocolysis or Arabin pessary having been used for prolonging pregnancy in laser treated TTTS pregnancies with short cervical length [102]. At present, there are no effective methods to prevent a preterm birth if started spontaneously with labor, but progesterone has been suggested to be used for primary prevention if there are risk factors for prematurity or for secondary prevention after tocolysis [103], although no benefit from progesterone has been demonstrated in twin pregnancies [104].

The results of this thesis demonstrate that despite a fetal cardiovascular distress when exposed to TTTS in utero, survivors of laser treated TTTS have a favorable long-term cardiac outcome. However, increasing perinatal survival after laser treatment is a challenge. In January 2013, a Swedish national workshop was held in Stockholm, aiming to increase the knowledge about TTTS and to suggest guidelines. A new website was presented to be used as an internet-based platform with information of diagnosis, surveillance, treatment, follow-up and current research.

Increased knowledge about how to characterize TTTS when assessing MC pregnancies with ultrasonography and Doppler, may improve possibilities for early treatment and close surveillance, and also to adequately inform prospective parents.

An extended follow-up program has been introduced for extremely preterm born children, including neurodevelopmental testing, blood pressure monitoring and evaluation of respiratory function. Based on the high perinatal morbidity, this program may be suggested for TTTS twins irrespective of gestational age at birth.

## 7 SVENSK SAMMANFATTNING

### *Bakgrund:*

Tvillinggraviditeter utgör 1-3 % av alla graviditeter och av dessa är en tredjedel enäggstvillingar och två tredjedelar tvåäggstvillingar. Om enäggstvillingarna delar samma moderkaka är risken 10 % att ett tvillingtransfusionssyndrom (TTS) utvecklas. Den ena tvillingen (givaren) kommer via gemensamma kärl i moderkakan att förlora en del av sin blodvolym. Blodmängden förs istället över till den andra tvillingen (mottagaren). Den ojämna blodfördelningen medför risker för såväl givare som mottagare att bli cirkulatoriskt påverkade och dö. Dödligheten vid obehandlad TTS är ca 80%, men idag behandlas tillståndet med laserkoagulering av kärlförbindelserna. Enäggstvillingar är genetiskt identiska, vilket ger en unik möjlighet att studera hur olikheter i fostermiljön kan påverka tvillingarnas utveckling, inte minst vad gäller hjärt-kärlfunktion. Denna avhandling har flera syften: 1) att utvärdera om det vid 8 års ålder finns skillnader i blodtryck och kärlfunktion mellan tvillingsyskon som haft olika födelsevikt pga olika fostertillväxt (delstudie I); 2) att studera om det finns tecken till långtidseffekter av TTS på hjärtats struktur och funktion (delstudier II och IV); 3) att rapportera överlevnad och sjuklighet i nyföddhetsperioden för den grupp kvinnor som genomgått laserbehandling i Sverige pga TTS (delstudie III).

### *Metoder och resultat:*

I delstudie I undersökte vi 31 tvillingpar i 8-årsåldern från enäggsgraviditeter med och utan TTS samt från tvåäggsgraviditeter. I varje tvillingpar var en tvilling född lätt-förtiden och den andra normalstor-förtiden. Vi fann i gruppen enäggstvillingar bl a att blodtrycket var högre hos den tvilling som haft den lägsta födelsevikten.

I delstudie II undersökte vi hjärtat med ultraljud hos elva 9-åriga TTS-par, som under fostertiden genomgått fostervattentappningar. Den enda skillnaden vi fann mellan respektive tvilling var att mottagarna hade något sämre kammarfyllnad jämfört med sina givarsyskon. När vi därefter i delstudie IV undersökte nitton TTS-par vid 4,5 års ålder, som genomgått laserbehandling under fostertiden, fann vi något mindre uttalade men snarlika skillnader i hjärtats kammarfyllnad. I delstudie III gick vi igenom journaldata från alla TTS-graviditeter som behandlats med laser (71 kvinnor). Vi fann bland annat att överlevnaden från behandling till ett års ålder var 46 %, den genomsnittliga graviditetslängden var 30 veckor och hälften av de levande födda tvillingarna krävde respiratorbehandling.

### *Slutsatser:*

Dålig fostertillväxt kan bidra till högre blodtryck, smalare blodkärl och nedsatt förmåga att vidga blodkärlen i barndomen (delarbete 1). Trots att TTS foster, i synnerhet mottagartvillingar, ofta har ett belastat hjärta med bland annat förtjockade hjärtmuskelväggar, hade TTS tvillingarna i våra studier väsentligen normal hjärtfunktion i tidig barndom. De milda tecken på sämre kammarfyllnad som förelåg bland mottagartvillingar som behandlats med fostervattentappningar, i jämförelse med givarsyskonen (delarbete II), var mindre uttalade i den laserbehandlade gruppen (delarbete IV). Ungefär hälften av de tvillingfoster som genomgår laserkoagulering av kärlförbindelser i moderkakan vid TTS lever vid ett års ålder. Om mycket för tidig förlossning är nödvändig, bör det mottagande teamet förbereda sig för att ta hand om två svårt sjuka nyfödda som ofta kräver respiratorvård (delarbete III).

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