Twin-to-twin transfusion syndrome : from placental anastomoses to long term outcome

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Twin-to-twin transfusion syndrome: from placental anastomoses to long-term outcome

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geboren te Rome in 1968

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La vita è bella…

...soprattutto con Stella

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Chapter 1

General introduction

Introduction

Twins, especially identical twins, have fascinated humanity over the centuries. In perinatology, however, fascination for the twinning process is often outweighed by concerns due to increased medical complications associated with twin pregnancies. Perinatal mortality and morbidity in twins is significantly increased compared to singletons, partly due to a higher incidence of prematurity and very low birth weight in twins[1]. Mortality is reported to be 3 to 7-fold higher in twins than in singletons[2], whereas morbidity, particularly risk of cerebral palsy, is 7-fold higher in twins than in singletons[1]. Risk of adverse outcome is particularly pronounced in monochorionic twins. Perinatal mortality in monochorionic twins is 2.5-fold higher than in dichorionic twins[3], whereas neurological morbidity is 7-fold higher in preterm monochorionic twins than in preterm dichorionic twins[4]. Adverse outcome in monochorionic twinning is due to complications associated with the presence of placental vascular anastomoses.

Vascular anastomoses are extremely rare in dichorionic placentas but ubiquitous in monochorionic placentas[5], and may lead, amongst other complications, to twin-to-twin transfusion syndrome (TTTS). TTTS occurs in approximately 15% of monochorionic twin pregnancies[6]. TTTS usually develops during the 2nd trimester of pregnancy, resulting in hypovolemia, oliguria and oligohydramnios in the donor twin and hypervolemia, polyuria and polyhydramnios in the recipient twin[6]. TTTS is associated with high perinatal mortality and morbidity. In the past, diagnosis of TTTS was based on neonatal criteria such as birth weight discordance and hemoglobin difference. Nowadays, diagnosis of TTTS is reached strictly on prenatal ultrasound criteria. The main diagnostic criterion for TTTS is the twin oligo-polyhydramnios sequence (TOPS). A significant evolution in prenatal care strategies and management options for patients with TTTS has occurred during the last decade. The two main therapeutic options in TTTS are serial amniodrainage and fetoscopic laser occlusion of communicating placental vascular anastomoses[6]. Fetoscopic laser surgery is a new treatment modality that has led to an increase in survival rates. In perinatology, a decrease in mortality rates may be associated with an increase in morbidity rates. Follow-up studies in infants with TTTS are shedding more

light on the wide range of morbidity associated with TTTS, such as neurological, cardiac and renal sequelae.
Since monochorionic twinning is known to occur worldwide in 1 in every 400 pregnancies[6], the estimated number of TTTS cases in The Netherlands is approximately 75 cases per year. The majority of TTTS cases in The Netherlands are referred to the Leiden University Medical Center (LUMC). The LUMC is a tertiary medical center and serves as the national referral center for fetal therapy, including fetoscopic laser surgery for TTTS. The laser surgery program at the LUMC started in August 2000.

Outline of the thesis

In June 2002 the Leiden's TTTS study (LETTS' study) was started. The aim of this prospective study was to investigate the short-term and long-term outcome in TTTS treated with fetoscopic laser surgery. With an intense and ongoing collaboration between the obstetric and neonatology departments of the LUMC, several other study projects associated with the pathogenesis, diagnosis and treatment of TTTS were also initiated. The aim of these studies can be summarized as follows:

Chapter 2 - Review of the literature on TTTS. This review analyzes the possible pathophysiologic mechanisms involved, discusses the latest findings in diagnosis, therapy and prognosis, and focuses on neonatal and pediatric morbidity associated with TTTS.

Chapter 3 - Description of a novel technique to determine the net feto-fetal blood flow through placental arterio-venous anastomoses in a unique case of TTTS.

Chapter 4 - Study on the role of velamentous umbilical cord insertion and discordant placental territories in the pathogenesis of TTTS by comparing monochorionic placentas with and without TTTS.

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Chapter 2

Twin-to-twin transfusion syndrome: from placental anastomoses to long-term neurodevelopmental outcome

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Introduction

Twin-to-twin transfusion syndrome (TTTS) is a complication of monochorionic twin gestations and is one of the most lethal conditions in perinatal medicine. TTTS is thought to result from an unbalanced net transfusion of blood between one twin, the donor, and the other twin, the recipient, via placental vascular anastomoses. In the 19th century, Friedrich Schatz, a German obstetrician, extensively studied the twinning process and was the first to speculate on the relationship between vascular anastomoses and the development of TTTS[7]. The number, size and type of these anastomoses appear to play an important role in the etiology of TTTS[8]. Despite a growing understanding of TTTS, the exact pathophysiology remains to be elucidated. Various new hypotheses question the validity of the classic concept of TTTS resulting mainly from inter-twin transfusion of blood. Recently, a staging system based on antenatal ultrasound findings has been introduced and appears to have prognostic significance[9]. Most importantly, this staging system may be used to compare various therapeutic modalities in TTTS. The two main current treatment options in TTTS are serial amnioreduction and fetoscopic laser occlusion of vascular anastomoses. Both treatments are associated with improved survival rates. The first randomized trial designed to compare the effectiveness of these two therapeutic options has recently been published and has shown that fetoscopic laser surgery is associated with less mortality and less short-term neurological morbidity than serial amnioreduction[10]. The results of other ongoing randomized trials are eagerly awaited. Despite the improvement in overall survival rates, mortality and morbidity rates remain strikingly high, especially in TTTS managed expectantly or with serial amnioreduction. A high risk of long-term neurodevelopmental disability has been reported, particularly after intrauterine fetal demise of a co-twin during the second or third trimester[11-16]. Severe cerebral damage in the surviving monochorionic twin may even occur during the first trimester[17]. In TTTS with double survival, recipient twins are at risk for life-threatening cardiovascular complications such as hypertrophic cardiomyopathy and right ventricular outflow tract obstruction[18;19], whereas donor twins are at risk for acute or chronic renal failure due to chronic renal hypoperfusion[20;21].

This review examines the various theories on the pathogenesis of TTTS, describes the diagnostic tools and criteria, discusses some of the randomized trials on the efficacy of various treatments and focuses on neonatal and pediatric morbidity associated with TTTS.

The twin-to-twin transfusion syndrome

Several forms of TTTS have been described: chronic, acute perimortem, acute perinatal and twin reversed arterial perfusion sequence[11]. The chronic form of TTTS is the most common form and complicates about 10 to 15% of monochorionic twin pregnancies[22-24]. Chronic TTTS usually becomes clinically apparent during the second or early third trimester of pregnancy. Recent reports suggest that chronic TTTS may occur even in the first trimester of pregnancy[25;26]. Clinical symptoms are due the rapid development of polyhydramnios, causing maternal discomfort, premature rupture of the membranes, or preterm labor. Chronic TTTS is then diagnosed if the oligo-poyhydramnios sequence is detected on ultrasound examination[27]. The donor twin has severe oligohydramnios due to hypovolemia and oliguria and appears tightly wrapped in his amniotic sac and "stuck" to the uterine wall despite changes in maternal position, hence referred to as the "stuck" twin. Conversely, the recipient twin has severe polyhydramnios due to hypervolemia and polyuria. In the most severe cases, the recipient may subsequently develop circulatory volume overload and fetal hydrops. Inter-twin growth discrepancy and hemoglobin discordance are often reported, but are not key criteria for the diagnosis of chronic TTTS[27-29]. Both fetuses are at risk for intrauterine fetal demise, either due to hypoxia and anemia in the donor, or cardiac failure in the recipient. Moreover, both fetuses may die as a result of preterm delivery. An acute form of TTTS, referred to as acute perimortem TTTS, occurs after intrauterine fetal demise of a co-twin and is due to acute exsanguination from the surviving twin into the low-pressure circulation of the demised co-twin through the vascular anastomoses[30-32]. Acute exsanguination often leads to co-twin death or may lead to severe hypoxic-ischemic damage in the surviving twin due to hypovolemia, hypotension and anemia, resulting in co-twin death, multicystic encephalomalacy and multi-organ damage[12-16;33].

Anecdotal reports suggest that acute TTTS may also occur during delivery[34-39]. This form of TTTS, often referred to as acute perinatal TTTS, may then lead to a difference in hemoglobin concentration at birth between the donor and the recipient twin without necessarily discordance in birth weight[40-44]. Acute shifts of blood from one twin to the other are thought to result from blood pressure differences due to uterine contractions or changes in fetal positions[36;44;45]. The donor twin has often signs of acute hemorrhagic hypovolemic shock and requires treatment with urgent blood transfusion or volume expanders[35-37]. Research data on acute perinatal TTTS is however extremely scarce. Hemoglobin differences between monochorionic twins at birth have also been shown to be related to birth order[35;38;46]. In a recent study in monochorionic twins without chronic TTTS, we reported that hemoglobin levels in second-born twins are significantly higher than in first-born twins[47]. Once the umbilical cord of the first-born twin is clamped, the second-born twin has the sole connection to the entire placenta and may not only receive blood from its own placenta but also from the placenta-share of the co-twin, through the vascular anastomoses[38;41].

The most extreme form of TTTS is acardiac twinning, which occurs in 1 out of 35 000 pregnancies[48]. Acardiac twinning, also named twin reversed arterial perfusion sequence, occurs when a large arterio-arterial and veno-venous shunt is present, causing reversed flow in the single umbilical artery of the acardiac fetus. The acardiac fetus is severely malformed without a functional heart. The donor twin is otherwise structurally normal and is referred to as the pump twin as it pumps blood both into its own circulation as well as directly into the circulation of the acardiac co-twin. The pump twin is therefore also at risk for intrauterine fetal demise due to high output cardiac failure.

Incidence

Twin gestations represent 1 to 2% of all pregnancies. Two thirds of twin gestations are dizygotic (therefore always dichorionic) and are not at risk for developing TTTS. One third of twin gestations are monozygotic and may give rise to either monochorionic or dichorionic placentation.

Chorionicity in monozygotic twinning depends upon the time interval between fertilization and cleavage of the embryo[49]. Cleavage within three days of fertilization results in a dichorionic placentation, whereas cleavage occurring within four to eight days of fertilization will lead to a monochorionic placenta[49]. Two third of monozygotic pregnancies are monochorionic. Only monochorionic twins are at risk for developing TTTS. Approximately 10 to 15% of all monochorionic twin pregnancies will eventually develop chronic TTTS[22-24]. The incidence of acute perinatal TTTS is not known.
Increased infertility treatments have led to a higher rate of multifetal gestations. Twinning rate in the United States was 31 per 1000 total live births in 2002, an increased twinning rate of 65% since 1980 and 38% since 1990[50]. To date, approximately one million children world-wide were conceived through assisted reproductive technology, with associated rates of multifetal gestations up to 50%[51;52]. Most twins born after assisted reproduction techniques are dizygotic. However, various studies have also shown a significant increase in monozygotic twinning after various forms of infertility treatment such as in vitro fertilization related techniques (in particular blastocyst transfer), ovulation induction and intracytoplasmic sperm injection, mostly due to alterations on the zona pellucida[53-56]. Monozygotic twinning is increased 2 to 6 times in babies born after in vitro fertilization[57-59]. Consequently, increased rates of multifetal gestations due to infertility treatments may theoretically also lead to increased rates of TTTS.

Pathogenesis

The presence of placental vascular anastomoses is a *conditio sine qua non* for the development of TTTS. Injection studies of twin placentas have shown that such anastomoses are almost invariably present in monochorionic twins and extremely rare in dichorionic twins[5]. Three types of anastomoses have been documented: from artery to artery, from vein to vein and from artery to vein. Arterio-venous anastomoses are unidirectional and are referred to as “deep” anastomoses since they proceed through a shared placental cotyledon, whereas arterio-arterial and veno-venous anastomoses

are bi-directional and are referred to as "superficial" since they lie on the chorionic plate (Figure 1). Injection studies of monochorionic placentas have demonstrated that arterio-arterial, arterio-venous and veno-venous anastomoses are present in 80%, 95% and 20% of monochorionic placentas, respectively [60-62].

The presence of at least one arterio-venous anastomosis has been shown to be essential for the development of chronic TTTS[26;61-63]. According to the classic pathophysiological concept, chronic TTTS is caused by net imbalance of blood flow between the twins due to arterio-venous anastomoses[7;8]. Blood from one twin (the donor) is pumped through an artery into the shared placental cotyledon and then drained through a vein into the circulation of the other twin (the recipient). Unless blood is pumped back from the recipient to the donor through oppositely directed

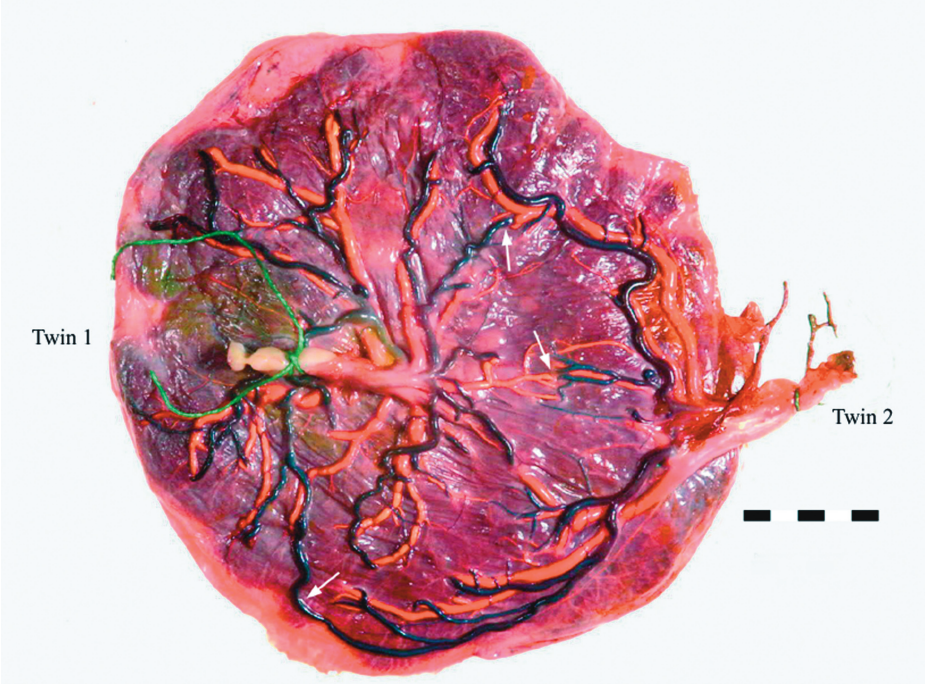

FIGURE 1 Placenta injection study with colored dye from a monochorionic twin gestation without chronic twin-to-twin transfusion syndrome delivered after 38 weeks of gestation. Arteries are injected with dark-blue dye and veins with orange dye. The arrow at the bottom of the picture indicates an arterio-arterial anastomosis. An arterio-venous anastomosis from twin 2 to twin 1 is pointed out in the center of the picture, whereas an arterio-venous anastomosis from twin 1 to twin 2 is indicated at the top of the picture. Twin 2 has a velamentous cord insertion.

deep arterio-venous anastomoses or through superficial anastosmoses, an imbalance of blood volumes occurs, gradually leading to the development of chronic TTTS. The presence of arterio-arterial anastomoses in particular is thought to give some protection against the development of chronic TTTS by compensating for the circulatory imbalance caused by the uni-directional arterio-venous anastomoses[7;8]. Various studies have demonstrated that these bi-directional arterio-arterial anastomoses occur more frequently in monochorionic placentas of twins without chronic TTTS compared to placentas of twins with chronic TTTS around 80% and 25%, respectively [26;64-67]. The antenatal detection of an arterio-arterial anastomosis with color Doppler ultrasound examination is associated with a 9-fold reduction in likelihood ratio of developing chronic TTTS[68]. This protective role of arterio-arterial anastomoses which was already proposed by Schatz in the 19th century[7], has recently also been demonstrated in a mathematical computer model of chronic TTTS[69]. Conversely, the pathogenesis of acute perimortem and acute perinatal TTTS is mainly mediated through large superficial low-resistance arterio-arterial or veno-venous anastomoses[13;29-32;44;70;71].

The validity of the traditional theory of unbalanced blood shunting from the donor to the recipient as the major factor causing the development of chronic TTTS has frequently been questioned. Several studies report the absence of differences in hemoglobin, erythropoietin and iron metabolism between donors and recipients[72-74]. However, these data are not in direct conflict with the classic pathophysiologic concept of inter-twin transfusion in TTTS and may simply reflect the inter-twin transfusion of blood components and various hormones through the vascular anastomoses. Several new hypotheses have been added to the classic pathophysiologic concept of TTTS and may also play a role in the pathogenesis of chronic TTTS (Table 1).

Significant higher concentrations of fetal atrial natriuretic peptide as well as brain natriuretic peptide have been found in recipient twins compared to donor twins[75-78]. The release of these peptides is stimulated by cardiac overload and causes increased fetal urine production, leading consequently to polyhydramnios and to the further development of chronic TTTS. The fetal renin-angiotensin system is reported to be up-regulated in donor twins due to hypovolemia and decreased renal perfusion[79-81]. Although

TABLE 1 Summary of theories on pathogenesis of chronic TTTS.

Author (year)	Pathogenesis
Schatz (1882)[7]	Donor "pumps" blood into recipient through placental vascular anastomoses
Schatz (1882)[7]	Absent compensatory arterio-arterial anastomoses
Nageotte (1989)[75]	Increased atriopeptin in recipient
Wieacker (1992)[77]	Increased atriopeptin and decreased antidiuretic hormone in recipient
Wax (1992)[92]	Compression of placental vessels of donor
Fries (1992)[89]	Compression of velamentous inserted cord of donor
Bajoria (1999)[84]	Increased endothelin concentrations in recipient
Mahieu-Caputo (2000)[80]	Up-regulation of renin-angiotensin system in donor
Bajoria (2000)[88]	Reduced amino-acids levels in donor
Sooranna (2001)[87]	Reduced leptin levels in donor
Bajoria (2001)[86]	Reduced insulin-like growth factor II levels in donor
Bajoria (2004)[82]	Increased vasopressin concentrations in donor

up-regulation of renin synthesis may be necessary to restore euvolemia in the donor, the activation of the renin-angiotensin system also leads to vasoconstriction and may further aggravate renal hypoperfusion and oligohydramnios in the donor twin. Similarly, raised concentrations of vasopressin were recently found in donor twins, suggesting that oligohydramnios may also result from the antidiuretic and vasoconstricitve activity of vasopressin[82]. Furthermore, vasoconstriction may contribute to placental dysfunction and aggravate intrauterine growth restriction in the donor. Activation of the renin-angiotensin system may also have a paradoxical effect in the recipient twin. Transfer of renin and angiotensin II through the vascular anastomoses from the donor into the hypervolemic recipient may increase the blood pressure and aggravate cardiac dysfunction in the recipient. Increased blood pressure has indeed often been reported in recipient twins after birth[12;83] and may also be due to other vasoconstrictive substances circulating in the recipient twin. Various studies have demonstrated a significantly higher concentration of endothelin-1 in recipient twins compared to donor twins[78;84;85]. Endothelin-1, a potent vasoconstrictor, may cause hypertension and therefore also aggravate cardiac dysfunction in the recipient.

Several other hormones, such as insulin-like growth factor-II and leptin may also contribute to the development of chronic TTTS. Levels of insulin-like growth factor-II and leptin, both playing a role in intrauterine growth restriction, were recently reported to be significantly lower in donor twins, suggesting that growth restriction in the donor twin might result from abnormal placental development[86;87]. Similarly, lower essential and non-essential amino acids have been reported in growth restricted donor twins suggesting that fetal undernutrition may be partly due to impaired placental transport of amino acids[88].

Velamentous cord insertion is reported to be more common in pregnancies affected by chronic TTTS compared to monochorionic gestations without chronic TTTS, and occur principally in donor twins[89-91]. It is suggested that a velamentous inserted cord may be more easily compressed resulting in reduced blood flow through the umbilical vein to one twin[89]. A vicious circle may then be established as less blood flows to one twin (the donor), more blood will flow to the other twin (the recipient) through the placental anastomoses. The consequent development of polyhydramnios in the recipient twin may then compress the umbilical vein of the donor twin even further, ultimately leading to the development of chronic TTTS. A comparable mechanism is advocated by other authors who speculated that polyhydramnios may partially compress the venous placental vessels of the donor twin, leading, through a similar vicious circle, to the development of chronic TTTS[92]. However, these theories do not explain why chronic TTTS also occurs in twins without velamentous inserted cords. Moreover, similar rates of velamentous insertion between monochorionic pregnancies with chronic TTTS compared to monochorionic pregnancies without chronic TTTS have recently been reported[93]. We are currently performing a prospective study on the rate of velamentous cord insertion and type of placental vascular anastomoses in monochorionic twins with chronic TTTS compared to monochorionic twins without chronic TTTS.

In summary, the development of TTTS is associated with a multitude of complex hormonal and hemodynamic mechanisms. More research is needed in order to determine which of these mechanisms is the most important. However, monochorionic twinning does not occur in the animal world except in humans and in armadillos[94], and animal models for studying the pathogenesis of TTTS are not available. Lack of a suitable

experimental animal model may hamper further investigations on the pathogenesis of TTTS. Nevertheless, computer modeling studies may help to further elucidate the exact etiology of TTTS and test the various hypotheses[95-101].

Diagnosis

The diagnostic criteria of TTTS have dramatically changed since the advent of prenatal ultrasonography. In the past, the definition of chronic TTTS was based on two main neonatal criteria: a birth weight discordance > 20% and a hemoglobin difference > 5 g/dL between the two infants. These traditional criteria have been abandoned because similar differences in hemoglobin and birth weight exist also in dichorionic twins and in monochorionic without chronic TTTS[44;71]. Moreover, studies with fetal blood sampling in chronic TTTS pregnancies have shown that the donor twin does not necessarily have a lower hemoglobin concentration than the recipient twin[72;102;103]. Absence of significant inter-twin hemoglobin differences may be due to compensatory increased hematopoiesis in the donor twin[103;104]. However, erythropoietin concentrations have recently been shown to be similar between donors and recipients, questioning the validity of the hypothesis on discordant hematopoiesis between twins with chronic TTTS[73].

The diagnosis of chronic TTTS is currently defined by prenatal ultrasound criteria[27;28;105;106]. Firstly, since TTTS occurs only in monochorionic twins, chorionicity must be determined through prenatal ultrasound scanning in the first (or early second) trimester of pregnancy. Ultrasound criteria for monochorionicity are the absence of a lambda-sign at the inter-twin membrane junction and the presence of a thin dividing membrane with a T-sign. Assessment of monochorionicity must imply a higher level of alertness from doctors and parents in order to detect the first clinical symptoms related to chronic TTTS, such as excessive uterine growth and premature contractions due to polyhydramnios. Prenatal diagnosis of chronic TTTS is then reached by demonstrating the characteristic oligo-polyhydramnios sequence on ultrasound. Oligohydramnios is present in the donor who is often also stuck to the uterine wall and tightly wrapped in his amniotic

sac, whereas polyhydramnios is found in the recipient. Oligohydramnios is defined as a deepest vertical amniotic fluid pool ≤ 2cm in the donor's sac, and polyhydramnios as a deepest vertical pool ≤ 8cm in the recipient's sac until 20 weeks of gestation and ≥ 10 cm after 20 weeks of gestation[105;106]. A staging system according to Quintero has recently been introduced and stratifies chronic TTTS in 5 stages based on ultrasound criteria (Table 2)[9]. This staging system allows comparison of different management strategies in chronic TTTS and is also useful in monitoring disease progression[9;12;107]. Several sonographic signs, such as increased nuchal translucency, folding of the inter-twin membrane, presence of a velamentous insertion of the umbilical cord and absence of arterio-arterial anastomoses are associated with a higher probability of the development of chronic TTTS[66;89;108]. However, accurate prediction of chronic TTTS is not yet possible and more research on the diagnostic value of early ultrasound findings is necessary. A study on early ultrasound predictors in monochorionic twins without chronic TTTS has been performed at our institution[109].

Diagnostic criteria for acute perinatal TTTS vary among studies, but usually include an hemoglobin difference > 5 g/dL at birth[34;35;38;39;44]. Most studies also rely on the absence of birth weight discordance to exclude chronic TTTS. However, birth weight discordance is not a reliable criterion to exclude chronic TTTS[27;28;44;71;93]. Moreover, rapid shifts of blood during labor can occur in any monochorionic twins with patent vascular anastomoses, with or without birth weight discordance[44]. Diagnostic

TABLE 2 Staging classification of chronic TTTS according to Quintero[9].

Stage	Sonographic criteria
stage I	Bladder of the donor twin still visible, Doppler studies are normal
stage II	Bladder of the donor twin not visible, Doppler studies are still normal
stage III	Doppler studies are critically abnormal*
stage IV	Ascites, pericardial effusion or pleural effusion, scalp edema, or overt hydrops are present
stage V	One or both twins are dead

** reversed flow in the ductus venosus or pulsatile umbilical venous flow in the recipient, and/or absent or reversed end-diastolic flow in the umbilical artery of the donor*

criteria of acute perinatal TTTS should therefore include an hemoglobin difference > 5 g/dL, with or without birth discordance[44]. Furthermore, diagnosis of chronic TTTS based on antenatal ultrasound examination and acute perimortem TTTS, should first be excluded.

Therapy

The intrauterine management of chronic TTTS remains a significant challenge in perinatal medicine. In severe TTTS, conservative treatment is associated with mortality rates from 73 to 100%[110-114]. Therefore, a wide variety of aggressive, sometimes even desperate treatment modalities have been attempted. Some reported treatments are not considered to be effective first-line treatments, including maternal treatment with digoxin to treat cardiac failure in the recipient[115], maternal treatment with indomethacin in order to reduce polyhydramnios[116] and fetal blood-letting from the hypervolemic recipient[117]. Other treatments are considered to be more effective, such as selective feticide by occlusion of the umbilical cord through ligation or bipolar diathermy to order to save one infant[118], inter-twin septostomy by creating a hole in the dividing membrane aiming at equilibrating the amniotic fluid between the donor's sac and the recipient's sac[119], serial amnioreduction[120] and fetoscopic laser occlusion of vascular anastomoses[121;122]. The latest two treatment options, serial amnioreduction and fetoscopic laser coagulation of vascular anastomoses, are the two main current treatment options in chronic TTTS. Although chorioamnionitis, rupture of the membranes, preterm labor and placental abruption are potential risks associated with these procedures, both treatments are widely reported to be associated with improved outcome. The aim of serial amnioreduction is to prevent uterine stretch and preterm labor by reducing the intrauterine pressure. Usually, repeated drainage procedures of the recipient's sac are needed as amniotic fluid re-accumulates leading to a relapse of polyhydramnios. The major advantage of treatment with amnioreduction is that it is widely available. Overall perinatal survival rates in case series treated with serial amnioreduction are reported to range from 47 to 80%[12;123-130]. However, serial amnioreduction does not treat the underlying pathology and is therefore mainly a symptomatic treatment.

Fetoscopic laser coagulation of vascular anastomoses was first described by De Lia *et al* in 1990[121]. The aim of fetoscopic laser surgery is to occlude the anastomosing vessels along the vascular equator of the chorionic plate in order to interrupt the inter-twin transfusion of blood, as shown in Figure 2. After fetoscopic laser coagulation of the anastomoses, the amniotic sac of the recipient is drained a single time to reduce the polyhydramnios. Fetoscopic laser surgery is therefore a combination of a symptomatic and a causal treatment. Overall perinatal survival rates in case series treated with fetoscopic laser coagulation of vascular anastomoses vary from 48 to 71%[9;121-124;131-137]. The true efficacy of the various treatments can only be assessed with prospective, randomized controlled trials designed not only to establish the survival rate but also the long-term neurodevelopmental sequelae. Assessment of the stage of chronic TTTS before treatment is crucial to be able to compare various

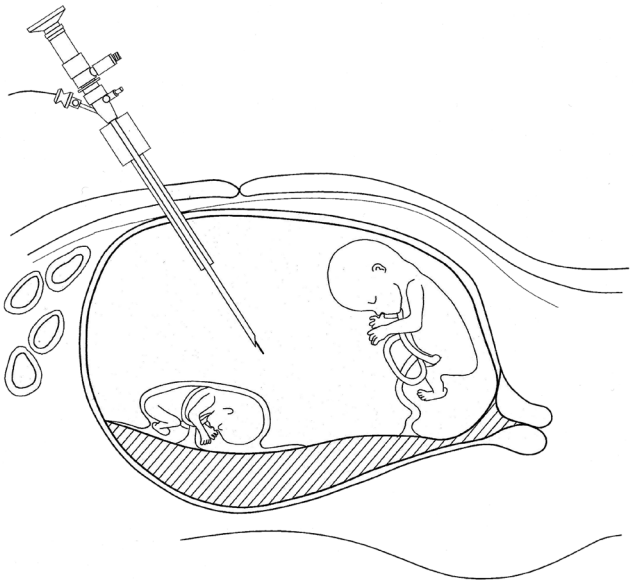

FIGURE 2 Schematic image of fetoscopic laser occlusion of vascular anastomoses in chronic twin-to-twin transfusion syndrome. The image shows a saggital view of the uterus with a posterior placenta. A fetoscope (2mm diameter) is placed under ultrasound guidance through the maternal abdominal wall into the recipient's sac with polyhydramnios. After studying the anatomy/angioarchitecture of the placenta vessels, a 0.7-mm neodymium:yttrium-aluminium-garnet (Nd:YAG) laser fiber is inserted in the fetoscope to ablate the anastomoses (from Deprest J, with permission).

treatments. Recently, Senat *et al* showed in a large multicenter randomized trial comparing fetoscopic laser surgery with serial amnioreduction that laser treatment leads to better survival rates and fewer neurological abnormalities in the neonate than serial amnioreduction[10]. The overall survival rate in the laser group was significantly higher compared to the amnioreduction group, 57% versus 41%, respectively (p = 0.01). Cystic periventricular leucomalacia grade III or IV was detected in 6% of the children in the laser group compared to 14% in the children in the amnioreduction group at 6 months of age (p = 0.02), but long-term neurodevelopmental outcome of the surviving children has not yet been reported. Two other ongoing multicenter randomized trials, one in the United Kingdom[138] and one in the United States (http://fetalsurgery.chop.edu), are also comparing serial amnioreduction with fetoscopic laser surgery. Whether fetoscopic laser coagulation of vascular anastomoses will emerge as the treatment of choice for chronic TTTS depends also on the results of these other trials. Some authors also suggest a stage-based treatment of chronic TTTS, in which milder TTTS cases are managed with amnioreduction and severe TTTS cases with laser surgery. Whether a tailored approach to management of chronic TTTS will improve outcome is currently under investigation[139].

Lastly, an international multicenter randomized controlled trial for the evaluation of septostomy versus serial amnioreduction has been performed[140]. No difference in overall survival was found between both treatments. The benefit as well as the rationale of septostomy has frequently been questioned[27;141]. Septostomy may result in complete disruption of the dividing membrane and the creation of iatrogenic monoamniotic twins[27;142]. Monoamniotic twins are known to be at risk for cord entanglement and are associated with severe perinatal morbidity and mortality. Moreover, results of a study with a mathematical model for chronic TTTS suggest that septostomy is unlikely to offer significant therapeutic efficacy[98].

A flow-chart showing the current management protocol for TTTS at our institution is presented in Figure 3.

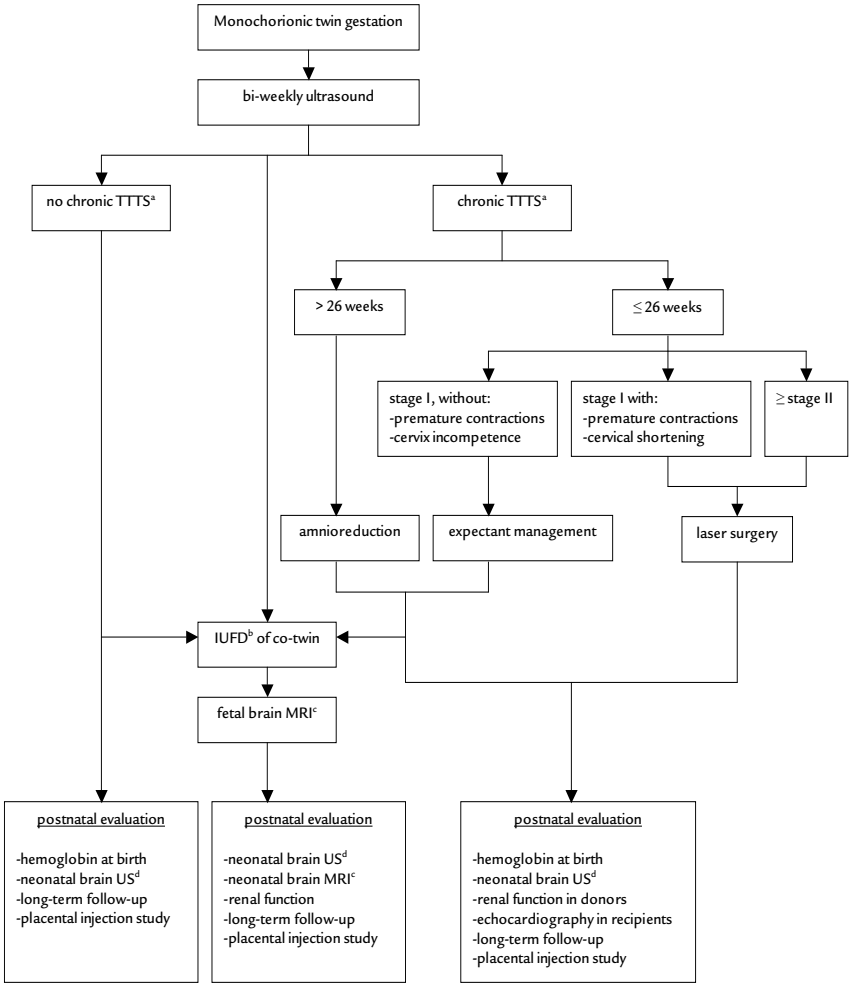

FIGURE 3 Flow-chart of antenatal and postnatal management in monochorionic twin gestations at our institution ([a]TTTS: Twin-to-twin transfusion syndrome, [b]IUFD: Intrauterine fetal demise, [c]MRI: Magnetic Resonance Imaging, [d]US: UltraSound)

Neonatal mortality and morbidity

Multifetal pregnancies have a 2 to 5 times higher risk of perinatal mortality compared to singleton pregnancies[143]. Higher mortality rates are mostly due to the higher proportion of infants with preterm delivery and low birth weights. Since preterm deliveries occur more often in multifetal gestations,

twins are also at risk for morbidity associated with prematurity such as chronic lung disease, periventricular leucomalacia, and intraventricular hemorrhage. Worse perinatal outcome is also specifically related to monochorionicity. Monochorionic twins have a significantly higher risk of adverse outcome than dichorionic twins (RR 2.5; 95% CI, 1.1-2.5)[3]. Additional morbidity found in surviving twins with TTTS includes neurological, cardiovascular and renal complications. Hypoxic-ischemic lesions to limbs, intestines and liver caused by infarctions have also been reported in TTTS survivors. Morbidity is known to be particularly high in surviving twins after intrauterine fetal death of their co-twin.

Neurological morbidity

Cerebral palsy is estimated to occur 7 times more often in twins than in singletons and is due to the higher incidence of prematurity and very low birth weight in twins[1]. The higher risk for cerebral damage in twins is also related to monochorionicity. Neurological morbidity is 7-fold higher in monochorionic twins compared to dichorionic twins[4] and is associated with selective intrauterine growth restriction[144], intrauterine fetal demise of a co-twin[145] and chronic TTTS[11;146]. Cerebral damage in chronic TTTS is presumed to be partly of antenatal origin and is related to hemodynamic disturbances[70;146]. The exact pathogenesis of cerebral damage is not fully understood yet. Hypoxic-ischemic damage caused by cerebral hypoperfusion is probably the major cause for cerebral injury in donor twins, whereas hyperviscosity and polycythemia causing vascular sludging may be an important cause for cerebral injury in the recipient[93]. Donors and recipients are equally at risk for adverse neurological outcome[12;129;146]. Early neonatal brain scans show that antenatally acquired cerebral white-matter lesions occur in 30 to 35% of survivors of chronic TTTS compared to only 3% in dichorionic twins[70;146]. Abnormalities on neonatal brain scans may also be secondary to postnatal injury associated with severe prematurity, which predisposes to periventricular leucomalacia and intraventricular hemorrhage. The incidence of major abnormal neonatal brain scans in chronic TTTS ranges from 17 to 41%[12;128;147;148]. The large difference in incidence is due to different definitions of abnormal scans

and relatively small sample sizes (inherent to the rarity of TTTS). Cerebral lesions include periventricular leucomalacia, cerebral white-matter cysts, severe intraventricular hemorrhage, cerebral infarct, ventricular dilatation and cerebral atrophy[12;128;147;148]. Minor lesions such as subependymal pseudocysts and lenticostriate vasculopathy have also been described in monochorionic twins with chronic TTTS[146;149]. Abnormal neonatal brain scans are reported less frequently in survivors of chronic TTTS treated with fetoscopic laser occlusion of vascular anastomoses than in survivors of chronic TTTS treated with serial amnioreduction[10].
Various studies have reported the long-term neurodevelopmental outcome in chronic TTTS. Rates of major neurodevelopmental deficiencies in children born after serial amnioreduction range from 5% to 25%[12;125;147;148;150]. Major neurodevelopmental sequelea in children born after fetoscopic laser coagulation of vascular anastomoses occur less frequently, and range from 2 to 11%[122;131;133;151;152]. However, results of the long-term outcome of the previously mentioned randomized trial form Senat *et al*[10] must be awaited before conclusions can be drawn on the possible beneficial aspects of laser surgery. Regardless of the antenatal treatment, the reported risks of neurological impairment in chronic TTTS remain high. Careful neurodevelopmental follow-up of all TTTS survivors is therefore of major importance to rule out cerebral palsy and global developmental delay. Whether neurological disabilities are mainly due to chronic TTTS or also partly caused by hemodynamic disturbances which may also occur in monochorionic twins without chronic TTTS, remains unclear. To address this issue, we are currently assessing the differences between long-term neurodevelopmental outcome between monochorionic twins with chronic TTTS treated with fetoscopic laser occlusion of vascular anastomoses and monochorionic twins without chronic TTTS[153].

Cardiovascular morbidity

Cardiovascular complications occur more frequently in monochorionic twins than in singletons, respectively 3.8% versus 0.6% and are mostly due to cardiac dysfunction occurring in monochorionic chronic TTTS gestations[19]. Congenital heart disease occurs 12 times more frequently in

monochorionic twins with chronic TTTS than in the general population and is found mainly in recipient twins[19]. Reported cardiovascular morbidity can be transient, progressive and sometimes persist beyond the neonatal period and includes fetal hypertension[83;154], hypertrophic cardiomyopathy[18;19;155], tricuspid regurgitation[156], left chamber myocardial infarction[157], pulmonary artery calcification[158] and right ventricular outflow tract obstruction[18;159;160].

Two theories have been postulated to explain the pathogenesis of cardiovascular morbidity in recipients with chronic TTTS. The first theory suggests that cardiovascular complications are a consequence of increased preload due to chronic hypervolemia causing cardiac hypertrophy. The second theory states that cardiovascular morbidity is due to an increased afterload induced by elevated levels of vasoconstrictive substances such as endothelin-1 found in recipients[84]. Higher rates of hypertension reported in recipients may support this second theory[12;83;154]. Whether cardiomyopathy in the recipient is primarily due to increased afterload or increased preload, is still open for debate.

Biventricular hypertrophy with prevalent left ventricular hypertrophic cardiomyopathy may be present in 40 to 100% of the recipient twins depending on the criteria[18;155;156;161]. Fetal hypertrophic cardiomyopathy may lead to hydrops fetalis in 10 to 15% of the recipient twins[161]. Nevertheless, hypertrophic cardiomyopathy appears to be reversible in most cases after delivery[155]. Cardiac hypertrophy may also lead to a functional obstruction of the outflow tract of the right ventricle, due to valvular or subvalvular pulmonary stenosis. Right ventricular outflow tract obstruction occurs in 5 to 11% of the recipients and is associated with high mortality rates[19;159;162]. Right ventricular outflow tract obstruction may be progressive and require urgent treatment with pulmonary balloon valvuloplasty or surgery after birth[18;159;163]

Improvement of cardiac function after delivery is also often reported in recipient twins, suggesting that removal of the causal factors helps the heart function to recover[155]. Whether removal of the causal factors by fetoscopic laser occlusion of the vascular anastomoses also results in a lower incidence of cardiac dysfunction in chronic TTTS is not yet known. We are currently performing a prospective study on echocardiographic abnormalities in monochorionic twins with chronic TTTS treated with laser

surgery compared to a control group of monochorionic twins without chronic TTTS[153].

Renal morbidity

Various renal complications have been reported in donor twins in TTTS and include renal cortical necrosis and fibrosis[164], transient renal insufficiency and hematuria[20], acute renal failure requiring long-term peritoneal dialysis[165], or permanent tubular dysfunction with polyuria due to renal tubular dysgenesis[21;166]. Autopsy studies in chronic TTTS report that renal tubular dysgenesis, characterized by loss of proximal convoluted tubules, is found in almost 50% of donor twins[21]. The pathogenesis of glomerular and tubular injury is probably secondary to hypoxic-ischemic injury due to chronic prenatal renal hypoperfusion in donor twins. Even though oliguric renal failure occurs frequently in donor twins, complete recovery of adequate renal function is usually reported[148]. Renal injury in donor twins may also lead, sporadically, to the development of chronic renal insufficiency requiring dialysis and kidney transplantation[12;165]. Renal function should be monitored carefully at birth in donor twins with TTTS by measuring urine output and serial serum creatinin levels to rule out renal insufficiency.

Antenatal hypoxic-ischemic lesions

Antenatal hypoxic-ischemic injury to various organs has been described mainly in recipient twins with TTTS, and includes lower limb necrosis[167-169], intestinal injury such as ileal atresia[170], and hepatic infarctions[171]. Most pathophysiologic mechanisms proposed to explain these injuries are based on hemodynamic disturbances. Necrotic tissue injury and gangrene can result from vascular sledging and peripheral ischemia due to the polycythemia-hyperviscosity syndrome since most of these injuries occur in recipient twins[167;168]. Idiopathic thrombosis, thrombotic injury induced by laser-surgery, anomalous vasculature and umbilical artery steal phenomenon have also been proposed as possible mechanisms[167;170;172].

Morbidity due to intrauterine fetal demise of co-twin

Risk of death or severe cerebral damage in the surviving twin after intrauterine fetal demise of a co-twin is 3 to 4-fold higher in monochorionic than dichorionic twins[14;32;173]. In the past, damage was thought to occur due to the release of thromboplastin and necrotic emboli from the demised fetus into the circulation of the surviving twin, causing disseminated intravascular coagulation, embolization and infarction[174-176]. However, current opinion is that damage results from acute exsanguination of the surviving twin into the low-pressure circulation of the demised co-twin, causing severe hypoxic-ischemic damage[30;32;70;173].
Severe neurological sequelae, such as multicystic encephalomalacy, occur in 20 to 40% of surviving twins after intrauterine fetal death of a co-twin[11;13-16]. Careful antenatal radiological evaluation by ultrasound or fetal magnetic resonance imaging should be performed after intrauterine fetal demise of a co-twin in a monochorionic twin gestation. Fetal magnetic resonance imaging detects brain damage earlier and with better definition of the brain abnormalities than ultrasound[177]. A multitude of abnormalities on neuro-imaging studies have been reported, including multicystic encephalomalacy, white-matter infarctions, hydranencephaly, holoprosencephaly, lisencephaly, polymicrogyria, and cortical atrophy associated with ventriculomegaly[178;179]. The type of cerebral abnormalities probably depends on the timing of intrauterine fetal demise of the co-twin. Intrauterine fetal demise of the co-twin in the early second trimester may lead to abnormalities related to disturbances in neuronal migration, such as polymicrogyria, whereas demise of the co-twin later during the third trimester may result in abnormalities typically associated with cerebral ischemic injury occurring in late gestation, such as white-matter infarctions or multicystic encephalomalacy[178;180].
Spontaneous fetal reductions in twin pregnancies diagnosed in the first trimester are not uncommon[181]. Approximately 30% of twin pregnancies will ultimately result in singletons[181]. This so-called “vanishing” twin phenomenon in monochorionic twins may also lead to severe cerebral damage[182]. Some authors hypothesize that a significant proportion of singletons with cerebral palsy of unknown etiology may in fact be surviving co-twins of undetected, initial monochorionic twin pregnancies[183].

A recent publication of a case report on multicystic encephalomalacy after first-trimester intrauterine fetal death in a monochorionic twin pregnancy, supports this hypothesis, and concludes that obstetricians and pediatricians should be aware that severe cerebral injury may occur earlier than previously thought[17]. Intrauterine fetal demise of a co-twin can also lead to renal cortical necrosis and subsequent renal failure, as well as splenic and hepatic infarctions in the surviving co-twin[174;184].

Conclusion

Though recent research has shed more light on the development of TTTS, the multitude of complex pathophysiologic mechanisms involved in the development of TTTS needs further exploration. The intrauterine management of TTTS remains a significant challenge in perinatal medicine. Although significant improvement in perinatal survival in TTTS has been achieved, this syndrome remains one of the most lethal conditions in perinatal medicine. Ongoing randomized controlled trials comparing different therapeutic regimens may eventually lead to universally accepted treatment protocols.
Considering the high incidence of cerebral white-matter damage, early neonatal brain scans and careful neurodevelopmental follow-up should be performed in all surviving twins to rule out severe neurological disabilities. Awareness of cardiovascular complications occurring in recipient twins and renal complications in donor twins may help improve neonatal care for these children. Surviving twins after intrauterine fetal demise of their co-twin are at high risk for developing severe cerebral and renal sequelae and require therefore adequate and complete work-up after birth.
Lastly, continuing close collaboration between obstetricians and neonatologists is crucial in order to improve the care of infants with TTTS.

Part 1
Placental anastomoses

Chapter 3

Assessment of feto-fetal transfusion flow through placental arterio-venous anastomoses in a unique case of twin-to-twin transfusion syndrome

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Placenta, Epub ahead of print

Abstract

Objective: In vivo measurements of blood flow through arterio-venous anastomoses in monochorionic twin placentas have recently been attempted with Doppler ultrasound, but the accuracy is questionable. We present a new method to determine the arterio-venous anastomotic blood flow.

Methods: Detailed description of a unique twin-to-twin transfusion syndrome case treated with fetoscopic laser surgery and subsequently with an intrauterine blood transfusion. Prospective measurements of decreasing hemoglobin levels between the intrauterine transfusion and birth allowed us to assess the net blood flow through the residual anastomoses.

Results: A case of twin-to-twin transfusion syndrome was treated with fetoscopic laser surgery at 27 weeks' gestation. The ex-recipient subsequently became severely anemic and was treated with an intrauterine blood transfusion at 29 weeks' gestation. After birth, a placental injection study identified five residual unidirectional arterio-venous anastomoses from the ex-recipient to the ex-donor without arterio-venous anastomoses in the opposite direction. The net feto-fetal blood flow through the five residual arterio-venous anastomoses was determined to be 27.9 mL/24h.

Conclusions: We found the blood flow across a single arterio-venous anastomosis at 29 weeks' gestation to be 5.6 mL/24h, much lower than previously measured with Doppler ultrasound. This finding may also explain the inaccuracy of Doppler flow measurements, as such low flow velocities cannot possibly be detected with current Doppler techniques.

Introduction

Twin-to-twin transfusion syndrome (TTTS) is the most common complication in monochorionic twin pregnancies and is associated with high perinatal morbidity and mortality rates[186]. TTTS is attributed to imbalanced inter-twin blood transfusion through placental anastomoses, leading to hypovolemia and oligohydramnios in the donor twin and hypervolemia and polyhydramnios in the recipient twin. Despite major advances in this field, the exact pathogenesis of TTTS remains incompletely understood[186]. Lack of a suitable experimental animal model has hampered further investigation on the development of TTTS. Computer modeling for TTTS has helped to elucidate several pathophysiological mechanisms[187]. *In vivo* assessment of blood flow through an arterio-venous (AV) anastomosis would help to further unravel the complex pathophysiology of TTTS. Recently, several attempts have been made to measure anastomotic blood flow using Doppler ultrasound[188;189]. However, the results of these studies are highly discordant and lead to a fierce debate on the "correct" blood flow down an AV anastomosis[190].
In a case of TTTS treated with fetoscopic laser surgery, the ex-recipient became severely anemic due to residual AV anastomoses and required an intrauterine blood transfusion 48 hours before emergency delivery. This offered the opportunity to quantify the inter-twin blood flow through the unidirectional AV anastomoses with a novel method by analyzing the decrease in hemoglobin (Hb) concentrations at different time points.

Case presentation

A 31-year-old gravida 1 para 0 was referred to our institution at 27 + 0 weeks' gestation with signs of TTTS (Quintero stage II) and was treated with fetoscopic laser coagulation of vascular anastomoses. Serial ultrasound examinations after laser surgery showed normalization of amniotic fluid in both sacs and normal bladder filling in both fetuses. However, Doppler measurement of the middle cerebral artery peak systolic velocity (MCA-PSV) in the ex-recipient gradually increased up to 120 cm/sec (> 2 Multiples of Median)[191] suggesting the presence of severe fetal

anemia. An intrauterine blood transfusion was performed at 29 + 3 weeks' gestation. A total volume of 53 ml of blood with an Hb concentration of 27.2 g/dL was transfused during 30 minutes. Hb concentrations in the ex-recipient before and after the transfusion were 3.0 g/dL and 11.2 g/dL, respectively. However, within 48 hours after the intrauterine transfusion, MCA-PSV Doppler studies showed again signs of severe fetal anemia and cardiotocography demonstrated a sinusoidal pattern. A caesarean section was performed 48 hours after the intrauterine transfusion. The first-born twin (ex-donor) was plethoric and weighed 1210 g. The second-born twin (ex-recipient) was pale and weighed 1527 g. Hb concentrations in twin 1 and twin 2 were 24.3 g/dL and 7.7 g/dL, respectively. Blood pressures at birth in twin 1 and twin 2 were 53/32 mmHg and 47/27 mmHg, respectively.

Macroscopic examination of the placenta revealed a fetoscopic hole in the membranes of twin 2, confirming that the second-born twin was the ex-recipient. Injection with color-latex showed four small residual unidirectional AV anastomoses from the ex-recipient to the ex-donor with a diameter of about 0.5 mm (Figure 1). A residual arterio-arterial (AA) anastomosis was also detected. However, this anastomosis was initially not detected after dye injection, but became patent only after injection with increased pressure and forced manual compression of the dye. Placental casting showed additionally a shared cotyledon underneath the placental surface connecting an ex-recipient chorionic artery with an ex-donor chorionic vein, hence a fifth unidirectional AV anastomosis.

Calculation of blood transfusion

As a result of the intrauterine transfusion, the ex-recipient received 53 mL blood of 27.2 g/dL, which equals 14.4 g of Hb, resulting in an increase in Hb concentration from 3.0 g/dL to 11.2 g/dL. We used the method described by Hoogeveen *et al* to calculate the dilution of fetal Hb[192], with a post-transfusion feto-placental blood volume supposed to return to the pre-transfusion volume, i.e. 149 mL. The AV transfusion rate can then be calculated from the known amount of Hb transfused from ex-recipient to ex-donor, assuming that the AV flow and the ex-recipient's blood volume

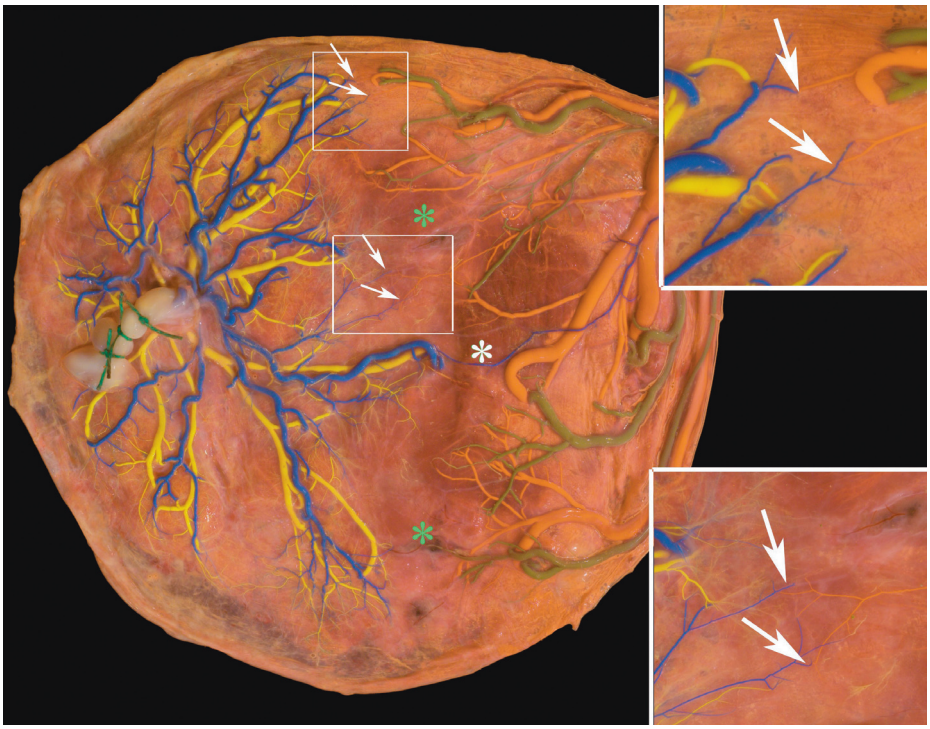

FIGURE 1 Monochorionic placenta after dye-injection (blue or green for arteries and orange or yellow for veins). The white arrows indicate the residual AV anastomoses from the ex-recipient (left side) to the ex-donor (right side). Details of the four residual anastomoses between arteries of the ex-recipient (blue) and veins of the ex-donor (orange) are shown in the top-right and bottom-right corner. The green stars show the obliterated anastomoses after laser coagulation. The white star indicates the obliterated but during dye-injection fiercely re-opened arterio-arterial anastomosis.

remain unaltered during the 48 hours following intrauterine transfusion. This is by standard physics as follows:
The change of the ex-recipient's Hb concentration, $d[Hb]_t$, at time t (t is between the moment of transfusion, at t = 0, and birth, at t = 48 hours), in an infinitesimal short period of time, dt, equals the amount of ex-recipient Hb concentration $[Hb]_t$ transfused to the other twin by the AV in time period dt. The ex-recipient's decrease in Hb concentration is the grams of Hb transfused, i.e. $[Hb]_t$ times AV_{flow} times dt, divided by the ex-recipient's blood volume, or

$$d[Hb]_t = -\frac{AV_{flow} = \cdot [Hb]_t}{BloodVol} \cdot dt \quad (1)$$

The minus sign just implies that the increase in $[Hb]_t$ which is $d[Hb]_t$ is negative. This equation actually represents the following standard differential equation (i.e. dividing by dt)

$$\frac{d[Hb]_t}{dt} = -\frac{AV_{flow}}{BloodVol} \cdot [Hb]_t \quad (2)$$

with solution

$$[Hb]_t = [Hb]_{t=0} \exp\left[-\frac{AV_{flow}}{BloodVol} t\right] \quad (3)$$

Dividing by $[Hb]_{t=0}$, taking the natural logarithm of both sides, and solving for the AV_{flow} (in mL/h) gives

$$AV_{flow} = \frac{BloodVol}{-48} \cdot \ln\left(\frac{[Hb]_{t=48}}{[Hb]_{t=0}}\right) \quad (4)$$

When the begin-Hb and end-Hb concentrations, i.e. at the moment of transfusion $[Hb]_{t=0}$ and at birth $[Hb]_{t=48}$, and the blood volume are substituted in equation 4, i.e. $[Hb]_{t=0}$ = 11.2, $[Hb]_{t=48}$ = 7.7, $BloodVol$ = 1.49 dL, the solution of the combined AV flow yields

$$AV_{flow} = 27.9 \text{ mL/24h} \quad (5)$$

Assuming that the blood flow through each of the five AV anastomoses was equal, the net transfusion flow through a single AV anastomosis was 5.6 mL/24h.

Comment

This study reports a unique case of TTTS that allowed us to assess the anastomotic blood flow through the placental anastomoses. Unfortunately, we did not measure the percentage of transfused adult red cells or adult Hb in the ex-donor after birth. This would have given us yet another method to calculate the feto-fetal transfusion during the last 48 hours before birth and thus to confirm the above calculations. Through the calculations in this paper, we found the blood flow across a single AV anastomosis at 29 weeks to be 5.6 mL/24 h.

This flow is in amazing agreement with the flow through an arterio-arterial anastomosis at 28 weeks ($7.6 \pm 4.0 \times 10^{-8}$ L/s, which equals 6.6 ± 4.2 mL/24h)[188;189], which approximately equals the oppositely directed AV transfusion during steady state[100]. In contrast, Nakata *et al* measured in vivo AV blood flows of up to 25 mL/min (36 L/24 h) using Doppler flow during a fetoscopic procedure[189]. Feto-fetal blood flow of this magnitude, however, would lead to fatal acute hemorrhagic shock in the donor fetus within a few minutes and is thus physiologically implausible[190]. Previously, using micro-bubble contrast angiography, Denbow et al. measured an inter-twin transit time of 65 s[193]. in a placenta that included an anastomotic pattern of two AVs and one AA (Dr Mark L Denbow, personal communication). In our case of unidirectional AVs, assuming a 4 cm anastomotic length and a diameter of 0.5 mm, the transit time would be about 120 s. Interestingly, our TTTS mathematical model predicts AV flows that cause severe, i.e. Quintero stage IV, TTTS of about 10 to 15 mL/24 h[187], in excellent agreement with the value reported in this paper.

In conclusion, by direct measurement of Hb concentration decrease in a unique case of TTTS with known anastomotic pattern, we were able to estimate the feto-fetal transfusion flow through placental arterio-venous anastomoses. This information can now be used to further develop improved computer models and to help elucidate the pathophysiology of TTTS.

Chapter 4

Velamentous cord insertion and unequal placental territories in monochorionic twins with and without twin-to-twin transfusion syndrome

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Submitted

Abstract

Objective: To determine the incidence of velamentous cord insertion and placental territory discordancy in monochorionic twins with and without TTTS.

Methods: All consecutive placentas of monochorionic twins delivered at our center between June 2002 and April 2006 were studied with vascular injection of the umbilical vessels with colored dyes. Velamentous cord insertions were recorded and placental territories were calculated by computer analysis.

Results: A total of 76 monochorionic placentas with TTTS and 63 monochorionic placentas without TTTS were studied. The incidence of velamentous cord insertion (per fetus) in the TTTS group and the no-TTTS group was 13% (20/152) and 14% (18/126), respectively ($p = 0.79$). Placental territory discordancy in the TTTS group and the no-TTTS group was 20% and 20% ($p = 0.83$). In the TTTS group, donor twins had more often a velamentous cord insertion than recipient twins (24% and 3%, respectively, $p < 0.001$) and smaller placental territories (44% and 56%, respectively, $p < 0.001$).

Conclusions: Our findings suggest that velamentous cord insertion and placental territory discordancy are not critical factors for the development of TTTS.

Introduction

Chronic twin-to-twin transfusion syndrome (TTTS) is a complication of monochorionic twin pregnancies and results from inter-twin blood transfusion via placental vascular anastomoses. Although vascular anastomoses are invariably found in almost all monochorionic placentas, only 10-15% of monochorionic twins will eventually develop TTTS[6;27]. Differences in angio-architecture, amongst those the absence of arterio-arterial anastomoses, are one of the major factors involved in the development of TTTS[6;27;186;194]. However, angio-architecture alone does not fully explain the pathophysiology of TTTS[6;27;186;194]. Several other hypotheses on the pathophysiology of TTTS have been proposed, including utero-placental insufficiency and paradoxical activation of fetal vasoactive and humoral factors[186;194].

Several authors found a higher incidence of velamentous cord insertions in TTTS placentas and hypothesized that velamentous cord insertion and unequal placental territories may lead to utero-placental insufficiency, subsequently establishing a vicious cycle resulting in the development of TTTS[89-91;113]. However, these hypotheses were mostly unsubstantiated or based on small studies[89-91;113]. Moreover, two recent reports show that the incidence of velamentous or marginal cord insertion is similar in monochorionic twins with and without TTTS[195;196].

The objective of this study was to determine the incidence of velamentous cord insertions and placental territory discordancy in monochorionic twins with and without TTTS.

Materials and methods

All consecutive placentas of monochorionic twin pregnancies examined at our center between June 2002 and April 2006 were included in this study. Monochorionicity was confirmed after delivery by gross examination of the dividing membrane and/or histopathological examination of the placenta and the dividing membrane. Placentas were divided in a group with TTTS and a group without TTTS. TTTS was diagnosed using standard antenatal ultrasound criteria[105]. The Leiden University Medical Center is a tertiary

medical center and it is the national referral center for fetal therapy in the Netherlands, including laser treatment for TTTS. Most TTTS cases referred to our center were therefore treated with laser.
During prenatal ultrasound in TTTS twin pairs, great care was taken to define which fetus, donor or recipient, would be born first. At delivery, umbilical cords were labeled to identify the first and second-born twin. The type of abnormal umbilical cord insertion, velamentous or marginal insertion (within 1 cm of placental margin), was recorded. Placental angio-architecture was studied by injecting the umbilical vessels of both cords with different colored dyes. Arteries were injected with (dark-) blue dye whereas veins were injected with orange or yellow dye. Placentas were then photographed in a plane view, and the picture was saved for computer analysis. Each fetal territory was measured by following the margins demarcated by the presence of color specific dye. Individual placental territories were measured using Image Tool for Windows version 3.0 (Image Tool, San Antonio, Texas, USA) and expressed as a percentage of the total area. The percentage of individual placental territory was calculated by dividing each individual placental territory by the sum of both territories. Placental territory discordancy was then determined by subtracting the percentage of individual placental territory from one fetus with the percentage of individual placental territory from the other fetus. The same formulas were used to determine the percentage of birth weight share per infant and inter-twin birth weight discordancy. Placentas with intrauterine fetal demise were excluded when placental maceration prohibited accurate evaluation of type of umbilical cord insertion and placental territory discordancy. Placentas of monochorionic monoamniotic pregnancies and higher multiple pregnancies were also excluded from the study.
Results of categorical variables were compared using Chi-squared test. Continuous variables were analyzed with the Independent Samples T- test. For comparisons between donors and recipients, the Paired Samples T-Test and Mc Nemar test was used. A p-value < 0.05 was considered to indicate statistical significance. Statistical analysis was performed with SPSS for Windows version 11.0 (SPSS, Inc., Chicago, Illinois, USA).

Results

The number of consecutive monochorionic diamniotic placentas delivered and examined at our center during the study period was 161. The data required for this study could not be recorded completely for 22 placentas (nine in the TTTS-group and thirteen in the no-TTTS group) because of placental maceration caused by intrauterine fetal demise (n = 9), placental fragmentation (n = 3), placenta fixation in formalin (n=1), loss or destruction of the placenta after delivery (n = 9). These 22 cases were excluded from further analysis. A total of 76 monochorionic placentas with TTTS and 63 monochorionic placentas without TTTS were included in the study. Mean gestational age at birth in the TTTS group and no-TTTS group was 30.8 weeks (range: 17 to 38 weeks) and 33.8 weeks (range: 25 to 38 weeks), respectively. The monochorionic pregnancies with TTTS were treated with fetoscopic laser coagulation (n = 61), amniodrainage (n = 10) or without intrauterine intervention (n = 5).
The overall incidence of velamentous cord insertion per fetus in all monochorionic twin pregnancies was 13% (37/278). Type of umbilical cord insertion and difference in placental territories between the TTTS group and no-TTTS group are presented in Table 1. Examples of monochorionic placentas with velamentous cord insertion and unequal placental territories are shown in figures 1, 2, 3 and 4 (pictures are taken after colored dye injection).

TABLE 1 Type of umbilical cord insertion and placental territory discordancy in monochorionic pregnancies with and without TTTS.

	TTTS group (n = 76 placentas)	no-TTTS group (n = 63 placentas)	p-value
Velamentous cord insertion – no (%)[a]	20 (13%)	18 (14%)	0.79
Marginal cord insertion – no (%)[a]	49 (33%)	33 (26%)	0.27
Velamentous or marginal cord insertion – no (%)[a]	69 (45%)	52 (41%)	0.49
Placental territory discordancy - %[b]	20 ± 14	20 ± 15	0.83
Placental territory discordancy > 20% - no (%)	31 (41%)	25 (40%)	0.64

[a] *Refers to the type of cord insertion per fetus*
[b] *Value given as mean ± SD*

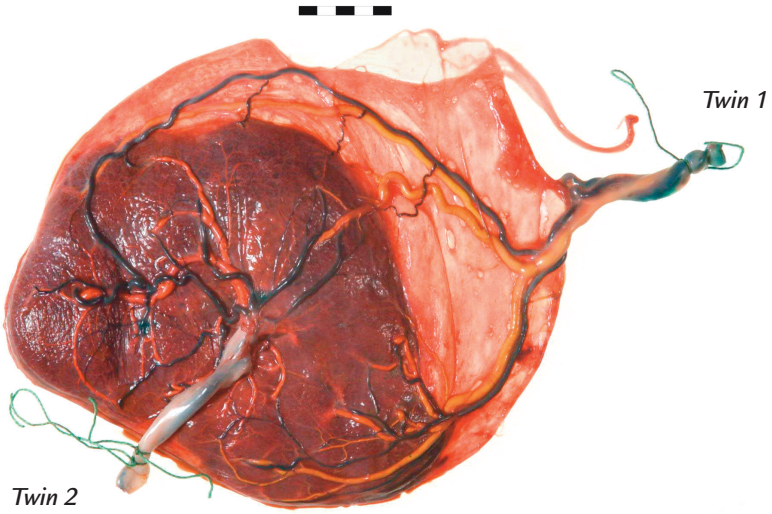

FIGURE 1 Monochorionic placenta without TTTS. Twin 1 has a velamentous cord insertion and a placental territory of 36%, whereas twin 2 has a central cord insertion and a placental territory of 64%.

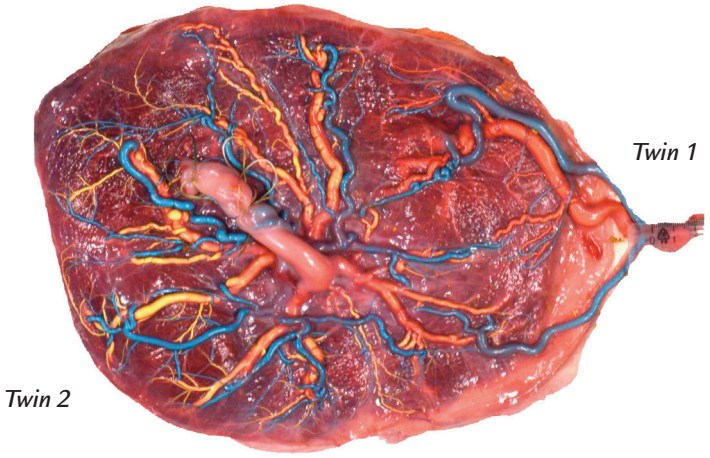

FIGURE 2 Monochorionic placenta without TTTS. Twin 1 has a velamentous cord insertion and a placental territory of 21%, whereas twin 2 has a paracentral cord insertion and a placental territory of 79%.

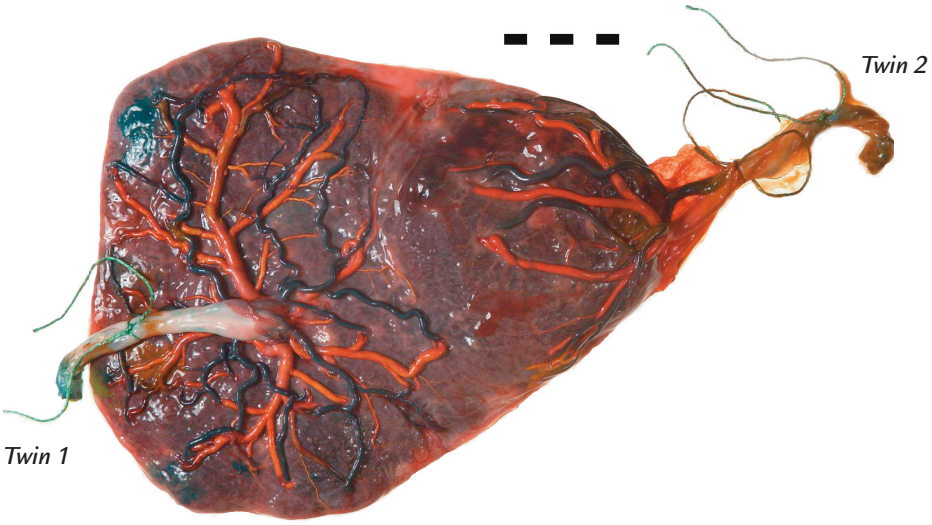

FIGURE 3 Monochorionic placenta with TTTS treated with fetoscopic laser surgery. Twin 1 (ex-recipient) has a central cord insertion and a placental territory of 73%. Twin 2 (ex-donor) has a velamentous cord insertion and a placental territory of 27%.

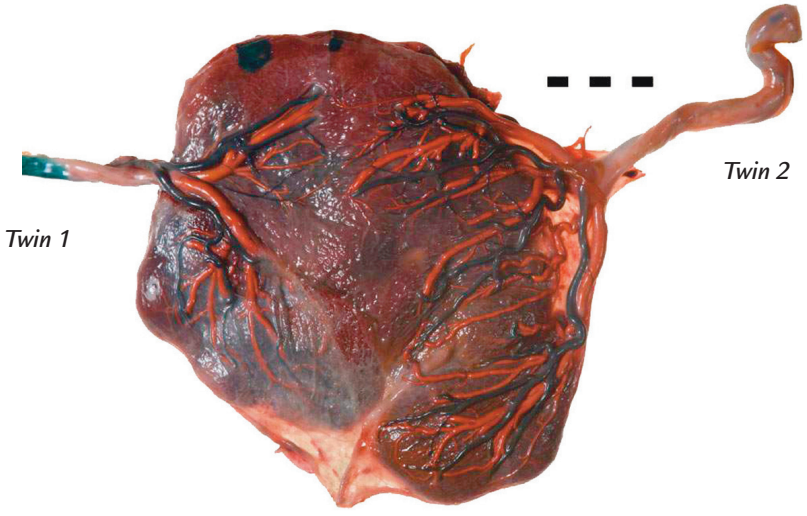

FIGURE 4 Monochorionic placenta with TTTS treated with amniodrainage. Twin 1 (donor) has a marginal cord insertion and a placental territory of 47%, whereas twin 2 (recipient) has a velamentous cord insertion and a placental territory of 53%.

Type of abnormal umbilical cord insertion, placental territory discordancy and birth weight difference between donor and recipient twins in the TTTS group are presented in Table 2.
Differences between monochorionic twins with and without velamentous cord insertion are presented in Table 3.

TABLE 2 Type of umbilical cord insertion, birth weight and individual placental territories in donor and recipient twins with TTTS.

	Donor (n = 76)	Recipient (n = 63)	p-value
Velamentous cord insertion – no (%)[a]	18 (24%)	2 (3%)	< 0.001
Marginal cord insertion – no (%)[a]	30 (39%)	19 (25%)	0.06
Velamentous or marginal cord insertion – no (%)[a]	48 (63%)	21 (28%)	< 0.001
Birth weight – g[b]	1547 ± 723	1763 ± 739	< 0.001
Birth weight share – %[b]	46 ± 6	54 ± 6	< 0.001
Individual placental territory - %[b]	44 ± 11	56 ± 11	< 0.001

[a] *Refers to the type of cord insertion per fetus*
[b] *Value given as mean ± SD*

TABLE 3 Differences between twins with and without velamentous cord insertion.

	Velamentous insertion[a] (n = 38)	No velamentous insertion[a] (n = 240)	p-value
Gestational age at birth– weeks[b]	30.7 ± 5.1	32.4 ± 4.4	0.036
Birth weight – g[b]	1454 ± 672	1914 ± 755	0.001
Individual placental territory - %[b]	36 ± 10	52 ± 11	< 0.001

[a] *Refers to the type of cord insertion per fetus*
[b] *Value given as mean ± SD*

Discussion

In this large single center study we report on the difference in velamentous cord insertion and discordant placental territories between monochorionic twin placentas with and without TTTS. We found no difference in velamentous cord insertion between monochorionic placentas with and without TTTS.

Velamentous cord insertions are rare in singleton placentas (2%) and far more common in dichorionic (7%) and particularly in monochorionic twin placentas (12%)[197]. The high incidence of velamentous cord insertions in monochorionic twin placentas is thought to result from a "battle" for space between each twin's placental shares, a competition process also called trophotropism[26;198;199]. Velamentous cord insertions are associated with smaller placental mass and lower birth weights[26;198;200;201].

In monochorionic twinning, velamentous cord insertions have also been related to the development of TTTS. In a study of 38 monochorionic placentas, Fries *et al*[89] reported a significantly higher prevalence of velamentous cord insertion in TTTS placentas than in no-TTTS placentas, 32% (7/22) and 9% (5/54), respectively ($p < 0.01$). In view of this finding, Fries *et al* proposed an etiologic role for velamentous cord insertion in the development of TTTS[89]. As a velamentous inserted cord can be easily compressed, Fries *et al* suggested that TTTS could result from hemodynamic instability due to reduced blood flow to the donor twin with a velamentous inserted cord. However, the number of placentas studied was small. Moreover, 3 of the 38 (8%) monochorionic pregnancies were monoamniotic. This probably represents a selection bias, as placental angio-architecture, type of umbilical cord insertion and incidence of TTTS are known to be different in monoamniotic and diamniotic monochorionic pregnancies[60]. In a study of 60 monochorionic placentas, Machin[90] reported that roughly 30% of twins with velamentous or marginal cord insertion have TTTS, whereas only 6% (1/17) of twins without velamentous or marginal cord insertion develop TTTS. However, exact data on the number of placentas with TTTS was not mentioned. Contrarily, in a study of 58 monochorionic twin pregnancies, Bajoria report similar frequencies of velamentous cord insertion in TTTS and no-TTTS twins (16% and 19%, respectively)[196]. In a recent study of 89 consecutive

monochorionic placentas, De Paepe *et al* also found a similar prevalence of velamentous or marginal cord insertion in TTTS and no-TTTS placentas (37% and 36%, respectively)[195]. In another (unpublished) series of 90 monochorionic placentas, Taylor *et al* also found equally high incidence of velamentous cord insertion in TTTS and no-TTTS placentas (53% and 52%, respectively)[93]. Our findings are in agreement with the findings of Bajoria, De Paepe *et al* and Taylor *et al* and challenge the existence of a causative relationship between velamentous cord insertion and the development of TTTS.

This study also shows a similar frequency of placental territory discordancy in monochorionic placentas with and without TTTS. Only a few studies have expanded on the relationship between placental asymmetry and development of TTTS. In a small uncontrolled study of 9 monochorionic pregnancies, Bruner *et al* speculated that TTTS may result from asymmetrical placental insufficiency[89-91;113]. According to this hypothesis, placental vascular anastomoses may become functional and lead to TTTS when resistance to placental perfusion reaches a threshold. In a study of 89 monochorionic placentas, De Paepe *et al* found uneven placental distribution (defined as greater than 25% discordance between the two placental territories) more frequently in TTTS placentas than in no-TTTS placentas (73% and 24%, respectively, $p < 0.005$)[195]. However, in the same study, De Paepe *et al* found a similar incidence in abnormal cord insertion[195]. We, as well as others, have found that velamentous cord insertions are associated with smaller placental territories and smaller birth weights[26;90;198;201]. Also, the method used to determine the percentage of each twin's portion of the placenta was not described in de study from De Paepe *et al*[195]. If estimations of placental territories were performed by rough approximation, an obvious bias may have been introduced. In contrast, we used computer analysis to calculate placental territories. Our findings are in agreement with a recent study of 133 monochorionic placentas from Quintero *et al*[202], showing similar frequencies of placental territory discordancy in monochorionic placentas with TTTS and without TTTS. Similar placental territory discordancy in TTTS and no-TTTS placentas suggests that a causal relationship between unequal placental territories and the development of TTTS is highly improbable.

Finally, this study shows a significant difference in placentation between

donor twins and recipient twins with TTTS. We found that donor twins are more likely to have a velamentous or marginal cord insertion and smaller placental territories than recipient twins. In a study of 71 TTTS placentas, Quintero *et al* also found significant differences in individual placental territories between donor twins and recipient twins (44% versus 55%, respectively, $p < 0.001$)[202], in agreement with our results. In contrast, in a smaller study of 32 TTTS placentas, Bajoria found no difference in velamentous or marginal cord insertions between donor and recipient twins (65% versus 59%, respectively)[196].

Our findings suggest an association between abnormal cord insertion, smaller placental territories and being a donor in TTTS. The cause of this high frequency of abnormal cord insertion and smaller placental territory in donor twins is not known. Placental formation is directly related to placental angiogenesis. Various factors, such as vascular endothelial growth factors and fibroblast growth factors, as well as vascular flow are suggested to play an important role in placental vascularization[195;203]. We speculate that abnormal cord insertion and smaller placental territories in donors are not a cause but a consequence of TTTS. As the donor twin becomes hypovolemic, vascular perfusion of its placental territory diminishes resulting in a decrease in villous and capillary surface areas. This process may then lead to a reduced expansion of placental cotyledons, particularly those on the outer-side of the donor's placenta, resulting in a smaller placenta with a marginal or velamentous inserted cord. Cotyledons from the donor's placenta that are also perfused through anastomoses by the recipient have a greater probability to remain intact. Fetal hypovolemia and placental hypoperfusion in donors may also explain the difference in vascular distribution patterns between donors and recipients found in the study by De Paepe *et al*[195].

In conclusion, our findings show that the frequency of velamentous cord insertion and placental territory discordancy is similar in TTTS and no-TTTS monochorionic twins, challenging the notion of a causative relationship with the development of TTTS.

Chapter 5

Residual anastomoses after fetoscopic laser surgery in twin-to-twin transfusion syndrome: frequency, associated risks and outcome

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Abstract

Objective: To study the incidence and clinical implications of residual anastomoses in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery

Methods: We examined all placentas treated with fetoscopic laser surgery and delivered at our center between June 2002 and December 2005 with vascular injection using colored dyes. Presence of residual anastomoses was studied in association with adverse outcome and inter-twin hemoglobin difference at birth. Adverse outcome was defined as fetal demise, neonatal death or severe cerebral injury. The relation between residual anastomoses and placental localization (anterior or posterior uterine wall) was evaluated.

Results: A total of 52 laser-treated placentas were studied. Residual anastomoses were detected in 33% (17/52) of placentas. Adverse outcome was similar in the groups with and without residual anastomoses, 18% (6/34) and 29% (20/70), respectively ($p = 0.23$). Large inter-twin hemoglobin differences (> 5 g/dL) were found in 65% (11/17) of cases with residual anastomoses and 20% (7/35) of cases without residual anastomoses ($p < 0.01$). Anterior placental localization was not associated with a more frequent presence of residual anastomoses.

Conclusions: Residual anastomoses at our institution are seen in one third of monochorionic placentas treated with fetoscopic laser surgery. Although residual anastomoses in our study were not associated with adverse outcome, they were often associated with neonatal hematological complications.

Introduction

Twin-to-twin transfusion syndrome (TTTS) presenting as oligo-polyhydramnios sequence is a major complication of monochorionic twin pregnancies and is due to inter-twin blood transfusion via placental vascular anastomoses. Fetoscopic laser coagulation of placental vascular anastomoses is nowadays considered to be the treatment of choice in severe second trimester TTTS. Fetoscopic laser surgery in TTTS is a causative treatment and is associated with significantly improved outcome compared to serial amniodrainage[10].

The aim of laser coagulation of vascular anastomoses is to completely separate the inter-twin placental circulation. Careful examination of the placenta after birth through injection studies is required to determine whether all vascular connections have been adequately coagulated or whether residual anastomoses (RA) are still present.

Although the first report of fetoscopic laser surgery was published more than 15 years ago[121], only few studies have since reported on the placental findings and incidence of RA after laser surgery[135;202;204;205]. Most importantly, the results of these studies are highly discordant and the incidence or RA varied from 0% to 75%[135;202;204;205].

The objective of our study was to determine the incidence and clinical implications of RA in a large series of placentas treated with fetoscopic laser surgery. We hypothesized that the presence of RA may lead to a higher incidence of adverse outcome and larger inter-twin hemoglobin difference at birth. Furthermore, we tested the hypothesis that RA may be found more frequently in anterior placentas due to the more complex approach in these cases.

Material and methods

All consecutive placentas of monochorionic twin pregnancies with TTTS treated with fetoscopic laser surgery and delivered at our center between June 2002 and December 2005 were included in the study. The Leiden University Medical Center is the national referral center for laser treatment for TTTS in the Netherlands. TTTS was diagnosed using standard antenatal

ultrasound criteria[105]. The fetoscopic laser surgery technique used was described in detail previously[137] and is similar to the method reported by Hecher *et al*[123] and Senat *et al*[10].
After birth, presence of RA was studied by placental colored dye injection. The umbilical vessels of both cords were injected with different-colored dyes (blue or green for arteries and orange or yellow for veins). Injection was continued until dye was seen to flow through the distal end of the vascular tree and into the placental substance. Placentas were then photographed in a plane view, and the picture was saved in a computerized data base. Placentas were divided in two groups, a group with RA and a group without RA. Placentas of TTTS pregnancies with single or double intrauterine fetal demise were excluded when placental maceration prohibited accurate evaluation of RA.
Adverse outcome was defined as intrauterine fetal demise, neonatal death or severe cerebral injury on neonatal ultrasound examination. The criteria used for determining severe cerebral injury were published in a previous study[206]. Hemoglobin was measured at birth from cord blood. Anemia and polycythemia at birth were defined as previously described[207].
Results of categorical variables were compared using Chi-squared test. Continuous variables were analyzed with the Independent Samples T-test. A p-value < 0.05 was considered to indicate a statistical significance. Statistical analysis was performed with SPSS version 11.0 (SPSS, Inc., Chicago, Illinois, USA).

Results

A total of 61 monochorionic placentas of TTTS pregnancies treated with laser were examined at our center during the study period. Overall perinatal mortality was 21% (26/122: intrauterine fetal demise, n= 18; neonatal death, n = 8). The data required for this study could not be recorded completely for nine placentas due to maceration caused by single intrauterine fetal demise (n = 8) or placental fragmentation (n = 1). These nine cases were excluded from further analysis. Fifty-two placentas were thus included in the study. RA were found in 33% (17/52) of laser treated placentas. A total of 39 RA were detected, with an average of

2.3 (±1.7) RA per placenta. The type of RA detected were arterio-venous anastomoses from donor to recipient (n = 16), arterio-venous anastomoses from recipient to donor (n = 16), arterio-arterial anastomoses (n = 4) and veno-venous anastomoses (n = 3). Some placentas with RA (5/17) had multiple types of anastomoses. Twenty-five of the 39 (64%) RA were very small (< 1 mm diameter) (example shown in Figure 1 and 2). Superficial arterio-arterial or veno-venous anastomoses were found in 35% (6/17) of placentas with RA. Adverse outcome occurred in 8% (1/12) of infants with superficial RA compared to 23% (5/22) in infants with only deep arterio-venous RA (p = 0.39). Further details on placentas, vascular anastomoses

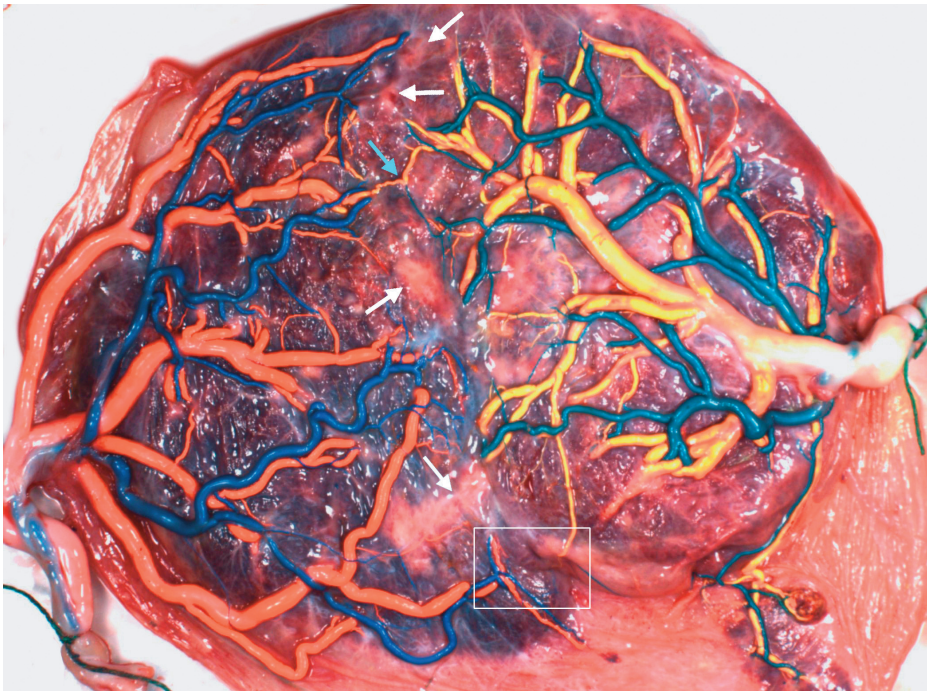

FIGURE 1 Placenta of TTTS-pregnancy treated with laser at 17 week's gestation, followed by spontaneous delivery of 2 healthy girls at 38 weeks. The placental share of the ex-donor (birth weight 3055 g) is on the left-side of the picture (arteries are blue, veins are orange). The placental share of the ex-recipient (birth weight 2915 g) is on the right-side (arteries are green, veins are yellow). Hemoglobin level in the ex-donor and ex-recipient were 13.2 g/dL and 19.2 g/dL respectively. The white arrows indicate the successfully coagulated anastomoses. The light blue arrow indicates a residual veno-venous anastomosis. A very small residual arterio-venous anastomosis from the ex-donor to the ex-recipient is in the white square that is enlarged in Figure 2.

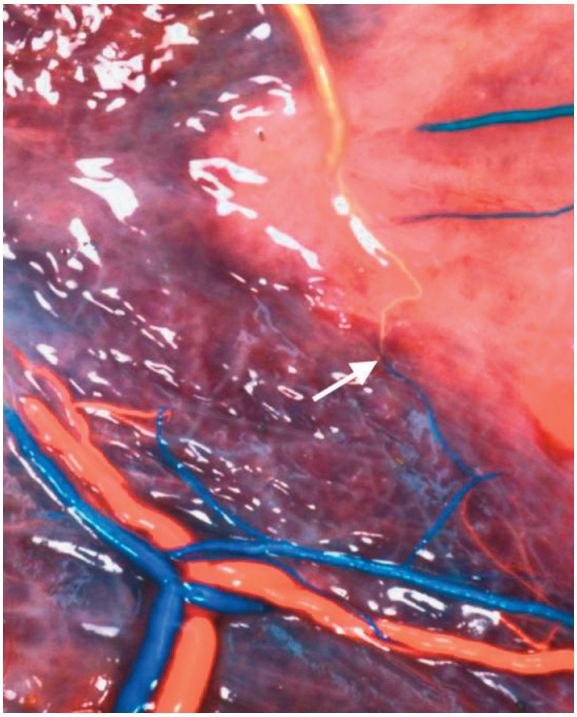

FIGURE 2 Detail of the very small residual anastomosis (diameter < 1 mm) between an artery (blue) from the ex-donor and a vein (yellow) from the ex-recipient.

TABLE 1 Characteristics of TTTS pregnancies with and without residual anastomoses.

	RA[e] group (n = 17 twin pairs)	no-RA[e] group (n = 35 twin pairs)	p-value
Gestational age at laser surgery – weeks[a]	19.9 ± 4.1	20.6 ± 3.2	0.51
Anterior placenta[b]	5 (29%)	11 (31%)	0.83
Number of anastomoses coagulated per placenta[a]	7.2 ± 4.5	6.0 ± 2.6	0.23
Gestational age at birth – weeks[a]	31.8 ± 3.2	31.1 ± 5.2	0.61
Birth weight – g[a,c]	1718 ± 608	1633 ± 855	0.60
Inter-twin birth weight difference > 20%[b]	7 (41%)	11 (31%)	0.49
Intrauterine fetal demise[b,c]	0 (0%)	10 (14%)	0.02
Neonatal death[b,c]	1 (3%)	6 (9%)	0.28
Severe cerebral injury[b,c]	6 (18%)	7 (10%)	0.27
Adverse outcome[b,c,d]	6 (18%)	20 (29%)	0.23

[a] *Value given as mean ± SD*
[b] *Percentages are between brackets*
[c] *Refers to single infants instead of twin pair*
[d] *Adverse outcome was defined as intrauterine fetal demise, neonatal death or severe cerebral injury*
[e] *RA: residual anastomoses*

and clinical outcomes in the RA group and no-RA group are presented in Table 1. The ten cases of intrauterine fetal demise in the no-RA group were all double demises: 4 pairs of twins died within 2 weeks of laser surgery and one pair of twins died 6 weeks after laser surgery.
In two of the 17 (12%) cases in the RA group, the presence of RA had already been predicted by the fetoscopic operator. In the first case a large arterio-arterial anastomosis was detected during fetoscopy but could not be coagulated due to its size. In the second case, surgery was complicated by intra-amniotic hemorrhage impeding further intervention due to blood-stained amniotic fluid. In two other cases (12%), presence of RA was suspected during the weeks following intervention because of fetal Doppler measurements (high middle cerebral artery peak systolic velocity (MCA-PSV) in one fetus). In the first case there was persistence of feto-fetal transfusion, in the second case there was a reversal of feto-fetal transfusion. In the remaining 13 cases (76%), RA had not been suspected antenatally.
The results of hematological values at birth are presented in Table 2.

TABLE 2 Hemoglobin values at birth in TTTS pregnancies with and without residual anastomoses.

	RA[c] group (n = 17 twin pairs)	no-RA[c] group (n = 35 twin pairs)	p-value
Hemoglobin – g/dL[a]	15.4 ± 5.6	16.0 ± 3.8	0.60
Anemia in one twin[b]	9 (26%)	8 (11%)	0.06
Polycythemia in one twin[b]	4 (12%)	2 (3%)	0.07
Inter-twin hemoglobin difference > 5 g/dL[b]	11 (65%)	7 (20%)	< 0.01
Twin pairs with anemia or polycythemia[b]	10 (59%)	8 (23%)	0.01

[a] *Value given as mean ± SD*
[b] *Percentages are between brackets*
[c] *RA: residual anastomoses*

Discussion

This study is the first large single center study reporting on the frequency and clinical implications of RA after fetoscopic laser surgery. We found that RA are present in one third of laser treated placentas. In a small study, De Paepe *et al* found a much higher incidence of RA in laser treated placentas, namely 75%[204]. Quintero *et al*, on the other hand, described a much lower incidence of RA, ranging from 0% to 5%[135;202;205]. Such an extreme discrepancy between the various studies can be due to several factors, namely (1) number of placentas studied, (2) differences in laser technique and surgical results and (3) differences in placental injection techniques. Obviously, no reliable conclusions on incidence of RA can be drawn if the number of studied placentas is too small. De Paepe et al, for example, were able to study only 8 placentas[204]. The second factor, adequate identification and successful coagulation of vascular anastomosis, probably depends on the ability of each fetal surgeon. Hecher *et al* reported an association between improved outcome and growing experience in the laser technique, possibly attributable to an improved efficiency in laser surgery[123]. However, the relation between RA and inter-individual or inter-center variation in fetoscopic laser surgery has not been studied. Nevertheless, we have previously shown that the overall outcome after laser surgery in our center is similar to that in other large centers[137]. Finally, the third factor, differences in placental injection technique, may influence the results on the incidence of RA. Discordant opinions on the sensitivity of various injection methods have recently resulted in a fierce debate in the literature[208]. Quintero *et al* performed placental injection studies with air and claimed that this method has similar sensitivity compared to other methods[202;209]. Most other authors, as well as our group, advocate placental injection with colored dye[8;61;67;204;210-212]. Which of the two methods (air injection or colored dye injection) is superior in detecting RA is not known. However, in our experience, the vast majority of RA are very small (with diameters < 1mm) and therefore difficult to detect without accurate injection with colored dye.

Several reasons can be envisaged to explain the occurrence of RA, namely (1) anastomoses (particularly the minuscule ones) were not detected during fetoscopy, (2) anastomoses were not coagulated because surgery

was too selective in order to spare cotyledons, (3) placental vessels of the donor were collapsed due to hypovolemia and vasoconstriction preventing adequate detection, (4) insufficient coagulation led to temporary anastomotic flow obstruction, but revascularization occurred later, and (5) anastomoses were detected during fetoscopy, but were considered too large to tackle. These reasonings may have consequences for the laser surgery technique. If (1), (2) or (3) are true, RA can be avoided using a technique of complete coagulation (with a 5 mm width) of the entire vascular equator. If (4) is true, then RA can be avoided by creating sufficient placental damage using a technique of deeper, longer-lasting and more frequent coagulation.

This study shows that, despite the fact that laser surgery in anterior placentas is technically more complex, anterior placentas are not associated with increased incidence of RA. Furthermore, the overall outcome of TTTS pregnancies with RA in our study was similar to those in TTTS pregnancies without RA. Both hypotheses, that RA could be associated with anterior placentas and adverse outcome, were thus not confirmed in our study. Lack of association between RA and adverse outcome may partly be due to the frequent presence (35%) of residual superficial anastomoses. Superficial arterio-arterial anastomoses are known to protect against TTTS[186].

An important (but inevitable) bias in our study may have been introduced by the exclusion of nine cases in which placental fragmentation or postmortem placental changes prohibited the injection and demonstration of the vessels on the placental surface. Adverse outcome in the nine excluded pregnancies was 50% (9/18; intrauterine fetal demise: n = 8, severe cerebral injury: n = 1). If adverse outcome in these pregnancies would have been caused by RA, then the incidence of adverse outcome in the RA group would have increased from 18% (6/34) to 29% (15/52). This is still similar to the incidence of adverse outcome in the no-RA group (29%) (p = 0.97). Therefore, it seems reasonable to deduce that RA in our study were not associated with adverse outcome.

We have shown in this study that large inter-twin hemoglobin differences (> 5 g/dL) were significantly more frequent in the presence of RA compared to the group without RA. Therefore, the hypothesis related to the association between RA and larger inter-twin hemoglobin difference

seems legitimate. RA were also associated with a higher incidence of anemia or polycythemia at birth. Overall, RA were associated with isolated hemoglobin discordance at birth or recurrent TTTS in 11 of the 52 (21%) TTTS pregnancies treated with laser surgery. Our findings are in agreement with recent reports suggesting that incomplete coagulation results in isolated hemoglobin discordance or recurrent TTTS in up to 27% of double survivors after laser surgery[213;214]. Routine serial ultrasound examination with MCA-PSV measurement after laser surgery proved invaluable in the early detection of severe fetal hematological disorders and is nowadays strongly recommended[213;214]. Similarly, we also strongly recommend routine placental injection study in TTTS treated with laser because it may give equally invaluable information to perinatologists to understand the etiology of severe hematological disorders. Finally, placental injection studies are an important feedback source to individual fetoscopic surgeons. In conclusion, clinicians involved in the care for monochorionic twins should be aware that RA occur frequently after fetoscopic laser surgery in TTTS. Although RA are not clearly associated with adverse outcome, these anastomoses may be associated with severe hematological disorders in fetuses and neonates. Routine Doppler measurements after laser surgery and accurate placental injection studies after birth can help in detecting and understanding fetal and neonatal hematological complications.

Part 2
Related syndromes

Chapter 6

Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence

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Abstract

Objective: To report an uncommon form of chronic inter-twin transfusion, referred to as twin anemia-polycythemia sequence (TAPS), with severe anemia in one twin and polycythemia in the other, without the characteristically associated twin oligo-polyhydramnios sequence (TOPS) seen in the classical twin-to-twin transfusion syndrome (TTTS).

Methods: Description of the clinical course and placental characteristics of two pairs of monochorionic twins with TAPS.

Results: The two pairs of monochorionic twins were born at 33 and 34 weeks' gestation, respectively. Serial fetal ultrasound examinations revealed no signs of TOPS. At birth, both donor twins were severely anemic requiring blood transfusion and both recipients were polycythemic, one requiring partial volume exchange transfusions. Inter-twin difference in reticulocyte counts was extremely high, suggesting a chronic form of inter-twin blood transfusion. Placental injection studies revealed a preponderance of very small (< 1 mm) arterio-venous anastomoses in one direction.

Conclusions: Chronic inter-twin transfusion may lead to an uncommon form of inter-twin transfusion, named TAPS, not associated with TOPS, resulting in severe fetal or neonatal hematological complications. We hypothesize that TAPS is mediated through minuscule unidirectional anastomoses. TAPS can be diagnosed antenatally with Doppler studies and postnatally by hemoglobin and reticulocyte measurements.

Introduction

Twin-to-twin transfusion syndrome (TTTS) is a heterogeneous disease affecting monochorionic twin pregnancies and results from hemodynamic imbalance due to placental vascular anastomoses[27;186]. Various forms of TTTS have been described: acute perimortem TTTS, acute perinatal TTTS and chronic TTTS. Acute perimortem TTTS occurs after intrauterine fetal demise of a co-twin and is due to acute exsanguination from the surviving twin into the low-pressure circulation of the demising co-twin. Acute perinatal TTTS may occur during birth due to acute inter-twin shifts of blood resulting from blood pressure differences associated with uterine contractions or changes in fetal position. Both acute forms of TTTS are mediated through superficial arterio-arterial and veno-venous anastomoses[29;47;71]. The most common form of TTTS, referred to as chronic TTTS, occurs usually during the second trimester of pregnancy and affects 15% of monochorionic twin pregnancies. Chronic TTTS results from chronic inter-twin transfusion of blood mediated through arterio-venous anastomoses[186]. Chronic TTTS itself is also a highly heterogeneous disease and may be staged using Quintero's classification based on ultrasound criteria[9]. Chronic TTTS is diagnosed when antenatal sonographic evidence of twin oligo-polyhydramnios sequence (TOPS) is found[6;27;28;93;102;186]. TOPS is defined by deepest vertical pool of ≤ 2 cm and ≥ 8 cm in the donor's and recipient's amniotic sac, respectively[27;28;93;186].
We report two cases of monochorionic twins with an unusual form of chronic TTTS. Both cases had hematological evidence of severe chronic inter-twin transfusion but no antenatal sonographic signs of TOPS. Placental injection studies revealed a preponderance of very small (< 1 mm) arterio-venous anastomoses in one direction.

Case presentation

Case 1

A 35-year-old gravida 2 para 1 was referred at 27 + 1 weeks' gestation with a dichorionic-triamniotic triplet pregnancy after in vitro fertilization. Weekly ultrasound examination throughout pregnancy showed no signs

of TTTS (last ultrasound examination was performed at 33 + 1 weeks' gestation). Doppler measurement of the middle cerebral artery peak systolic velocity (MCA-PSV) showed a mild increase (1.2 multiples of median) in MCA-PSV in one of the monochorionic fetuses (maximum velocity of 58 cm/sec at 33 + 1 weeks' gestation). Labor was induced and three girls were born vaginally at 34 + 0 weeks' gestation. The last two girls were the monochorionic twin pair. The second-born infant was pale and weighed 1725 g (between 9th and 25th percentile for gestational age). The third-born infant was plethoric and weighed 1695 g (9th percentile for gestational age). Birth weight discordance was 2%. Apgar scores for the second-born and third-born infant were 8/9/10 and 7/8/8, respectively. Hemoglobin values for twin 2 and twin 3 were 7.6 g/dL (< 5th percentile for gestational age) and 21.7 g/dL (> 95th percentile for gestational age) with reticulocyte counts of 21% (absolute reticulocyte count 399 x 10^3/mm^3) and 4% (absolute reticulocyte count 181 x 10^3/mm^3), respectively. The anemic infant, who was also the fetus with increased MCA-PSV, required a blood transfusion on day 1. No signs of acute hemorrhagic hypovolemic shock at birth (heart rate 140 bpm, blood pressure 44/35 mmHg) were present in the anemic infant. No evidence of placental abruption was found and none of the infants were hypoxic at birth. Kleihauer-Betke test was not done. The hematocrit value in the co-twin increased up to 69% on day 1, but the infant remained asymptomatic and did not require a partial volume exchange transfusion. Cranial ultrasound examination in both twins was normal and further neonatal course was uneventful.
Macroscopic placental examination showed a velamentous insertion of the umbilical cord of twin 3 and an injection study with colored dye revealed five very small (< 1 mm) arterio-venous anastomoses (< 1 mm) from twin 2 to twin 3 (Figures 1 and 2).

Case 2

A 31-year-old gravida 5 para 3 was referred to our center at 16 weeks' gestation with a twin pregnancy. The twin pregnancy had been diagnosed as dichorionic in the referring hospital based on an ultrasound examination at 10 weeks' gestation. The course of the pregnancy was uneventful and no signs suggestive of TTTS were found on monthly ultrasound examination.

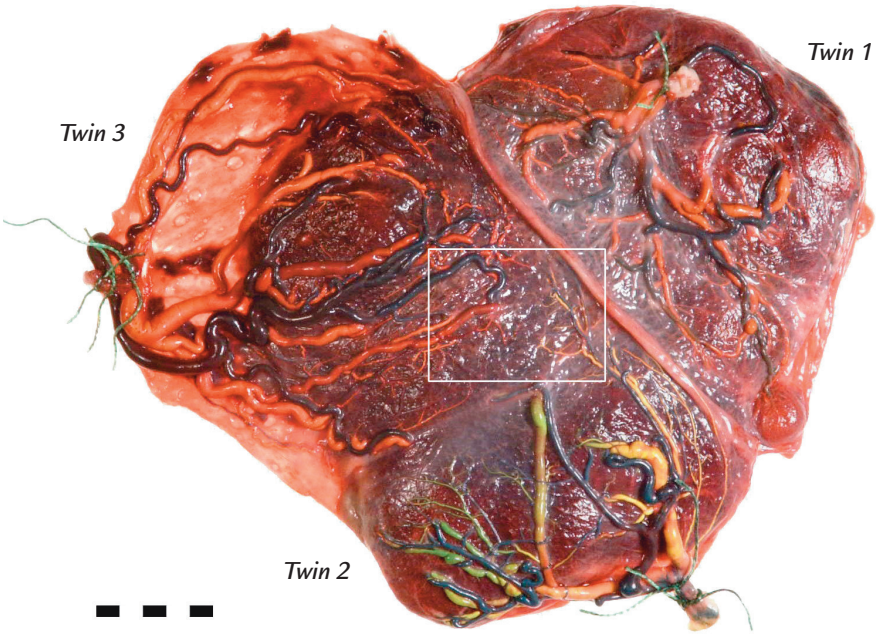

FIGURE 1 Case 1. Triplet placenta after injection with colored dye. There is a monochorionic placenta (left-bottom side of the picture) and a fused dichorionic placenta (top-right) (blue for arteries and orange or yellow for veins). The five anastomoses from donor to recipient are in the white square that is enlarged in figure 2.

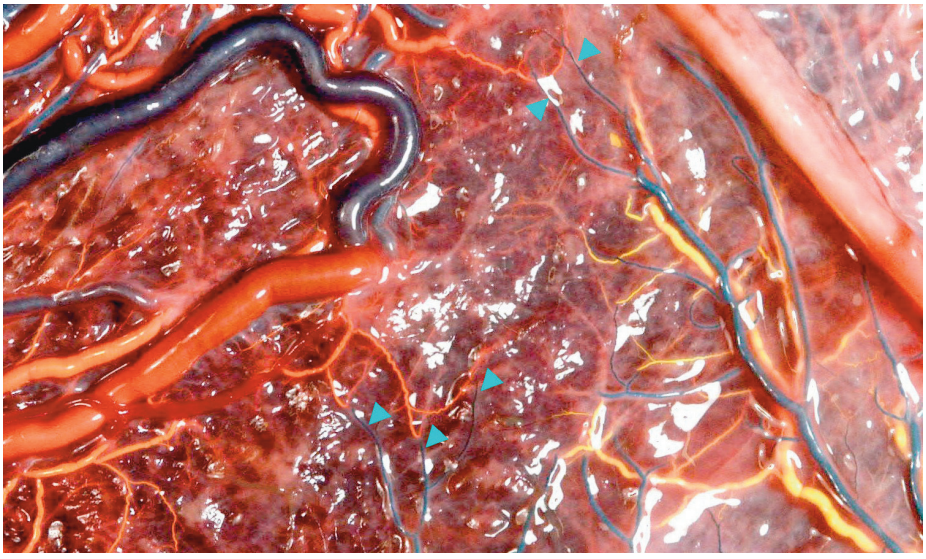

FIGURE 2 Case 1. The sky-blue arrows indicate five very small (< 1 mm) arterio-venous anastomoses from twin 2 (donor) to twin 3 (recipient).

MCA-PSV measurements were not done. One day after the last ultrasound examination at 33 + 3 weeks' gestation, spontaneous rupture of membranes occurred and was followed by the birth of two boys. The first-born infant was pale and weighed 1880 g (between 9th and 25th percentile for gestational age). The second-born twin was extremely plethoric and weighed 1980 g (between 25th and 50th percentile for gestational age). Birth weight discordance was 5%. Apgar scores were 8/9/9 and 7/8/8 for the first-born and second-born infant, respectively. Hemoglobin values for twin 1 and twin 2 were 6.0 g/dL (< 5th percentile for gestational age) and 26.3 g/dL (> 95th percentile for gestational age) with reticulocyte counts of 30% (absolute reticulocyte count 525 x 10^3/mm^3) and 3% (absolute reticulocyte count 192 x 10^3/mm^3), respectively. The anemic infant had no signs of acute hemorrhagic hypovolemic shock at birth (heart rate 120 bpm; blood pressure 51/37 mmHg) but required a blood transfusion on day 1. No clinical or laboratory evidence of placental abruption was found. Kleihauer-Betke test was not performed. The second-born twin required oxygen administration and ventilatory support with CPAP due to cyanosis and respiratory failure. Severe hypoglycemia was corrected by intravenous glucose infusions. The hematocrit increased up to 90% on day 1 and two partial volume exchange transfusions were performed because of symptomatic polycythemia-hyperviscosity syndrome. Cranial ultrasound showed persistent bilateral periventricular echodensities in both twins. At macroscopic examination, the placenta was monochorionic-diamniotic. Colored-dye injection showed four very small (< 1 mm) arterio-venous anastomoses from twin 1 to twin 2 and one very small (< 1 mm) arterio-venous anastomosis from twin 2 to twin 1 (Figures 3 and 4). Microscopic examination showed that the dividing membrane was diamniotic.

Comment

In this study, we found evidence that monochorionic twin pregnancies without signs of TOPS can still be affected by chronic inter-twin transfusion resulting in severe fetal or neonatal hematological complications. We have named this condition twin anemia-polycythemia sequence (TAPS). Differential diagnosis in monochorionic twins with highly discordant

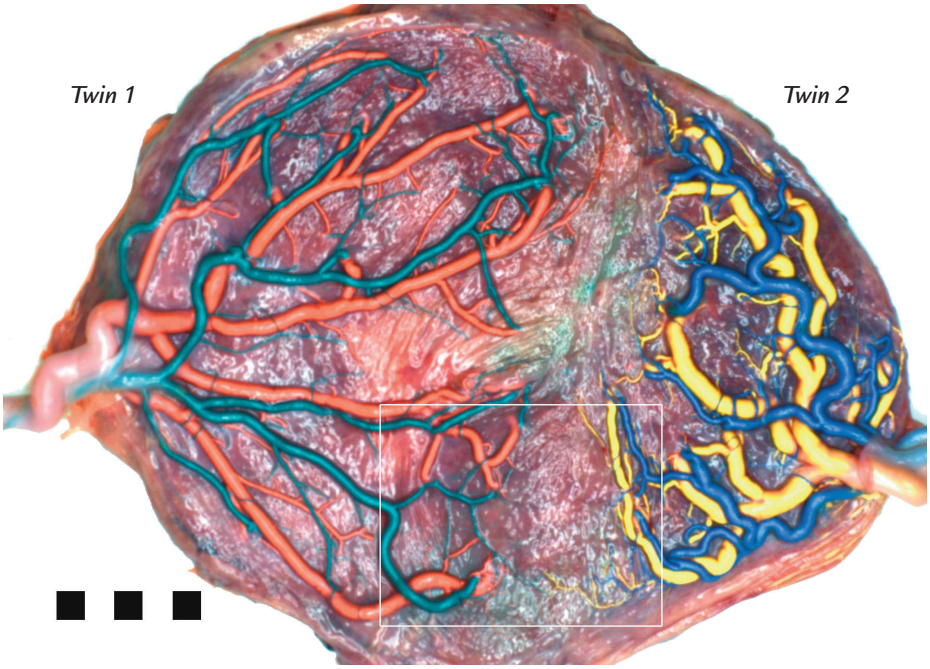

FIGURE 3 Case 2. Monochorionic placenta after injection with colored dye (blue or green for arteries and orange or yellow for veins). The five small arterio-venous anastomoses are only visible after enlargement. The four anastomoses from donor to recipient are in the white square that is enlarged in figure 4 (the single anastomosis from recipient to donor is not in the white square).

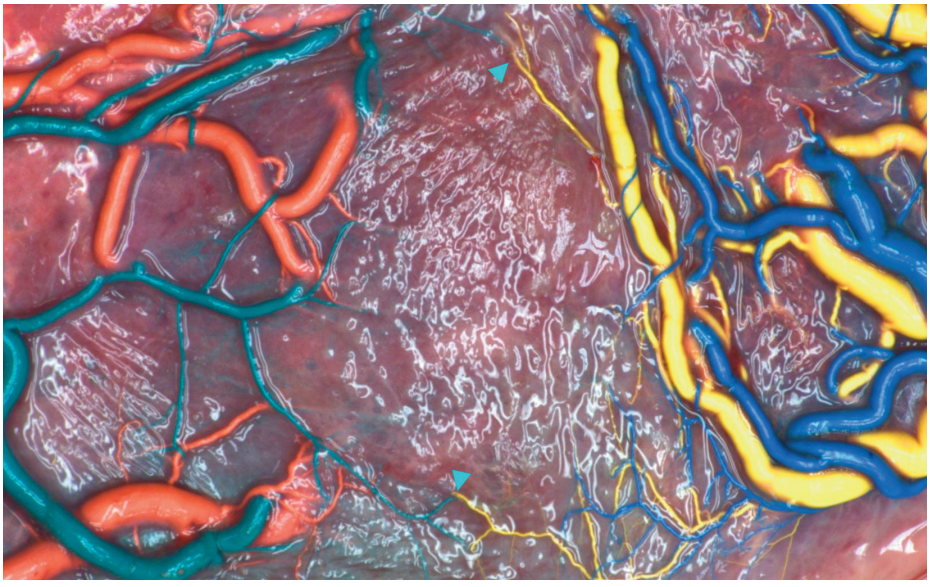

FIGURE 4 Case 2. The sky-blue arrows indicate four small arterio-venous anastomoses from twin 1 (donor) to twin 2 (recipient).

hemoglobin values at birth includes acute peripartum TTTS, chronic TTTS with TOPS and chronic TTTS with TAPS.
Other possible causes for isolated anemia or polycythemia should also be ruled out. Chronic anemia in newborns may result from partial placental abruption, infection or chronic feto-maternal hemorrhage. Unfortunately, Kleihauer-Betke test was not performed in our two cases, which is a limitation of our study. However, the associated combination with polycythemia in the co-twin clearly suggests a form of feto-fetal transfusion rather than feto-maternal transfusion. Common causes for polycythemia should also be excluded, such as chronic hypoxia associated with intrauterine growth retardation. Polycythemia due to chronic in utero hypoxia results from increased erythropoietin levels and reticulocytosis[215;216]. Reticulocyte counts in the polycythemic infants in our reported cases were normal for gestational age, suggesting that polycythemia resulted from chronic inter-twin transfusion rather than from increased erythropoiesis secondary to chronic hypoxia.
A first possible diagnosis in both reported cases is that of acute peripartum TTTS. However, the anemic infant in acute peripartum TTTS has clinical signs of hemorrhagic hypovolemic shock such as pallor, tachycardia and hypotension[47;186]. Also, reticulocyte counts after acute blood loss are typically not increased due to lack of time for compensatory hematopoiesis. Although it is difficult to completely rule out an acute peripartum TTTS event in our cases, acute peripartum TTTS should be disregarded for several reasons. First, no clinical signs of acute perinatal blood loss were found in the anemic infants. Second, very high reticulocyte counts were measured in both anemic infants, suggestive of chronic rather than acute blood loss. Finally, acute peripartum TTTS is mediated through (large) superficial anastomoses[47;71]. Placental injection studies in both reported cases showed only minuscule arterio-venous anastomoses without superficial anastomoses. Small-caliber anastomoses have a high vascular resistance and theoretically do not allow large amounts of blood volume to be transfused acutely from one twin to the other (according to Poisseuille's law of fluid dynamics).
A second possible diagnosis, that of the common form of chronic TTTS can be disregarded due to absence of the typical signs of TOPS. We suggest therefore that atypical forms of chronic TTTS without TOPS, but

associated with anemia in one twin and polycythemia in the other, be referred to as TAPS. TAPS differs significantly in terms of diagnosis and management from the typical form of chronic TTTS with the characteristic signs of TOPS. Therefore, a clear distinction between TAPS and TOPS is clinically more useful and reflects more accurately the heterogeneity of TTTS. TAPS may occur spontaneously (natural form) as reported in this study, but may also develop after laser surgery (iatrogenic form). Recent reports suggest that the iatrogenic form of TAPS may occur in up to 13% of cases after laser surgery[213;214]. Interestingly, in iatrogenic TAPS, it is usually the former recipient who becomes anemic, whereas the former donor becomes polycythemic[213;214].

Most authors consider the presence of TOPS to be the major diagnostic criterion for chronic TTTS[27;28;93;186]. Marked fetal growth discordances and inter-fetal hemoglobin differences may also be present but these features are not considered key elements in the diagnosis of chronic TTTS[27;93;102]. Doppler studies are a standard requirement to determine Quintero staging once chronic TTTS is diagnosed[27;28] and are also helpful in the diagnosis of fetal anemia after single intrauterine fetal death (acute perimortem TTTS)[33]. Routine Doppler studies are also recommended after laser surgery to rule out fetal anemia[213;214].

We suggest that Doppler studies and MCA-PSV measurements also be performed during each follow-up visit in all uncomplicated monochorionic twin pregnancies, even in the absence of TOPS. Signs of fetal anemia in a monochorionic twin should then alert the perinatologist of the possibility of TAPS. Fetal anemia may affect cerebral oxygenation and result in hypoxic-ischemic cerebral injury, whereas polycythemia has been suggested to cause cerebral injury in TTTS by vascular sludging[206]. Hypothetically, TAPS may also be responsible for some cases of unexplained fetal demise in monochorionic pregnancies without signs of chronic TTTS.

Whether TAPS is associated with increased mortality or morbidity is not known. If so, invasive procedures such as fetoscopic laser surgery, intrauterine blood transfusion or a combination of both, may improve outcome. At present, however, we think that invasive procedures are not indicated because the risk of procedure-related complications may outweigh the risk of mortality or morbidity.

There as several hypotheses on the origin of inter-twin amniotic fluid discordances in the common form of chronic TTTS with TOPS[186]. It is

however not yet clear why there are no inter-twin amniotic fluid discordances in TAPS. One of the common denominators between the two reported cases is the preponderance of very small (< 1 mm), arterio-venous anastomoses in one direction. Vascular resistance in small-caliber vessels is higher than in larger vessels and reduces the volume of fluid passage (Poisseuille's law). We hypothesize that if only a few very small placenta vascular anastomoses are present, inter-twin blood transfusion occurs very slowly and allows more time for hemodynamic compensatory mechanisms to take place. Activation or inactivation of the renin-angiotensin system may then induce the hypovolemic donor and the hypervolemic recipient to remain euvolemic, which in turn explains the absence of TOPS. However, compensatory hematopoiesis may not be sufficient to prevent severe anemia in the donor, despite the extremely high reticulocyte count. Another possible explanation is that the reported infants were born before TOPS had time to develop. Inter-twin hemoglobin discordance and amniotic fluid discordance varies with the state of progression of chronic TTTS[102]. It is conceivable that some twins with chronic TTTS may first develop TAPS and then TOPS. As placental blood vessels enlarge throughout pregnancy, these vessels may eventually become just large enough to produce TAPS at a certain point in gestation, but not before this time. Regardless of the various hypotheses, our understanding of TAPS is clearly still incomplete.

In conclusion, perinatologists involved in the care of monochorionic twins should maintain a high index of suspicion, as antenatal ultrasound examination without Doppler studies does not rule out a chronic form of inter-twin transfusion. TAPS can occur in the absence of the characteristic inter-twin discordance in amniotic fluid volumes. TAPS should be diagnosed when large inter-twin discordance in fetal or neonatal hemoglobin levels and reticulocyte counts are found, in absence of TOPS. Placental injection studies may then reveal a preponderance of very small arterio-venous anastomoses in one direction.

Chapter 7

Hemoglobin differences at birth in monochorionic twins without chronic twin-to-twin transfusion syndrome

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Abstract

Objectives: To determine the hemoglobin (Hb) differences at birth in monochorionic (MC) twins without chronic twin-to-twin transfusion syndrome (TTTS) in relation to birth order and placental vascular anatomy.

Methods: All consecutive cases of MC twins without chronic TTTS and dichorionic (DC) twins delivered at our center and admitted to our neonatal nursery between June 2002 and December 2004 were included in our study. We analyzed Hb differences at birth and on day 2, in relation to birth order and placental vascular anatomy.

Results: Forty-five pairs of MC twins and 71 pairs of DC twins were included. Mean Hb differences at birth in MC and DC twins were similar, 1.5 and 1.4 g/dL, respectively. Hb differences > 5 g/dL at birth were found in 2% (1/45) of MC twins compared to 8% (6/71) in DC twins. On day 2, mean Hb differences in MC twins increased to 3.8 g/dL, and the rate of MC twins with Hb differences > 5 g/dL increased to 27% (12/45) ($p < 0.001$). Mean Hb differences and the percentage of twins with Hb differences > 5 g/dL in DC twins did not change on day 2.
In MC twins, Hb levels measured on day 2 were significantly higher in second-born twins than in first-born twins, 17.7 and 15.5 g/dL, respectively ($p = 0.002$). Hb differences on day 2 were significantly higher in MC twins with superficial vascular anastomoses than those without superficial anastomoses, 4.0 g/dL and 1.5 g/dL, respectively ($p = 0.036$).

Conclusions: Hb differences occur more frequently in MC twins without chronic TTTS than in DC twins, but only when measured on the second day of life. Hb differences in MC twins are associated with birth order and superficial vascular anastomoses.

Introduction

Placental vascular anastomoses are almost invariably present in monochorionic (MC) twin gestations, but are extremely rare in dichorionic (DC) twin gestations[5]. In contrast to DC twins, MC twins are therefore at risk for twin-to-twin transfusion syndrome (TTTS). Several forms of TTTS have been described: chronic TTTS, acute perimortem TTTS and acute perinatal TTTS[11;30;42;45]. The chronic form of TTTS is the most common form and complicates about 15% of monochorionic twin pregnancies. Chronic TTTS occurs during the second or early third trimester of pregnancy due to unbalanced blood flow from one twin to the other through placental vascular anastomoses[8;108]. The recipient twin gradually becomes hypervolemic, polyuric, and develops polyhydramnios, whereas the donor twin becomes hypovolemic, oliguric and develops oligohydramnios. The pathogenesis of chronic TTTS is mainly mediated through deep arterio-venous anastomoses, in the absence of compensatory superficial arterio-arterial anastomoses[62]. Acute perimortem TTTS occurs in MC twins after intrauterine death of the co-twin. This form of acute TTTS results from acute exsanguination from the surviving twin into the low-pressure circulation of the demised co-twin through superficial arterio-arterial or veno-venous anastomoses and leads to severe hypoxic-ischemic injury in the surviving twin[13;30-32;70;217]. Risk of neurological handicap or death in the surviving twin is then extremely high [11;13;14]. Acute TTTS is also reported to occur in MC twins during labor, hence referred to as acute perinatal TTTS[34;40-44;218]. Acute perinatal TTTS is thought to result from uterine contractions or changes in fetal position leading to inter-twin blood pressure differences and inter-twin blood transfusion[36;44;45]. Rapid transfer of blood from the donor twin into the circulation of the recipient twin is probably mediated through superficial arterio-arterial or veno-venous anastomoses[71]. Acute perinatal TTTS may then lead to hemoglobin (Hb) differences at birth without necessarily discordance in birth weight[39;43]. So far, knowledge on acute perinatal TTTS is mainly speculative and based on case reports[34-39].

In 1972, Klebe and Ingomar hypothesized that Hb difference in MC twins at birth may be due to differences in placental blood transfusion in relation to birth order[38]. They postulated that once the umbilical cord of the first-born

twin is clamped, the second-born twin may not only receive blood from its own placenta but also from the placenta-share of the co-twin, through the patent vascular anastomoses. Other authors also share this opinion[35;41;44]. The aim of this study was to determine the Hb differences and hemodynamic disturbances at birth in MC twins without chronic TTTS and DC twins in relation to birth order, and establish if there was an association between Hb differences and the presence or absence of superficial vascular anastomoses.

Materials and methods

All consecutive cases of MC twins without chronic TTTS and DC twins delivered at our obstetrical department and admitted to our neonatal nursery between June 2002 and December 2004 were included in our study. The prenatal diagnosis of chronic TTTS was made by using standard ultrasound criteria[105]: 1.) Monochorionicity established by absence of a "twin peak" sign and the presence of a thin dividing membrane, 2.) Oligohydramnios (deepest vertical pocket ≤ 2cm) in the twin sac of one fetus, and 3.) Polyhydramnios (deepest vertical pocket ≥ 8cm before 20 weeks of gestation or ≥ 10cm after 20 weeks of gestation) in the twin sac of the other fetus. Monochorionicity was confirmed by histopathological examination of the placenta. Placental vascular anastomoses in MC placentas were studied by injection with colored dye (blue or green for arteries, yellow or orange for veins). Arterio-venous anastomoses were classified as deep anastomoses, whereas arterio-arterial and veno-venous anastomoses were classified as superficial anastomoses.
We excluded pregnancies complicated by maternal red blood cell alloimmunization, major fetal congenital malformations, higher multiples gestations, and twin gestations with intrauterine fetal death of a co-twin. The following obstetrical data were collected: gestational age at delivery, mode of delivery and time interval (in minutes) between delivery of twin 1 and twin 2. The following neonatal data were gathered: birth weight, Apgar score at 5 minutes, arterial blood pressure measured by Dinamap at birth and heart rate at birth. Renal function was assessed by measuring urine output during the first 24 hours after birth (in ml/kg/h). Growth

discordance was calculated by dividing the difference in birth weights by the birth weight of the larger twin. Hb levels were assessed at birth from umbilical cord blood. Since Hb levels are not reliable when measured shortly after acute shifts in blood volume, a second (venous) Hb level was analyzed on the second day of life, when clinically indicated[39;46;219;220]. Inter-twin Hb difference was defined as absolute Hb difference (higher Hb value minus lower Hb value). Inter-twin Hb differences were analyzed in relation to birth order (twin 1 versus twin 2) and in relation to birth weight (smaller twin versus larger twin). We recorded the use of blood transfusions during the first and second day of life, as well as the need for volume expanders and inotropic support during the first day of life. For the purpose of our study we defined acute perinatal TTTS in MC twins as an Hb difference of at least 5 g/dL at birth, without an obvious other cause for such difference. Acute hemorrhagic hypovolemic shock was defined as a combination of pallor, tachycardia (heart rate >160 bpm) and/or hypotension, requiring treatment with volume expanders, blood transfusion and/or inotropic support [220]. Hypotension was defined as a systolic blood pressure below the 3rd percentile for gestational age[221]. Serial cranial ultrasound scans were performed routinely in all neonates. Periventricular hemorrhage (grade classification according to Papile[222]) and cystic periventricular leucomalacia (grade classification according to de Vries[223]) were recorded. Other significant neonatal problems were also reviewed, including respiratory distress syndrome, chronic neonatal lung disease, patent ductus arteriosus, necrotizing enterocolitis, retinopathy of prematurity and hyperviscosity syndrome. We also recorded the total number of days during which phototherapy treatment was required.

Results of categorical variables were compared using Fisher's exact test or Chi-square test, as appropriate. McNemar's test was used for analysis of paired nominal data. The paired Student's *t* test was used to study normally distributed values within twin pairs, whereas the unpaired Student's *t* test was used to compare normally distributed values between two groups. The Spearman rank correlation was used to study the relationship between time-interval at birth between twin 1 and twin 2 and Hb differences between both infants. A p-value < 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 11 (SPSS, Inc., Chicago, Illinois, USA).

Results

During the study period 1699 neonates were admitted to our nursery, among which 21% (349/1699) were born from 174 multifetal gestations. 55% (95/174) of the multifetal pregnancies were MC, and 45% (79/174) were DC. The Leiden University Medical Center is a tertiary medical center and serves as the national referral center for fetal invasive therapy in the Netherlands, which explains the high percentage of MC multifetal gestations and red blood cell immunization compared to other academic centers. A total of 45 MC pregnancies without chronic TTTS and 71 DC pregnancies were included in our study. The derivation of the included and excluded twin pregnancies is shown in Figure 1. Reasons for admission to our neonatal nursery were all mainly related to prematurity. Perinatal death did not occur in any of the MC twins without chronic TTTS delivered at our center during the study period.
Patients' characteristics for both MC and DC infants are shown in Table 1. There were no significant differences in baseline characteristics between the two groups, except for a higher percentage of MC infants being delivered by cesarean section compared to DC infants, 38% (34/90) versus 23% (32/142), respectively (p = 0.012). The percentage of MC and DC twin pairs with birth weight discordance > 20% was similar, 27% (12/45) and 24% (17/71), respectively.

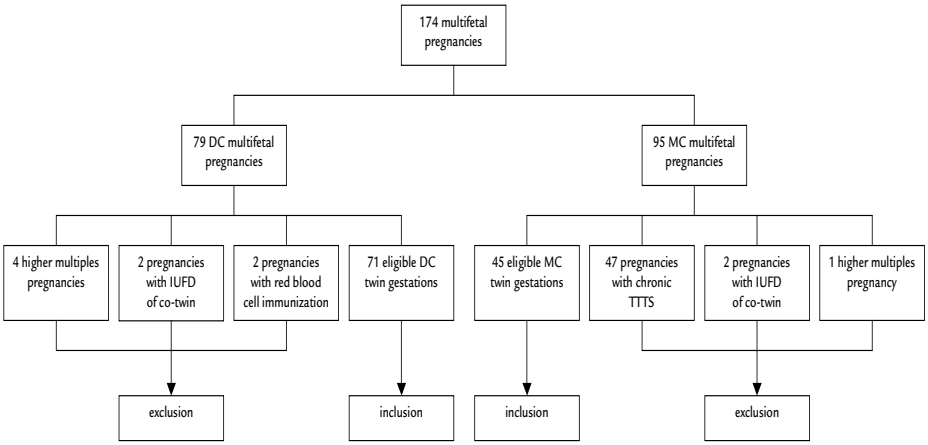

FIGURE 1 Flowchart showing the selection of our study population. (IUFD: intrauterine fetal demise)

TABLE 1 Baseline characteristics.

	MC infants (n = 90)	DC infants (n = 142)	p-value
Gestational age at birth – wk[a]	33.5 ± 3.4	32.6 ± 3.6	ns
Birth weight – g[a]	2040 ± 684	1885 ± 636	ns
Birth weight difference - %[a]	14 ± 11	13 ± 10	ns
Female – no. (%)	44 (49%)	72 (51%)	ns
Cesarean section – no. (%)[b]	34 (38%)	32 (23%)	0.012
Median Apgar score at 5 min (range)	9 (5-10)	9 (4-10)	ns
Neonatal death – no. (%)	3 (3%)	8 (6%)	ns
Respiratory distress syndrome – no. (%)	21 (23%)	46 (32%)	ns
Patent ductus arteriosus – no. (%)	5 (6%)	8 (6%)	ns
Periventricular hemorrhage grade 2-4 – no. (%)	2 (2%)	7 (5%)	ns

[a] *Value given as mean ± SD*
[b] *Refers to infants rather than mothers*

Hb values and hemodynamic parameters of MC and DC infants are presented in Table 2. Hb was measured at birth in 98% (88/90) and 93% (132/142) of MC and DC infants, respectively. On day 2, Hb measurements were available in 81% (73/90) and 85% (121/142) of MC and DC infants, respectively. Hb levels on day 2 were extracted from full blood count measurements, usually required to rule out perinatal infection associated with prematurity. Mean Hb differences at birth in MC twins and DC twins

TABLE 2 Hb values and hemodynamic parameters in MC and DC infants.

	MC infants (n = 90)	DC infants (n = 142)	p-value
Hb at birth[a]	16.5 ± 2.1	16.6 ± 2.3	ns
Hb difference between twins at birth[a]	1.5 ± 1.4	1.4 ± 1.7	ns
Hb on day 2[a]	16.6 ± 3.0	16.1 ± 2.6	ns
Hb difference between twins on day 2[a]	3.8 ± 2.5	1.7 ± 1.8	< 0.001
Hb difference >5 g/dl at birth – no. (%)	2 (2%)	12 (8%)	ns
Hb difference >5 g/dl on day 2 – no. (%)	24 (27%)	6 (4%)	< 0.001
Blood transfusion on day 1 or 2 – no. (%)	3 (3%)	9 (6%)	ns
Hypotensive at birth – no. (%)	9 (10%)	27 (19%)	ns
Volume expanders at birth – no. (%)	12 (13%)	31 (22%)	ns
Inotropic drugs – no. (%)	11 (12%)	14 (10%)	ns
Acute hemorrhagic hypovolemic shock – no. (%)	0 (0%)	0 (0%)	–

[a] *Value given as mean ± SD*

were similar, 1.5 and 1.4 g/dL, respectively. Hb differences > 5 g/dL at birth were found in 2% (1/45) of MC twin pairs compared to 8% (6/71) of DC twin pairs. On day 2, mean Hb differences in MC twins increased to 3.8 g/dL ($p < 0.001$). The rate of MC twin pairs with Hb differences > 5 g/dL increased up to 27% (12/45) ($p < 0.001$). In DC twins, mean Hb differences and percentage of twin pairs with Hb differences > 5 g/dL did not change on day 2.

Birth weight discordance > 20% and Hb difference > 5 g/dL at birth was detected in one pair (2%) of MC twins and in one pair (1%) of DC twins, in which the higher Hb level was found in the larger infant. In another set of DC twins with birth weight discordance > 20% and Hb difference > 5 g/dL at birth, the higher Hb level was found in the smaller twin. No difference in hemodynamic parameters, including blood pressure, heart rate, diuresis, or treatment with volume expanders, inotropics or blood transfusion, was found between MC and DC twins. None of the twins in our study suffered from acute hemorrhagic hypovolemic shock at birth. Serial cranial ultrasound scans showed a similar rate of periventricular hemorrhage (grade 2 to 4) in MC and DC twins, 2% (2/90) and 5% (7/142), respectively. None of the twins in both groups had cystic periventricular leucomalacia. One second-born infant of a preterm MC pregnancy required an exchange transfusion for hyperbilirubinemia on day 2 (serum bilirubin level: 13.9 mg/dL) despite phototherapy and adequate fluid administration. Routine work-up for hyperbilirubinemia showed an increase in Hb level from 14.3 g/dL at birth to 18.4 g/dL on day 2 without evidence of hemolysis and dehydration, suggesting that hyperbilirubinemia may have resulted from a high red cell volume.

Differences in Hb values and hemodynamic parameters in relation to birth order are presented in Table 3. A fall in Hb between birth and day 2 was found in first-born MC twins (- 0.9 g/dL), first-born DC twins (- 0.6 g/dL) and second-born DC twins (- 0.3 g/dL). A rise in Hb between birth and day 2 was found only in second-born MC twins (+ 1.1 g/dL). Mean Hb concentrations on day 2 in second-born MC twins on day 2 were significantly higher than in first-born MC twins, 17.7 and 15.5 g/dL, respectively ($p = 0.002$). No association between birth order and Hb differences was detected in DC twins. The fall in Hb between birth and day 2 in first-born MC twins was of similar magnitude as the fall in Hb in first-

TABLE 3 Hb values and hemodynamic parameters in MC and DC twin pairs in relation to birth order.

	MC twin pairs (n = 45)			*DC twin pairs (n = 71)*		
	Twin 1	Twin 2	p-value	Twin 1	Twin 2	p-value
Hb at birth[a]	16.4 ± 1.8	16.6 ± 2.4	ns	16.6 ± 2.4	16.6 ± 2.3	ns
Hb on day 2[a]	15.5 ± 2.2	17.7 ± 3.3	0.002	16.0 ± 2.6	16.3 ± 2.5	ns
Heart frequency at birth (bpm)[a]	150 ± 15	146 ± 19	ns	151 ± 18	151 ± 13	ns
Mean blood pressure at birth (mmHg)[a]	39.2 ± 7.4	38.8 ±7.9	ns	36.7 ± 7.7	35.3 ± 7.5	ns
Urine output on day 1 (ml/kg/h)[a]	2.7 ± 1.3	2.9 ± 1.3	ns	2.2 ± 1.1	2.3 ± 1.1	ns
Blood transfusion on day 1 or 2 – no. (%)	1 (2%)	2 (4%)	ns	6 (4%)	3 (2%)	ns
Volume expanders on day 1 – no. (%)	6 (13%)	6 (13%)	ns	16 (11%)	15 (11%)	ns
Inotropic drugs on day 1 – no. (%)	5 (11%)	6 (13%)	ns	7 (5%)	7 (5%)	ns
Phototherapy days[a]	1.7 ± 2.5	2.1 ± 2.7	ns	2.5 ± 3.0	2.5 ± 2.9	ns

[a] *Value given as mean ± SD*

born DC twins (p = 0.129), but was of greater magnitude compared to the fall in Hb in second-born DC twins (p = 0.028).

In MC twins, mean Hb values in the smaller and larger twin were respectively 16.3 and 16.8 g/dL (p = 0.065) at birth, and 16.7 and 16.4 g/dL (p = 0.753) on day 2. In DC twins, mean Hb values in the smaller and larger twin were respectively 16.8 and 16.4 g/dL (p = 0.148) at birth, and 16.3 and 16.0 g/dL (p = 0.303) on day 2.

Histopathological examination of MC placentas confirmed monochorionicity in all cases. Information on the type of vascular anastomoses was obtained in 80% (36/45) of the MC placentas. Arterio-arterial anastomoses, veno-venous anastomoses and arterio-venous anastomoses were present in 83% (30/36), 14% (5/36) and 83% (30/36) of placentas, respectively. Superficial vascular anastomoses were present in 83% (30/36) of MC placentas and absent in 17% (6/36) of MC placentas. Differences in Hb values in MC twin pairs with and without superficial vascular anastomoses are presented in Table 4. Mean Hb differences on day 2 were significantly higher in MC with superficial anastomoses than in MC twins without superficial vascular anastomoses, 4.0 g/dL and 1.5 g/dL, respectively (p = 0.036). Hb differences > 5 g/dL on day 2 were detected in 27% (8/30) of MC twin pairs with superficial anastomoses and 16% (1/6) of MC twin pairs without superficial anastomoses. There was no significant association between Hb differences > 5 g/dL and superficial vascular anastomoses probably because of the small number of cases.

TABLE 4 Hb values in MC twin pairs with and without superficial vascular anastomoses.

	Twin pairs with superficial anastomoses (n = 30)	Twin pairs without superficial anastomoses (n = 6)	p-value
Hb difference at birth[a]	1.3 ± 1.3	1.3 ± 1.3	ns
Hb difference on day 2[a]	4.0 ± 2.2	1.5 ± 1.6	0.036
Hb difference >5 g/dl at birth – no. (%)	0 (0%)	0 (0%)	–
Hb difference >5 g/dl on day 2 – no. (%)	8 (27%)	1 (16%)	ns

[a] *Value given as mean ± SD*

The median time interval between delivery of twin 1 and twin 2 in the sub-group of MC twins delivered vaginally was 5 minutes (range 1-40 minutes). We found a positive correlation between time interval between vaginal delivery of the first and second-born infants and Hb differences on day 2 (Spearman correlation coefficient = 0.402), but the increase in Hb differences was not significant (p = 0.079). No correlation was found between time interval between vaginal delivery of the first and second-born infants and Hb differences at birth. The difference in mean Hb concentrations between MC twins born vaginally and through caesarean section was not significant, respectively 1.7 versus 1.2 g/dL at birth (p = 0.355) and 3.7 versus 3.9 g/dL on day 2 (p = 0.778).

Comment

This study shows that Hb differences at birth between MC twins without chronic TTTS and DC twins are similar. Only a small number of twin pairs in both groups of twins have highly discordant Hb values (Hb difference > 5 g/dL) at birth. However, when Hb levels are measured on day 2, Hb differences between MC twins become more evident and one in four MC twin pairs has Hb differences > 5 g/dL. The different Hb values between birth and day 2 may be due to acute shifts in blood volume in MC twins during delivery[39]. Hb concentrations are known to be unreliable when measured shortly after acute shifts in blood volume. When acute blood loss occurs, Hb levels may initially remain within the normal range and not reflect the actual blood loss[46;219;220]. After equilibration between

intravascular and extravascular spaces has completed, the expected fall in Hb levels may appear. This compensatory mechanism of equilibration or hemodilution may take several hours to complete[39;220;224]. Hemodilution is due to a gradual increase in plasma volume and intravascular volume to compensate for the blood loss. Conversely, when an infant receives an acute transfusion of blood, the infant responds by gradually decreasing its plasma volume while the red cell mass remains unchanged[224;225]. After equilibration between intravascular and extravascular spaces is complete, the expected rise in Hb levels may appear.

This study also establishes a clear association between Hb difference and birth order in MC twins. We found that Hb levels on day 2 were significantly higher in second-born MC twins than in first-born MC twins. Our results are in agreement with previous studies[35;38;46]. Once the umbilical cord of the first-born twin is clamped, the second twin has sole access to the entire placenta and may therefore receive blood through the vascular anastomoses from both parts of the placenta [38;41]. To determine whether the Hb difference in MC twins was due to a relatively higher Hb concentration in second-born twins or a lower Hb concentration in first-born twins, we compared Hb values between MC and DC twins. The fall in Hb between birth and day 2 found in first-born MC is also detected in first and second-born DC twins and is probably due to factors related to prematurity, such as blood loss caused by repeated venepunctures and hemodilution caused by treatment with volume expanders. The rise in Hb between birth and day 2 in second-born MC twins was in striking contrast to the fall in Hb in all other infants. Thus, Hb differences between MC twins are probably due to a relatively higher Hb in second-born twins rather than a lower Hb in first-born twins. The rise in Hb levels in second-born twins on day 2 compared to day 1 may reflect the process of hemoconcentration after acute blood transfusion. High red cell volume can be potentially harmful in neonates and lead to polycythemia or severe hyperbilirubinemia[224;225] requiring an exchange transfusion, as occurred in one second-born twin in our study.

We also report that Hb differences in MC twins measured on day 2 are greater in the presence of superficial vascular anastomoses. Superficial arterio-arterial and veno-venous anastomoses are known to be responsible for acute transfusions of blood after intrauterine fetal death of one of the

twins[30;32;70]. In analogy to acute perimortem TTTS, it is conceivable that superficial vascular anastomoses may also be responsible for rapid inter-twin blood transfusion during delivery. Hypothetically, after clamping of the first-born's cord, uterine contractions may allow placental blood from the first twin's low-pressure placental bed to be transfused through the low-resistance superficial vascular anastomoses into the higher pressure circulation of the second twin.

Disparity in placental blood transfusion may also be responsible for Hb differences in MC twins[38]. The amount of placental blood transfused into the circulation of the newborn before clamping of the umbilical cord is a major factor determining Hb levels[225]. Assuming that the time taken to clamp each of the umbilical cords is not different between twin 1and 2, other factors may be related to Hb differences in MC twins, such as mode of delivery or time interval between birth of twin 1 and 2. We detected no association between mode of delivery (vaginal delivery or cesarean section) and Hb differences in MC twins. A positive, but statistically not significant correlation was found between Hb differences and time interval between vaginal delivery of twin 1 and twin 2.

A weakness of our study is that we excluded all uncomplicated term or near-term twin deliveries, as Hb is obviously not routinely taken on term infants. Risk of significant acute perinatal transfusion in term MC twins remains therefore unknown and needs to be investigated in future studies.

A handful of case reports of MC twins with acute perinatal TTTS have been reported in the literature[34-39]. Most donor twins had signs of acute hemorrhagic hypovolemic shock and required prompt refilling of the intravascular compartment with blood transfusion or volume expanders. In our study, only one of the 45 pairs of MC twins fulfilled our criteria for acute perinatal TTTS and the donor twin had no characteristic signs of acute hemorrhagic hypovolemic shock, such as pallor, tachycardia and/or hypotension. Diagnostic criteria for acute perinatal TTTS vary among the published case reports, but always include a significant Hb difference at birth. Most reports also rely on the absence of birth weight discordance between both twins to exclude chronic TTTS[34;35;38;39]. However, birth weight and Hb discordance are not appropriate diagnostic criteria for chronic TTTS[11;27;28;71;93]. As shown in this study, birth weight discordance and Hb difference can also be found in DC twins and in MC

twins without antenatal signs of chronic TTTS. Moreover, Hb discordance is only present in a minority of cases with chronic TTTS[102]. Diagnosis of TTTS is nowadays based on antenatal ultrasound finding of the characteristic oligo-polyhydramnios sequence[11;27-29;93]. Therefore, most case reports in which acute perinatal TTTS was diagnosed without antenatal ultrasound examinations, may have been cases of chronic TTTS, the most common cause for hematological and hemodynamic discordance in MC twins[34;35;38;39]. Furthermore, acute shifts of blood volume in acute perinatal TTTS or acute perimortem TTTS may occur in any MC twins with patent vascular anastomoses, with or without birth weight discordance.
Differential diagnosis of acute perinatal TTTS should not only include chronic TTTS, but also acute perimortem TTTS. In one case report of acute perinatal TTTS, the hyperperfused recipient twin died during delivery and the hypoperfused donor died postnatally due to acute renal failure[36]. However, pathologic examination showed chorioamnionitis and funisitis, and culture of the placenta grew a *Fusobacterium* species. Therefore, acute perimortem TTTS due to bacterial sepsis seems a more plausible explanation for the hematological and hemodynamic discordance at birth rather then acute perinatal TTTS.
The pathogenesis of acute perinatal TTTS remains unclear. Superficial vascular anastomoses have been suggested to be responsible for acute perinatal blood transfusions[71]. The association between Hb differences and superficial vascular anastomoses reported in this study is in agreement with this suggestion. Acute fetal blood loss from the donor twin into the circulation of the recipient twin may then occur as a result of variations in blood pressure due to uterine contractions or fetal positions, but strong evidence is not available. Birth order may also play a major role in the etiology of acute perinatal TTTS. Uotila and Tammela treated three cases with acute perinatal TTTS during a 2-year period[37]. In all three cases, the donor twin was the second-born twin. The authors therefore hypothesized that acute hemorrhagic hypovolemic shock may result from acute feto-placental transfusion from the second-born twin into the placenta due to decreased vascular resistance in the placenta-share of the first twin. Although the number of MC twins born at our hospital during the study period is almost certainly higher than the number reported by Uotila and Tammela, similar events were never recorded at our center. Moreover, most

authors suggest that after clamping the cord of the first-born twin, second-born twins are more likely to receive a large placental blood transfusion rather than lose blood into the placenta[35;38;41]. Our findings also support this hypothesis, since second-born twins had significantly higher Hb values than first-born twins.

We conclude that significant Hb differences are often found in MC twins without chronic TTTS, especially when Hb measurements are performed on day 2. Hb differences in MC twins are related to birth order and superficial vascular anastomoses.

Part 3
Neonatal outcome

Chapter 8

Neonatal outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser occlusion of vascular anastomoses

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Abstract

Objective: To determine neonatal mortality and morbidity in monochorionic twins with chronic twin-to-twin transfusion syndrome (TTTS) treated with fetoscopic laser occlusion of vascular anastomoses.

Methods: Prospective study of monochorionic twins delivered at our center between June 2002 and December 2004. Neonatal outcome was assessed in 40 monochorionic twin pairs with TTTS treated with laser compared to 46 monochorionic twin pairs without TTTS.

Results: Neonatal mortality in the TTTS and no-TTTS group was 8% (6/76) and 3% (3/90), respectively. The rate of severe cerebral lesions on ultrasound scan in the TTTS and no-TTTS group was 14% (10/72) and 6% (5/82), respectively. The incidence of adverse neonatal outcome (neonatal mortality, major neonatal morbidity or severe cerebral lesions) in the TTTS and no-TTTS group was 26% (20/76) and 13% (12/90), respectively (RR = 1.97, 95% CI = 1.03 to 3.77).

Conclusions: Although perinatal outcome in TTTS has improved after laser therapy, neonatal mortality and morbidity remain high. Relative risk for adverse neonatal outcome is twofold increased in TTTS treated with laser than in monochorionic twins without TTTS.

Introduction

Chronic twin-to-twin transfusion syndrome (TTTS) is the most common major complication of monochorionic twin pregnancies and is due to unbalanced inter-twin blood transfusion via placental vascular anastomoses. TTTS leads to hypovolemia, oliguria and oligohydramnios in the donor twin and hypervolemia, polyuria and polyhydramnios in the recipient twin. If untreated, perinatal mortality and morbidity rates ranges from 50% to 100%[110;111]. Improved prenatal care strategies and management options have led to a significant increase in perinatal survival, to 60-70%[27;28;93;129;137]. As a result of the decrease in perinatal mortality, attention is now shifting towards short-term and long-term morbidity. Some studies have focused on long-term neurological morbidity and usually report a high risk of cerebral palsy in TTTS survivors[12;147;151;152;226]. Only a few studies have focused on other characteristic morbidities in TTTS, such as cardiovascular and renal morbidity. Cardiac morbidity occurs mainly in recipients with chronic volume overload and includes biventricular hypertrophy, tricuspid regurgitation and right ventricular outflow tract obstruction,[19;159] whereas renal morbidity is especially reported in donor twins and is due to chronic volume depletion and renal hypoperfusion[21;148;165]. Ischemic limb injury has sporadically been reported in recipient twins due to polycythemia-hyperviscosity syndrome[167].
The two current treatment options in TTTS are serial amnioreduction and fetoscopic laser occlusion of vascular anastomoses[27;93;129;130]. Recently, a randomized trial demonstrated that the incidence of disease-free survival was higher after laser treatment than after serial amnioreduction[10]. Although fetoscopic laser occlusion of vascular anastomoses is advocated as the preferred treatment for TTTS, limited data are available on neonatal morbidity associated with this treatment.
The first objective of this study was to determine the incidence of adverse neonatal outcome in monochorionic twins with TTTS treated with laser and to characterize neonatal morbidity after laser treatment, including cerebral, cardiac, hematological, renal and limb disorders. The second objective was to determine neonatal differences in outcome between donors and recipients after laser treatment.

Methods

All consecutive cases of monochorionic twins with chronic TTTS treated with laser (TTTS group) and delivered at our center between June 2002 and December 2004 were included in this study. The Leiden University Medical Center is a tertiary medical center and is the national referral center for fetal therapy including laser treatment for TTTS in the Netherlands. The aim of fetoscopic laser surgery is to occlude the anastomosing vessels along the vascular equator of the placenta in order to interrupt the inter-twin transfusion of blood. After laser treatment, the amniotic sac of the recipient is drained a single time to reduce the polyhydramnios. Monochorionic twins with major fetal congenital abnormalities, acardiac twinning and triplet pregnancies were excluded from the study. The control group consisted of all monochorionic twins without TTTS (no-TTTS group) born at our center during the same study period. The institutional review board approved the study and all parents gave informed consent.
TTTS was diagnosed using standard prenatal ultrasound criteria[105]: 1.) Monochorionicity established by absence of a "twin peak" sign and presence of a thin dividing membrane, 2.) Oligohydramnios (deepest vertical pocket ≤ 2cm) in the twin sac of one fetus, and 3.) Polyhydramnios (deepest vertical pocket ≥ 8cm before 20 weeks of gestation or ≥ 10cm after 20 weeks of gestation) in the twin sac of the other fetus. TTTS pregnancies were staged prior to laser treatment according to the criteria of Quintero[9]. Monochorionicity was confirmed after delivery by histopathological examination of the placenta. Gestational age at laser treatment and delivery was recorded. A team composed of a sonographer specialized in fetal ultrasound (MS), a maternal fetal medicine specialist (JM) and a neonatologist (EL) met directly after delivery of each TTTS twin pair to determine which of the newborn infants was the donor and the recipient, using antenatal ultrasound measurements such as umbilical cord insertions, the positions of both fetuses before and after laser treatment and prior to delivery, estimated fetal weights and birth weights and careful examination of fetal membranes after birth to identify the fetoscopic defect in the recipient's membranes.
At birth, weight discordance was assessed and calculated as follows: ((birth weight larger twin – birth weight smaller twin)/ birth weight larger twin) x

100%. Significant birth weight discordance was defined as more than 20% difference in birth weight. Small for gestational age was defined as birth weight less than 10th percentile[227]. Hemoglobin levels were measured from umbilical cord blood. Reticulocyte count was measured only in the TTTS group. Anemia at birth was defined as hemoglobin level below the 3rd centile for gestational age requiring a blood transfusion during the first day of life. Polycythemia-hyperviscosity syndrome was defined as a hematocrit greater than 65% in symptomatic infants requiring treatment with partial exchange transfusion. Blood pressure was measured shortly after birth. Hypotension at birth was defined as a systolic blood pressure below the 3rd percentile for gestational age and requiring treatment with volume expanders and/or inotropic support during the first day of life[221]. Cerebral ultrasound scans were performed in all neonates on the first day of life and thereafter according to our unit protocol. The cerebral ultrasound protocol at our neonatal ward requires a minimum of 3 scans during the first week of life (day 1, 3 and 7), followed by weekly scans thereafter until discharge or transfer to another hospital. Intraventricular hemorrhage with or without parenchymal involvement was graded according to the classification of Volpe[228], and periventricular leucomalacia was graded according to the classification of de Vries *et al*[223]. Ventricular dilatation was diagnosed when measurement of the lateral ventricles exceeded the 97th percentile, using ventricular index measurements as described by Levene[229]. Severe cerebral lesions on ultrasound scans were defined as the presence of at least one of the following findings: intraventricular hemorrhage grade III, intraventricular hemorrhage with parenchymal involvement, cystic periventricular leucomalacia ≥ grade II, ventricular dilatation, porencephalic or parenchymal cysts or other severe cerebral lesions associated with adverse neurological outcome. We recorded the following neonatal morbidity: respiratory distress syndrome, chronic lung disease defined as oxygen dependency at 36 weeks postmenstrual age, symptomatic patent ductus arteriosus requiring indomethacin therapy or surgical closure, necrotizing enterocolitis ≥ stage II[230], retinopathy of prematurity ≥ stage III[231] and renal failure. Adverse neonatal outcome was defined as neonatal mortality or severe cerebral lesions or one of the following major neonatal disorders: chronic lung disease, necrotizing enterocolitis ≥ grade II, retinopathy of prematurity ≥ stage III, chronic renal

failure, major cardiac morbidity or major ischemic limb injury.
Data on neonatal morbidity and morbidity in monochorionic twins with TTTS, treated at our center but delivered at other centers, were reviewed retrospectively from medical records. The choice for in-institution or out-of-institution delivery depended on the clinical picture and the wish of the parents.
The primary outcome measure was adverse neonatal outcome. Secondary outcome measures were neonatal death, severe cerebral lesions and neonatal morbidity. Outcome was compared between the TTTS and the no-TTTS group, and between donors and recipients in the TTTS group.
Statistics: We calculated that group sizes of 69 infants were required to demonstrate a 15% difference in adverse neonatal outcome (20% versus 5%) with 0.05 significance and a power of 80%, by two-tailed analysis. Results of categorical variables were compared using Fisher's exact test or Chi-square test, as appropriate. Unpaired Student's *t* test was used to compare normally distributed values between two groups. For comparisons between donors and recipients, the paired Student t test was used for normally distributed continuous variables and the Mc Nemar test was used for analysis of paired nominal variables. A p-value < 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 11 (SPSS, Inc., Chicago, Illinois, USA).

Results

During the study period 85 women with TTTS were treated at our center with fetoscopic laser surgery. Laser treatment resulted in at least one survivor in 82% (70/85) of pregnancies. Intrauterine fetal demise occurred in 25% (42/170) of the fetuses. Neonatal death occurred in 7% (9/128) of life-born infants. Overall perinatal survival was thus 70% (119/170).
Forty TTTS pregnancies treated with laser were delivered at our center. All parents agreed to have their child participate in our study. The mean gestational age at laser treatment was 19.9 weeks (range 16-26 weeks). The median Quintero stage was II. Twenty percent (8/40) of pregnancies were stage I, 35% (14/40) stage II, 40% (16/40) stage III and 5% (2/40) stage IV. Two TTTS pregnancies were complicated: laser treatment was

attempted in a TTTS pregnancy presenting at 22 weeks of gestation with premature contractions and preterm premature rupture of the membranes. During fetoscopy, the donor twin appeared to be stuck on the placental surface hampering visualization of the placental vascular equator, resulting in incomplete laser treatment. After counseling, the parents opted for selective coagulation of the umbilical cord of the recipient twin. In another TTTS pregnancy, laser treatment was attempted at 22 weeks of gestation, although preterm labor due to massive polyhydramnios was at an advanced stage. After successful laser treatment, a cerclage was inserted to prevent premature delivery. Two weeks later, preterm premature rupture of the membranes occurred. Two girls were delivered at 25 weeks of gestation. Both pregnancies were included in this study on an intention to treat basis. Forty-six monochorionic pregnancies without TTTS were delivered at our center during the study period and all parents agreed to have their child participate in our study.

Intrauterine fetal death of one of the twins occurred in 4 cases in the TTTS group (2 donor twins and 2 recipient twins) and in 2 cases in the group without TTTS. The total number of twin infants included in the TTTS group and no-TTTS group was therefore 76 and 90, respectively. Patient's characteristics, inter-twin birth weight differences and hemoglobin differences at delivery in both groups are presented in Table 1.

Neonatal mortality and short-term morbidity in both groups are presented in Table 2. Adverse neonatal outcome occurred more often in the TTTS than in the no-TTTS group, 26% (20/75) and 13% (12/90), respectively (relative risk (RR) = 1.97, 95% confidence interval (CI) = 1.03 to 3.77). One donor twin in the TTTS group developed massive tricuspid regurgitation and severe hydrops after laser treatment. The infant died at delivery due to intractable cardiac failure. One recipient twin in the TTTS group was found to have right ventricular outflow tract obstruction and required balloon valvuloplasty. In another recipient twin, necrosis of the right hand and forearm was suspected antenatally at 24 weeks of gestations, eight weeks after laser treatment. Complete hypoplasia of the right hand and forearm was confirmed at delivery. One second-born twin in the TTTS group died at birth due to severe perinatal asphyxia, which was not related to TTTS. Transient renal failure with oliguria and high creatinin level (maximum serum creatinin level of 0.18 mmol/l on day 6) was present in one donor in

TABLE 1 Patients' characteristics, inter-twin birth weight differences and hemoglobin differences at delivery.

	TTTS group (n = 76)	No-TTTS group (n = 90)	p-value
Gestational age at birth – weeks[a]	32.6 ± 3.5	33.6 ± 3.3	0.07
Female – no. (%)	38 (48%)	46 (51%)	0.74
Vaginal delivery – no. (%)	51 (66%)	57 (63%)	0.61
Median Apgar score at 5 min (range)	8.5 (1-10)	9 (3-10)	< 0.01
BW – g[a]	1785 ± 676	2077 ± 681	< 0.01
BW difference – %[a]	19 ± 17	13 ± 11	0.07
BW difference > 20% – no. (%)	30 (39%)	22 (24%)	0.01
Hb at delivery – g/dL [a]	16.1 ± 4.2	16.1. ± 3.2	0.98
Hb difference – g/dL [a]	5.0 ± 5.1	1.4 ± 1.4	< 0.01
Hb difference >5 g/dL – no. (%)	28 (37%)	2 (2%)	< 0.01
BW difference > 20% + Hb difference > 5 g/dL – no. (%)[b]	6 (8%)	2 (2%)	0.19

[a] *Value given as mean ± SD*
[b] *Higher hemoglobin value in larger twin*
BW: Birth weight; Hb: Hemoglobin

TABLE 2 Neonatal mortality and morbidity rates.

	TTTS group (n = 76)	No-TTTS group (n = 90)	p-value
Small for gestational age – no. (%)	19 (25%)	17 (19%)	0.32
Respiratory distress syndrome – no. (%)	26 (34%)	19 (21%)	0.05
Chronic lung disease – no. (%)	5 (7%)	4 (4%)	0.53
Patent ductus arteriosus – no. (%)	5 (7%)	4 (4%)	0.53
Necrotizing enterocolitis – no. (%)	2 (3%)	2 (2%)	0.85
Hypotension at birth – no (%)	11 (14%)	9 (10%)	0.34
Hydrops – no. (%)	1 (1%)	1 (1%)	0.90
Anemia at birth – no. (%)	13 (17%)	4 (5%)	0.01
Polycythemia-hyperviscosity – no. (%)	4 (5%)	1 (1%)	0.11
Severe cerebral lesions – no. (%)	10 (14%)	5 (6%)	0.09
Neonatal death – no. (%)	6 (8%)	3 (3%)	0.20
Adverse neonatal outcome – no. (%)	20 (26%)	12 (13%)	0.03

the TTTS group. No infant developed chronic renal failure.
Cerebral ultrasound scans were performed in 93% (154/166) of the neonates. Abnormalities detected on cerebral ultrasound in both groups are presented in Table 3. Cystic periventricular leucomalacia grade III or IV was not detected. Incidence of severe cerebral lesions detected on ultrasound performed on day 1 in the TTTS group and no-TTTS group was 8% (6/72) and 2% (2/82), respectively (p = 0.147). Incidence of severe cerebral lesions at discharge in the TTTS group and no-TTTS group was 14% (10/72) and 6% (5/82), respectively (p = 0.089). One pair of twins in the no-TTTS group gradually developed progressive neurological disease during the first months after birth, associated with blindness, deafness and cerebral palsy. Diffuse cystic white matter disease was identified on cerebral ultrasound scans and magnetic resonance imaging. The combination of symptoms suggested a mitochondrial disorder, but this could not be confirmed. Although congenital anomalies occur more frequently in monochorionic twins[232], metabolic diseases or mitochondrial disorders are not directly associated with monochorionicity.

TABLE 3 Characterization of severe cerebral lesions detected on ultrasound scans.

	TTTS group (n = 72)	No-TTTS group (n = 82)
Intraventricular hemorrhage grade III – no. (%)	2 (3%)	1 (1%)
Periventricular hemorrhagic infarction – no. (%)	1 (1%)	0 (0%)
Cystic periventricular leucomalacia grade II – no. (%)	4 (6%)	1 (1%)
Ventriculomegaly – no. (%)	6 (8%)	2 (2%)
Porencephalic cyst – no. (%)	1 (1%)	0 (0%)
Diffuse cystic white matter disease – no. (%)	0 (0%)	2 (2%)

After delivery, all donors and recipients delivered at our center were identified without doubt. Differences in neonatal mortality and morbidity between donors and recipients in the TTTS group are summarized in Table 4. No difference in adverse neonatal outcome was found between donors and recipients. The incidence of respiratory distress syndrome, chronic lung disease, patent ductus arteriosus, necrotizing enterocolitis, major cardiac disease and renal failure was similar between donors and recipients.

TABLE 4 Neonatal mortality and morbidity rates in donors and recipients after laser treatment.

	Donors (n = 38)	Recipients (n = 38)	p-value
Birth weight – g[a]	1607 ± 625	1876 ± 657	0.001
Small for gestational age – no. (%)	15 (39%)	4 (11%)	0.01
Hemoglobin at birth – g/dL[a]	15.9 ± 4.6	16.3 ± 3.8	0.74
Anemia at birth – no. (%)	8 (21%)	4 (11%)	0.34
Polycythemia-hyperviscosity – no. (%)	4 (11%)	0 (0%)	0.12
Reticulocyte count – %[a]	7.2 ± 0.4	7.4 ± 0.3	0.82
Severe cerebral lesions – no. (%)	4 (11%)	6 (16%)	0.45
Neonatal death – no. (%)	2 (5%)	4 (11%)	0.62
Adverse neonatal outcome – no. (%)	8 (21%)	12 (32%)	0.34

[a]*Value given as mean ± SD*

A hemoglobin difference > 5 g/dL at birth was found in 35% (14/40) of twin pairs. In 8 of these pairs (57%) the highest hemoglobin level was found in the ex-recipient, and in the 6 remaining pairs (43%) the ex-donor had the highest hemoglobin level. Partial exchange transfusion for polycythemia-hyperviscosity syndrome was required in 4 cases, all ex-donors. In 2 of these 4 cases a residual arterio-venous anastomosis from recipient to donor was found after placenta injection with colored dye. No difference in reticulocyte count was found between donors and recipients. Reticulocyte count was significantly higher in the infants with lower hemoglobin levels compared to their co-twin with higher hemoglobin levels, 8.3% and 6.4%, respectively (p = 0.018).

During the study period, 45 life-born neonates with TTTS, treated with laser at our center, were delivered at other centers. Mean gestational age at delivery in the group of infants delivered at other centers was significantly higher than the mean gestational age in the group of infants delivered at our center, 34.4 and 32.6 weeks, respectively (p = 0.005). Median Quintero stage was similar in both groups (median: II; range I–IV). The incidence of adverse neonatal outcome in the group of neonates with TTTS delivered at other centers was 18% (8/45)(neonatal mortality: n = 3; severe neonatal morbidity: n = 3; severe cerebral lesions: n = 3). Outcome data in infants delivered at other centers was often incomplete. Cerebral ultrasound scans were performed in 44% (20/45) of the neonates. One

infant discharged without evidence of adverse neonatal outcome, gradually developed spastic hemiplegia. Cerebral ultrasound scan performed for the first time at 7 months of age showed large unilateral cystic white matter lesions consistent with middle cerebral artery infarction.

Discussion

This study is the first single center study reporting neonatal mortality and morbidity in TTTS after fetoscopic laser occlusion of vascular anastomoses. The data show that even though disease-free survival in TTTS has significantly increased with laser treatment[10], neonatal mortality and morbidity remain high. Adverse neonatal outcome was more than twice as likely to occur in TTTS survivors after laser treatment than in monochorionic twins without TTTS. Since monochorionicity is known to be associated with adverse outcome even in the absence of TTTS[4;144], selection of an adequate control group of monochorionic twins is crucial.
Various factors may be related to adverse outcome in TTTS after laser treatment. Differences in laser techniques or experience is known to influence outcomes[123]. In a previous study we reported that perinatal outcomes of TTTS treated at our center with laser treatment were similar to those published by major laser centers worldwide[137]. Differences in surgical technique are therefore unlikely to have influenced our results.
The most important factor leading to the high rate of adverse neonatal outcome in the TTTS group was the high rate of severe cerebral lesions. The 14% rate of severe cerebral lesions in TTTS survivors treated with laser found in this study was higher than the 7% rate reported in two other studies[10;124]. However, interpretation of the different results is difficult since inclusion criteria and definitions of cerebral lesions vary among the studies. In the study of Senat *et al*, severe cerebral lesions consisted only of severe cystic periventricular leucomalacia grade III to IV (n = 8) and IVH grade III to IV (n = 2)[10] whereas we also included cystic periventricular leucomalacia grade II and other major cerebral ultrasound abnormalities. Most importantly, the incidence of severe cerebral lesions in the study of Senat *et al* was related to the total number of fetuses and not the number of life-born infants[10]. The incidence of severe cerebral lesions in survivors in the

study of Senat would then be 12% (10/82) (periventricular leucomalacia grade III to IV: n = 8; IVH grade III to IV: n = 2)[10]. The incidence of cerebral lesions reported by Hecher *et al* does not include abnormal findings in the 6 patients with neonatal death[10;124], whereas the reported rate in this study concerns all life-born neonates regardless of neonatal survival. If we exclude the severe cerebral lesions found in the patients who died during the neonatal period, the incidence of severe cerebral lesions in this study is 10% (7/68). Nevertheless, rates of severe cerebral lesions found in this study are much lower than the reported rate of 18% to 55% in TTTS survivors not treated with laser[70;124;128;146;147]. Whether cerebral lesions in TTTS treated with laser are related to antenatal injury sustained before or after laser surgery, or are due to postnatal injury related to prematurity remains unclear. In the study by Banek *et al*, 2 fetuses had abnormal cerebral scans (ventriculomegaly, cystic defects) prior to laser treatment[151]. We found that severe cerebral lesions in the TTTS group were often detected on cerebral ultrasound examinations performed within 24 hours of delivery, suggesting antenatal origin of cerebral damage. However, presence or absence of severe cerebral lesions on ultrasound examination does not necessarily rule out a normal or adverse neuro-developmental outcome[233]. The lack of long-term follow-up data on neurological performance is an important limitation of the current study. We are therefore performing a prospective long-term follow-up study to determine the exact rate of cerebral palsy and cognitive deficits in both groups of twins.

Major cardiac disease occurred in two neonates in the TTTS group. One recipient had severe right ventricle outflow tract obstruction, and one donor twin had severe tricuspid regurgitation detected antenatally and died immediately after birth. Major renal morbidity was not detected and only one donor in the TTTS group had symptoms of transient mild renal failure. Even though the difference was not significant, gestational age in the TTTS group was slightly lower than in the no-TTTS group. This slight difference in gestational age may partially explain the lower birth weight and Apgar score in the TTTS group and the higher rate of respiratory distress syndrome. However, outcome in the TTTS group was also worse regarding items independent of gestational age, such as hematological discordance. Significant inter-twin hemoglobin differences were found in the TTTS-group after laser treatment resulting in a higher incidence of anemia and

polycythemia-hyperviscosity syndrome. Differences in hemoglobin levels in the TTTS group were not due to hemoglobin differences between donors and recipients. When a hemoglobin difference > 5 g/dL was present, the higher hemoglobin level was almost as likely to be present in donors as in recipients. Several factors may lead to significant higher hemoglobin levels in donors than in recipients after laser treatment. First, reversal of TTTS may occur after laser treatment when arterio-venous anastomoses from recipient to donor remain patent due to incomplete coagulation of all vascular connections or due to re-vascularization. Residual arterio-venous anastosmoses were found to be the cause of polycythemia-hyperviscosity syndrome in two of the four patients requiring treatment with partial exchange transfusion. Secondly, higher compensatory hematopoiesis in donors due to chronic lower hemoglobin levels may theoretically persist after laser treatment and lead to higher hemoglobin levels in the donor than in recipients. However, no difference in reticulocyte count between donors and recipients was found in this study.

In the past, diagnosis of TTTS was reached on the basis of two main neonatal criteria: 1.) inter-twin birth weight discordance > 20% and 2.) hemoglobin difference > 5 g/dL. These criteria have been abandoned since similar differences occur in dichorionic twins and monochorionic twins without TTTS. As shown in this study, these criteria were only present in 8% of TTTS twin pairs after laser treatment. Nowadays, diagnosis of TTTS is reached solely on prenatal ultrasound criteria when the characteristic oligo-polyhydramnios sequence is detected. In this study we found that even though mean hemoglobin levels were similar between donors and recipients, mean birth weight in donors was still significantly lower than in recipients, and donors were more often small for gestational age. When a birth weight difference > 20% was present, recipients were always the larger twin, except in one case where massive hydrops was present in the donor twin. With exception of birth weight discordance, no difference in neonatal mortality and major or minor morbidity was detected between donors and recipients.

The data in this study should be interpreted with care since a selection bias may have been introduced due to the specific nature of our tertiary referral center with neonatal intensive care facilities. It is conceivable that the more complicated or more premature TTTS cases may have been delivered

at our center whereas the less complicated or less premature cases were born elsewhere. Indeed, the mean gestational age at birth and incidence of disease-free survival in neonates with TTTS treated but not delivered at our center, was higher compared to the TTTS group delivered at our center. However, cerebral ultrasound scans were not routinely performed in neonates delivered at other centers. Therefore, the incidence of severe cerebral lesions and adverse neonatal outcome in the group of infants delivered at other centers may be underestimated. To overcome this type of bias we are currently performing a long term neuro-developmental follow-up study in all TTTS cases treated at our center included those delivered elsewhere.

In conclusion, even though perinatal outcome in TTTS has significantly improved after fetoscopic laser occlusion of vascular anastomoses, neonatal mortality and morbidity remain high. Neonates with TTTS after laser treatment therefore require close monitoring, donors and recipients alike.

Chapter 9

Incidence, origin and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery

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Abstract

Objectives: To determine the incidence, origin and character of cerebral lesions in monochorionic twins with twin-to-twin transfusion syndrome (TTTS) treated with fetoscopic laser surgery.

Methods: Prospective study of monochorionic twins with TTTS treated with fetoscopic laser surgery and monochorionic twins without TTTS delivered at our center between June 2002 and September 2005, using cranial ultrasonography.

Results: Incidence of antenatally acquired severe cerebral lesions in the TTTS group was 10% (8/84) and 2% (2/108) in the no-TTTS group ($p = 0.02$). Incidence of severe cerebral lesions at discharge was 14% (12/84) in the TTTS group and 6% (6/108) in the no-TTTS group ($p = 0.04$). Antenatal injury was responsible for severe cerebral lesions in 67% (8/12) of the TTTS group.

Conclusions: Incidence of severe cerebral lesions in TTTS treated with fetoscopic laser surgery is high and results mainly from antenatal injury.

Introduction

The risk of cerebral palsy in twins is 7 times higher than in singletons and mainly due to a higher incidence of prematurity and very low birth weight in twins[1]. Cerebral palsy in twins is specifically associated with monochorionicity. The risk of cerebral white matter lesions and subsequent cerebral palsy is 7 times higher in monochorionic than in dichorionic twins[4;234]. The origin of cerebral injury in monochorionic twins is related to the characteristic angioarchitecture of monochorionic placentas. Almost all monochorionic placentas have placental vascular anastomoses, whereas dichorionic placentas have none. Placental vascular anastomoses may cause unbalanced inter-twin blood transfusion and lead to twin-to-twin transfusion syndrome (TTTS). TTTS usually occurs during the 2nd trimester of pregnancy, resulting in hypovolemia, oliguria and oligohydramnios in the donor twin and hypervolemia, polyuria and polyhydramnios in the recipient twin. The higher rate of cerebral palsy in monochorionic twins results mainly from TTTS. Incidence of long-term neurological morbidity in TTTS ranges between 10 and 25%[186]. The pathogenesis of cerebral injury in TTTS is not clear. Cerebral injury in TTTS may result from antenatal injury secondary to hemodynamic and hematological disorders and/or from postnatal injury associated to prematurity and low birth weight[70;146;234]. Two previous cranial ultrasound studies in TTTS report a high incidence of antenatally acquired cerebral lesions[70;146]. However, these findings were based on small, retrospective, uncontrolled studies.

The primary objective of this study is to examine a large prospective series of monochorionic twins with TTTS treated with fetoscopic laser surgery and a control group of monochorionic twins without TTTS using cranial ultrasonography, and to determine the incidence, origin (antenatal or postnatal) and character of cerebral injury in relation to various risk factors.

Methods

Study population

This prospective study was conducted between June 2002 and September 2005 at the Leiden University Medical Center. The Leiden University Medical Center is a tertiary medical center and serves as the national referral center for intrauterine laser treatment in TTTS pregnancies in the Netherlands. All consecutive cases of monochorionic twins with TTTS treated with fetoscopic laser surgery (TTTS group) and monochorionic twins without TTTS (no-TTTS group) delivered at our center were included in this study. Pregnancies complicated by intrauterine fetal demise of both twins, major congenital anomalies, triplets and TTTS-pregnancies not treated with fetoscopic laser surgery were excluded from the study. The institutional review board of the Leiden University Medical Center approved the study and all parents gave written informed consent for their children. TTTS was diagnosed using standard prenatal ultrasound criteria[105], and staged according to the criteria of Quintero[9]. Monochorionicity was confirmed after delivery by histopathological examination of the placenta. Hemoglobin levels were measured at birth from umbilical cord blood. Anemia at birth was defined as a hemoglobin level below the 3rd percentile for gestational age requiring a blood transfusion during the first day of life. Polycythemia-hyperviscosity syndrome was defined as a hematocrit level greater than 65% in a symptomatic infant, requiring treatment with partial exchange transfusion. Inter-twin birth weight discordance was assessed and calculated as follows: ((birth weight larger twin – birth weight smaller twin)/ birth weight larger twin) x 100%. Birth weight discordance was defined as more than 20% difference in birth weight.

Cranial ultrasound

Cranial ultrasound scans were performed in all neonates within 24 hours after delivery and thereafter according to our clinical protocol. The cranial ultrasound protocol at our neonatal intensive care unit requires a minimum of 3 scans during the first week of life (day 1, 3 and 7), followed by at least 1 scan weekly until discharge. If cerebral abnormalities were detected, scanning frequency was intensified around the date of detection

and repeated at the time of the estimated date of confinement. In term infants, repeat cranial ultrasound scans were not performed if scans were normal at birth. Cranial ultrasound scans were performed with an Aloka 5000 scanner (Biomedic Nederland B.V., Almere, The Netherlands) with a multifrequency (5-10 MHz) transducer. The cerebral anatomy was visualized in the standard coronal and sagittal planes[235]. Experienced neonatologists performed all cranial ultrasound scans. Images were saved on magneto-optical disks and reviewed by two authors (EL and GvWM). Both authors were not blinded to the group to which the patients were assigned, since, being neonatologists, they were directly involved in the primary medical care of the neonates. Intraventricular hemorrhages (IVH) were classified according to Volpe[236] and periventricular leucomalacia (PVL) was graded according to de Vries *et al*[223]. Severe cerebral lesions on cranial ultrasound scans were defined as the presence of at least one of the following findings: IVH grade III, periventricular hemorrhagic infarction, PVL ≥ grade II, porencephalic cyst and ventricular dilatation. Ventricular dilatation was present when the width of one or both lateral ventricles exceeded the 97th percentile[229]. Other cerebral abnormalities associated with adverse neurological outcome were also recorded and classified as severe cerebral lesions. Severe cerebral lesions were considered to be of antenatal onset if present on the first cranial ultrasound scan on day 1. Periventricular white matter cysts detected within two weeks after birth were also considered to be of antenatal onset. PVL grade I, subependymal pseudocysts and lenticulostriate vasculopathy were classified as mild cerebral lesions. Lenticulostriate vasculopathy was defined as branching echogenic streaks in the basal ganglia.
The primary outcome measure was presence of severe cerebral lesions detected on cranial ultrasound scans. Outcome of the TTTS group was compared to the no-TTTS group.

Statistics

We calculated that group sizes of 69 infants were required to demonstrate a 15% difference in severe cerebral lesions (20% versus 5%) with a significance of 0.05 and a power of 80%, by two-tailed analysis. Results of categorical variables were compared using Fisher's exact test or

Chi-square test, as appropriate. Unpaired Student's *t* test was used to compare normally distributed values between two groups. For comparisons between donors and recipients, the paired Student t test was used for normally distributed continuous variables and the Mc Nemar test for analysis of paired nominal variables. Multiple logistic regression analysis with "random twin effect" was used to measure the independent effects of potential prognostic factors on outcome. A model with "random twin effect" was applied to adjust for possible correlated effects within twins. The results of the logistic models were expressed as an odds ratio (OR) and 95% confidence intervals (CI). A p-value < 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 11 (SPSS Inc., Chicago, IL, USA). Multiple logistic regression analysis was performed with EGRET version 2.0.1 for Windows (Cytel Software Corporation, Cambridge, Massachusetts, USA).

Results

During the study period, a total of 48 monochorionic twin pregnancies with TTTS treated with fetoscopic laser surgery and 60 monochorionic twin pregnancies without TTTS were delivered at our center. Patient's characteristics in both groups are presented in Table 1. The median Quintero stage in the TTTS-pregnancies was II. Seventeen percent (8/48) of pregnancies were stage I, 35% (17/48) stage II, 44% (21/48) stage III and 4% (2/48) stage IV.

A total of 206 live-born neonates were eligible for this study. The neonatal outcome of monochorionic twins delivered at our center between June 2002 and December 2004 has been published before[207]. Cranial ultrasound scans were performed in 93% (192/206) of neonates. Two neonates in the TTTS group died soon after birth before an ultrasound scan could be performed. Six pairs of twins (one twin pair in the TTTS group and five twin pairs in the no-TTTS group) were discharged from the hospital immediately after delivery before an ultrasound scan could be performed, all were term neonates in good clinical condition.

The overall incidence of antenatally acquired severe cerebral lesions monochorionic twins was 5% (10/192). The incidence of antenatally

TABLE 1 Baseline characteristics in the TTTS group and no-TTTS group.

	TTTS group (n = 48 pregnancies; 96 infants)	No-TTTS group (n = 60 pregnancies; 120 infants)	p-value
Gestational age at birth – wk[a]	32.1 ± 3.7	33.6 ± 3.1	< 0.01
Female – no. (%)	50 (52%)	64 (53%)	0.85
Vaginal delivery – no. (%)	59 (61%)	69 (57%)	0.54
Birth weight – g[a]	1710 ± 683	2074 ± 667	< 0.01
Birth weight difference – %[a]	18 ± 16	13 ± 11	0.07
Small for gestational age – no. (%)	21 (22%)	22 (18%)	0.33
Hemoglobin at birth – g/dL[a]	15.9 ± 4.5	16.1 ± 3.7	0.71
Hemoglobin difference at birth – g/dL[a]	5.8 ± 5.6	2.1 ± 3.2	< 0.01
Anemia at birth – no. (%)	18 (19%)	5 (4%)	< 0.01
Polycythemia – no. (%)	5 (5%)	2 (2%)	0.12
Intrauterine fetal death – no. (%)	8 (8%)	2 (2%)	0.02
Neonatal death – no. (%)	7 (7%)	3 (3%)	0.10

[a]*Value given as mean ± SD*

acquired severe cerebral lesions in the TTTS group and no-TTTS group was 10% (8/84) and 2% (2/108), respectively (OR: 5.58, 95% CI: 1.05 to 39.21, p = 0.02). Details of the antenatally acquired cerebral lesions are presented in Table 2. Small cysts located in the fronto-parietal periventricular white matter (PVL grade II) developed in three infants in the TTTS group within 2 weeks of birth. A large right-sided porecencephalic cyst in the temporo-parieto-occipital area was detected at birth in one donor twin. This was

TABLE 2 Antenatally acquired cerebral lesions detected by cranial ultrasound.

	TTTS group (n = 84 infants)	No-TTTS group (n = 108 infants)	p-value
IVH grade I – no. (%)	6 (7%)	2 (2%)	0.07
IVH grade II – no. (%)	3 (4%)	1 (1%)	0.20
IVH grade III – no. (%)	1 (1%)	1 (1%)	0.86
Periventricular hemorrhagic infarction – no. (%)	1 (1%)	0 (0%)	0.26
PVL grade II – no. (%)	3 (4%)	0 (0%)	0.05
PVL grade III – no. (%)	0 (0%)	1 (1%)	0.38
Lenticulostriate vasculopathy – no. (%)	10 (12%)	2 (2%)	< 0.01
Subependymal pseudocysts – no. (%)	3 (4%)	1 (1%)	0.20
Ventricular dilatation – no. (%)	3 (4%)	1 (1%)	0.20
Porencephalic cyst – no. (%)	1 (1%)	0 (0%)	0.26
Severe cerebral lesions – no. (%)	8 (10%)	2 (2%)	0.02

thought to be the end-stage of a middle cerebral artery infarction or a large periventricular hemorrhagic venous infarction. In the no-TTTS group, one infant was born 2 weeks after intrauterine fetal demise of its co-twin. On day 1, large cysts were found in the parieto-occipital periventricular white matter (PVL grade III).

The incidence of severe cerebral lesions at discharge in the TTTS group and no-TTTS group was 14% (12/84) and 6% (6/108), respectively (OR: 2.83, 95% CI: 0.93 to 8.94, p = 0.04). Findings on cranial ultrasound scans at discharge are summarized in Table 3. One recipient twin in the TTTS group developed extensive unilateral cystic white matter lesions six weeks after delivery. On MRI, this lesion was diagnosed as a middle cerebral artery infarction. One pair of twins in the no-TTTS group developed cerebral palsy, blindness and deafness and had diffuse cystic white matter disease on MRI, suggestive of a mitochondrial disorder. The proportion of severe cerebral lesions of antenatal origin versus severe cerebral lesions at discharge was 67% (8/12) in the TTTS group and 33% (2/6) in the no-TTTS group. Multiple logistic regression was carried out to measure the independent associations between severe cerebral lesions at discharge and

TABLE 3 Findings on cranial ultrasound scans at discharge.

	TTTS group (n = 84 infants)	No-TTTS group (n = 108 infants)	p-value
IVH grade I – no. (%)	6 (7%)	3 (3%)	0.16
IVH grade II – no. (%)	4 (5%)	2 (2%)	0.25
IVH grade III – no. (%)	2 (2%)	1 (1%)	0.42
Periventricular hemorrhagic infarction – no. (%)	2 (2%)	1 (1%)	0.42
PVL grade I – no. (%)	42 (40%)	25 (25%)	0.04
PVL grade II – no. (%)	5 (6%)	0 (0%)	0.01
PVL grade III – no. (%)	0 (0%)	1 (1%)	0.38
Lenticulostriate vasculopathy – no. (%)	10 (12%)	2 (2%)	< 0.01
Subependymal pseudocysts – no. (%)	3 (3%)	1 (1%)	0.20
Ventricular dilatation – no. (%)	6 (7%)	2 (2%)	0.07
Porencephalic cyst – no. (%)	1 (1%)	0 (0%)	0.32
Other severe cerebral lesions – no. (%)	1[a] (1%)	2[b] (2%)	0.59
Severe cerebral lesions – no. (%)	12 (14%)	6 (6%)	0.04

[a] *Middle cerebral artery infarction*

[b] *Diffuse cystic white matter disease*

various clinical parameters (TTTS, gestational age at birth, birth weight, inter-twin birth weight discordance, hemoglobin level at birth, anemia, polycythemia and inter-twin hemoglobin discordance). Only decreasing birth weight was independently associated with severe cerebral lesions at discharge (OR: 1.3 for each 100 gr decrease; 95% CI: 1.1 to 1.5, $p < 0.01$). The same analysis was performed in the TTTS group and showed a similar association between decreasing birth weight and severe cerebral lesions at discharge (OR: 1.2 for each 100 gr decrease; 95% CI: 1.1 to 1.3, $p < 0.01$). In the TTTS group, no difference was found in incidence of severe cerebral lesions at discharge was similar between donors and recipients, 15% (5/40) and 16% (7/44), respectively ($p = 0.51$). The incidence of lenticulostriate vasculopathy was also similar in recipient and donor twins, 16% (7/44) and 8% (3/40), respectively ($p = 0.13$), as well as between TTTS infants with and without polycythemia-hyperviscosity syndrome, 0% (0/5) and 13% (10/79), respectively ($p = 0.39$).

Comment

In this study, we found that 14% of survivors with TTTS treated with fetoscopic laser surgery have severe cerebral lesions on cranial ultrasound scans. The majority of severe cerebral lesions in TTTS in our study population were already detected at birth, suggesting that the onset of brain injury in infants with TTTS is more often of antenatal than of postnatal origin. The incidence of antenatally acquired severe cerebral injury in TTTS was almost six-fold higher than in the control group without TTTS. Two previous cranial ultrasound studies reported an even higher incidence of antenatally acquired cerebral lesions in TTTS survivors[70;146]. Bejar *et al* found an incidence of antenatal white matter necrosis of respectively 55% (5/9) and 26% (7/27) in monochorionic twin infants with and without TTTS[70]. However, their findings were based on only 9 neonates with TTTS. In a small uncontrolled series, Denbow *et al* report a 35% (11/31) incidence of antenatally acquired cerebral abnormalities in TTTS[146]. Minor cerebral lesions such as subependymal pseudocysts, lenticulostriate vasculopathy and mild ventricular dilatation were also included in their final analysis. After we excluded the cases with minor cerebral lesions, the

incidence of severe cerebral lesions in that study was 16% (5/31; cystic PVL: n = 4, periventricular hemorrhagic infarction: n = 1)[146]. In both reported studies, TTTS was not treated with fetoscopic laser surgery. This may explain the higher incidence of antenatally acquired severe cerebral lesions as cerebral injury in TTTS is known to be related to the type of antenatal treatment. In a randomized controlled trial comparing serial amnioreduction and laser treatment, severe cerebral lesions were diagnosed significantly more often after amnioreduction[10].

The exact mechanism responsible for antenatal cerebral injury in TTTS is not fully understood. Based on the ultrasound findings encountered in our study, most cerebral lesions could be classified in hemorrhagic injury (IVH and periventricular hemorrhagic infarction) or ischemic white-matter injury (PVL). Both types of injury have been reported to occur antenatally and are related to cerebral blood flow disorders[236-238]. Impaired cerebral perfusion in TTTS may result from hemodynamic imbalance and inter-twin shifts of blood through the vascular anastomoses and lead to hypoxic-ischemic insults that may have occurred prior to fetoscopic laser surgery. Indeed, cerebral lesions in TTTS have been detected prior to laser treatment[151]. However, so far it is not clear when cerebral injury occurs in TTTS treated with fetoscopic laser surgery: before, during or after laser treatment. Fetal neuro-imaging studies performed before and after laser treatment may shed more light on this issue.

Besides type of antenatal treatment, other risk factors have been reported to be related to cerebral lesions in TTTS, such as single intrauterine fetal demise, anemia or polycythemia and type of vascular anastomoses[10;70;186]. Cerebral injury in single intrauterine fetal demise is the result of hypoxic-ischemia due to acute exsanguination from the surviving twin into the low-pressure circulation of the demised co-twin. Acute inter-twin exsanguination can only occur if the placental vascular anastomoses are still functional. In our study, severe cerebral lesions were detected in one of the two survivors in the no-TTTS group born after spontaneous co-twin demise in the presence of functional vascular anastomoses. Conversely, severe cerebral lesions occurred in only one of the eight infants in the TTTS-group whose co-twin died in utero after interruption of the inter-twin placental vascular connections with laser surgery. Fetal anemia may negatively influence cerebral oxygenation and result in hypoxic-ischemic

cerebral injury[238]. Polycythemia has also been suggested to cause cerebral ischemic injury in TTTS by intravascular sludging[167;238]. However, in this study neither anemia nor polycythemia was independently associated with cerebral injury. Decreasing birth weight was the only independent predictor for cerebral injury in this study. The association between decreasing birth weight and severe cerebral lesions may be due to injury caused by very low birth weight as such or selective intrauterine growth restriction. Various studies have reported on the increased risk of cerebral injury in association with decreasing birth weight[239].

We also detected rare cerebral lesions in the TTTS group, such as middle cerebral artery infarction. Neonatal cerebral artery infarction is in some cases associated with hypoxic-ischemia and/or coagulopathy disorders[240]. The etiology of the focal ischemic stroke in the recipient twin in our study is still obscure, but could theoretically be related to sludging of polycythemic blood.

Apart from severe cerebral lesions, we also found a high incidence of milder cerebral lesions in the TTTS group, such as subependymal pseudocysts and lenticulostriate vasculopathy. Both type of lesions have been reported to be associated with miscellaneous conditions such as chromosomal disorders, congenital infection and metabolic disorders[241;242]. Neurological outcome in patients with isolated subependymal pseudocysts and lenticulostriate vasculopathy is usually not impaired[241;242]. Lenticulostriate vasculopathy was shown in previous studies to occur especially in recipient twins[149;243]. Nevertheless, in our study lenticulostriate vasculopathy was also detected in donor twins. The etiology of lenticulostriate vasculopathy is unclear and may be related to polycythemia in recipient twins[149]. However, in this study no association between lenticulostriate vasculopathy and polycythemia was found.

In conclusion, TTTS treated with fetoscopic laser surgery is associated with a high incidence of severe cerebral injury, mainly of antenatal origin. The high incidence of severe cerebral lesions warrants neonatal follow-up with serial cranial ultrasonography in all survivors of TTTS. Serial neuro-imaging studies performed before and after laser treatment are needed to evaluate the precise timing of cerebral injury in TTTS treated with fetoscopic laser surgery.

Chapter 10

Congenital heart disease in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery

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Submitted

Abstract

Objectives: To determine the incidence of congenital heart disease (CHD) and right ventricular outflow tract obstruction (RVOTO) in twin-to-twin transfusion syndrome (TTTS) treated with fetoscopic laser surgery and evaluate the role of increased afterload by determining the difference in blood pressure and endothelin-1 at birth between donor and recipient twins.

Methods: All consecutive cases of monochorionic twins with TTTS treated with laser (n = 46 twin pairs) and monochorionic twins without TTTS (n = 55 twin pairs) delivered at our center between June 2002 and June 2005 were included in the study. Echocardiography was performed within one week after delivery. At birth, blood pressure was measured in all survivors and endothelin-1 was determined in umbilical cord blood. Data on RVOTO in TTTS treated with laser surgery at our center but delivered elsewhere were reviewed retrospectively from medical records.

Results: The incidence of CHD in the TTTS group and no-TTTS group was 5.4% (4/74) and 2.3% (2/87), respectively (p = 0.42). RVOTO was diagnosed in one recipient twin delivered at our center and two recipient twins delivered elsewhere. The incidence of RVOTO in recipients was 4% (3/75). Mean systolic blood pressure at birth was similar in donor and recipient twins, 53 mmHg versus 56 mmHg, respectively (p = 0.42). Mean endothelin-1 level at birth was also similar between donors and recipients, 14.3 ng/L and 13.2 ng/L, respectively (p = 0.64).

Conclusions: The incidence of CHD in TTTS treated with fetoscopic laser surgery is higher than in the general population (5.4% versus 0.5%). We found no difference in afterload parameters between donors and recipients after laser treatment.

Introduction

Congenital heart disease (CHD) occurs 12 times more frequently in monochorionic twins with twin-to-twin transfusion syndrome (TTTS) than in the general population[19]. TTTS is a severe complication of monochorionic twinning and affects 15% of monochorionic twin gestations[11]. TTTS results from unbalanced inter-twin blood transfusion via placental vascular anastomoses leading to hypovolemia, oliguria and oligohydramnios in the donor twin and hypervolemia, polyuria and polyhydramnios in the recipient twin[11]. Recipient twins are especially at risk for cardiovascular disorders. The etiology of cardiovascular disorders in recipients is still unclear and may result either from increased preload due to chronic hypervolemia[162] or increased afterload due to high levels of vasoconstrictive substances such as endothelin-1[84]. Reported cardiovascular abnormalities in recipient twins include hypertension[12;83;154], (bi-)ventricular hypertrophic cardiomyopathy[18;19;155], tricuspid regurgitation[156] and most importantly right ventricular outflow tract obstruction (RVOTO)[19;159]. RVOTO may occur at subvalvular, valvular or supravalvular level and the severity of the obstruction determines the necessity of treatment[244].
The first objective of this study was to determine the incidence of CHD, and particularly of RVOTO, in monochorionic twins with TTTS treated with laser compared to a control group of monochorionic twins without TTTS. The second objective was to study the potential role of increased afterload in CHD in TTTS after laser treatment by measuring endothelin-1 and blood pressure at birth.

Patients and methods

All consecutive cases of monochorionic twins with TTTS treated with laser (TTTS group) and monochorionic twins without TTTS (no-TTTS group) delivered at our center between June 2002 and June 2005 were prospectively included in our study. Pregnancies complicated by intrauterine fetal demise of both twins, major congenital non-cardiac anomalies, triplets and TTTS-pregnancies not treated with fetoscopic

laser surgery were excluded from the study. The Leiden University Medical Center is a tertiary medical center and is the national referral center for fetal therapy including laser treatment for TTTS in the Netherlands. The institutional review board of the Leiden University Medical Center approved the study and all parents gave written informed consent for their children. TTTS was diagnosed using standard prenatal ultrasound criteria[105] and staged according to the criteria of Quintero[9]. Monochorionicity was confirmed after delivery by histopathological examination of the placenta. Postnatal trans-thoracic echocardiography was performed in all surviving infants by experienced pediatric cardiologists within one week of delivery. Standard echocardiography included two-dimensional echocardiography, M-Mode and color Doppler studies. All examinations were performed with an Aloka 5000 scanner (Biomedic Nederland B.V., Almere, The Netherlands) with 8 MHz transducers.

We recorded the presence of the following findings: atrial septal defect (ASD), ventricular septal defect (VSD), RVOTO, right or left ventricle hypertrophy, atrio-ventricular regurgitation and decreased shortening fraction. Patent ductus arteriosus and patent foramen ovale were not recorded as pathological cardiac findings. RVOTO was diagnosed in the presence of subvalvular, valvular or supravalvular obstruction[245]. Gradients were calculated using the modified Bernoulli formula[245]. Right or left ventricular hypertrophy was diagnosed if the anterior wall thickness of the right or left ventricle was more than the 95% confidence limits for estimated gestational age[245]. The presence of atrioventricular regurgitation was determined by color Doppler and classified as absent, mild-to-moderate and severe[245]. Left ventricular shortening fraction was calculated as the end-diastolic diameter minus the end-systolic diameter divided by the end-diastolic diameter[245]. Left ventricular shortening fraction was reported as decreased if less than 25%.

After birth, endothelin-1 was measured from umbilical cord blood. Endothelin was measured with a radioimmunoassay after C18 extraction and concentration. The interassay coefficient of variation ranged from 6.8 to 9.7 % at different levels (Nichols Institute, San Juan Capistrano, CA 92675, USA). Blood pressure was measured in each neonate on the right arm by Dinamap (Model XL, Critikon, Inc) with an appropriately sized cuff between 4 and 24 hours after birth, while the baby was supine and

quiet or asleep. A minimum of two blood pressure readings were taken and averaged. High systolic blood pressure at birth was defined as a systolic blood pressure above the 97th percentile for gestational age[246].
To assure that exclusion of out born cases does not create a bias on the incidence of RVOTO, we also reviewed the medical records of all twins with TTTS treated at our center between August 2000 (start of laser treatment program at the Leiden University Medical Center) and June 2005, including those delivered at other centers. The choice for in-institution or out-of-institution delivery depended on the clinical picture and the wish of the parents.
The primary outcome measures were CHD and RVOTO. The secondary outcome measures were blood pressure and endothelin-1 levels at birth. Results were compared between the TTTS and the no-TTTS group and between donors and recipients in the TTTS group.

Statistics

Results of categorical variables were compared using Fisher's exact test or Chi-square test, as appropriate. Unpaired Student's t test was used to compare normally distributed values between two groups. For comparisons between donors and recipients, the paired Student t test was used for normally distributed continuous variables and the Mc Nemar test for analysis of paired nominal variables. A p-value < 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 11 (SPSS, Inc., Chicago, Illinois, USA).

Results

Over the 3-year study period, 46 monochorionic pregnancies with TTTS treated with laser and 55 monochorionic pregnancies without TTTS were delivered at our center. All parents agreed to have their children participate in our study. Patient's characteristics are presented in Table 1. The median Quintero stage in the TTTS group was II. Seventeen percent (8/46) of pregnancies were stage I, 37% (17/46) stage II, 41% (19/46) stage III and 4% (2/46) stage IV. The overall neonatal outcome of monochorionic twins

TABLE 1 Baseline characteristics.

	TTTS group (n = 64 pregnancies; 92 fetuses)	No-TTTS group (n = 55 pregnancies; 110 fetuses)
Gestational age at birth – weeks[a]	32.0 ± 3.7	33.6 ± 3.1
Female – no. (%)	44 (48%)	60 (55%)
Birth weight – grams[a]	1706 ± 679	2075 ± 670
Intrauterine fetal demise – no. (%)	6 (7%)	2 (2%)
Neonatal death – no. (%)	7 (8%)	3 (3%)
Hydrops – no. (%)	2 (2%)	0 (0%)

[a]*Value given as mean ± SD*

delivered at our center has been published before[207].
A total of 194 live-born infants were eligible for the study. Echocardiography was performed in 86% (74/86) of infants in the TTTS group and 81% (87/108) of infants in the no-TTTS group. Two neonates in the TTTS group died shortly after birth before echocardiography could be performed. One neonate, a recipient twin, died due to severe perinatal asphyxia. The other neonate, a donor twin, developed severe fetal hydrops after laser treatment in association with massive tricuspid regurgitation and died at delivery due to intractable cardiac failure. The parents refused to authorize autopsy. Echocardiography was also not performed in one premature neonate in the no-TTTS group who died on day 1 due to early onset sepsis. We were not able to perform echocardiograms in 5 pairs of twins in the TTTS group and 10 twin pairs in the no-TTTS group due to early discharge from the hospital after delivery or transfer to

TABLE 2 Findings on postnatal echocardiography.

	TTTS group (n = 74 infants)	No-TTTS group (n = 87 infants)
Right ventricular hypertrophy – no. (%)	5 (7%)	2 (2%)
Left ventricular hypertrophy – no. (%)	1 (1%)	0 (0%)
Tricuspid regurgitation mild-moderate – no. (%)	4 (5%)	4 (5%)
ASD – no. (%)	1 (1%)	0 (0%)
VSD – no. (%)	2 (3%)	2 (2%)
RVOTO – no. (%)	1 (1%)	0 (0%)
Overall CHD – no. (%)	4 (5%)	2 (2%)

another hospital. These 15 pairs of twins were all alive and well at birth. Abnormalities detected on echocardiography are presented in Table 2. The overall incidence of CHD in monochorionic twins was 3.7% (6/161). The incidence of CHD in the TTTS group was 5.4% (4/74) (RVOTO, n = 1; VSD, n = 2; ASD, n = 1). The incidence of CHD in the no-TTTS group was 2.3% (2/87) (VSD, n = 2). The incidence of CHD in the sub-group of recipient twins in the TTTS group was 7.9% (3/38). No significant difference was found in incidence of CHD between the TTTS group and no-TTTS group (p = 0.42) or between recipient twins and infants in the no-TTTS group (p = 0.14).

RVOTO was diagnosed in one recipient twin born at 29 weeks of gestation. This patient has been reported earlier[207]. Routine echocardiography performed on day 1 showed pulmonary valve stenosis. The degree of pulmonary valve stenosis increased during neonatal life from moderate to severe. At one month of age the Doppler-gradient at the valvular level increased up to 125 mm Hg requiring balloon valvuloplasty. Serial echocardiographic examinations until one year of age showed no residual pulmonary valve stenosis and a mild pulmonary valve regurgitation.

Since the start of the laser treatment program in August 2000, a total of 112 TTTS twin pairs treated with fetoscopic laser surgery at our center were born. Overall perinatal survival was 70% (156/224) (intrauterine fetal demise: n = 58; neonatal death: n =10). Eighty-one (52%) survivors were donor twins and 75 (48%) were recipient twins. RVOTO (valvular pulmonary stenosis, n = 2, supravalvular pulmonary stenosis, n =1) was diagnosed in three TTTS survivors, including the previously reported patient and 2 other patients born elsewhere after fetoscopic laser treatment at our center. All patients with RVOTO were recipient twins. The overall rate of RVOTO in recipient twins with TTTS treated with laser was therefore 4% (3/75). Detailed information on the three recipients with RVOTO is presented in Table 3.

Results of blood pressure measurements and endothelin-1 concentrations in the TTTS group and no-TTTS group are shown in Table 4. We found no significant differences in blood pressure and endothelin-1 levels at birth between both groups or between donor and recipient twins. None of the neonates had persistent hypertension or required treatment for hypertension at the time of study.

TABLE 3 Prenatal and postnatal findings in the 3 recipients (1 born at our center and 2 born elsewhere) with RVOTO after laser therapy.

GA at laser (wk)	Quintero stage	Recipient fetal echocardiography	GA at birth (wk)	Postnatal cardiac diagnosis and course
15	2	Evidence of RVOTO AP max 4.5 m/s	29	Progression from moderate to severe valvular PS Balloon valvuloplasty at 1 month of age No residual PS. Mild PI
22	2	No evidence of RVOTO	37	Systolic heart murmur detected at 2 months of age Severe supravalvular PS detected at 2 months of age Surgical repair at 2 months of age No residual PS. Mild PI
18	2	No evidence of RVOTO	33	Severe respiratory failure at birth requiring ECMO Severe valvular PS detected at 1 week of age Balloon valvuloplasty at the age of 1 week Repeat balloon valvuloplasty at the age of 4 months No residual PS. Mild PI

GA, gestational age; RVOTO, right ventricular outflow tract obstruction; AP, pulmonary artery flow velocity; PS, pulmonary stenosis; PI, pulmonary valve insufficiency; ECMO, extra corporeal membrane oxygenation

TABLE 4 Systolic blood pressure and endothelin-1 levels at birth in donor and recipient twins.

All monochorionic twins	TTTS group (n = 86 infants)	No-TTTS group (n = 108 infants)	p-value
Systolic blood pressure[a] – mm Hg	55 ± 13	55 ± 10	0.91
Systolic blood pressure > 97th centile – no. (%)	8 (9%)	5 (4%)	0.08
Endothelin-1[a] – ng/L	15.3 ± 7.7	15.3 ± 8.8	0.97
TTTS group	Donors (n = 41 infants)	Recipients (n = 45 infants)	p-value
Systolic blood pressure[a] – mm Hg	53 ± 14	56 ± 11	0.21
Systolic blood pressure > 97th centile – no. (%)	5 (12%)	5 (11%)	1.0
Endothelin-1[a] – ng/L	15.6 ± 7.7	15.1 ± 8.2	0.64

[a]*Value given as mean ± SD*

Discussion

In this study, we report a high incidence of CHD in TTTS survivors (5.4%) after fetoscopic laser surgery and particularly in recipient twins (7.9%). The actual incidence of CHD in TTTS in this study is probably underestimated as prenatal findings of fetuses who died in utero or were aborted were not included, and postnatal cardiac evaluation was not assessed in one hydropic infant who died at birth due to intractable cardiac failure. Overall, the reported rates of CHD in TTTS are higher than in the general population (0.5%)[247]. These results are in accordance with two previous studies on CHD in TTTS survivors[19;248]. Karatza *et al* reported an overall prevalence of CHD of 6.9% (6/87) in TTTS and 11.9% (5/42) in recipients[19], whereas Herberg *et al* reported a prevalence of CHD of 11.2% (10/89) in TTTS and 13.7% (7/51) in recipients[248]. The overall incidence of RVOTO in all TTTS survivors treated with laser at our center was 4%. The RVOTO was hemodinamically significant in all patients requiring surgical or catheter-interventional treatment. As echocardiographic examination is not routinely performed in TTTS survivors born in other hospitals, however, mild RVOTO may not have been recognized. Herberg *et al* reported a 7.8% postnatal rate of RVOTO (4/51) in recipient twins with TTTS treated with fetoscopic laser surgery, but also included 2 cases with moderate and mild RVOTO treated expectantly[248]. The prenatal and postnatal rate of RVOTO in recipient twins with TTTS not treated with laser ranges from 4.8% to 11.3%[19;159;162]. Whether cardiovascular morbidity in recipient twins, and in particular RVOTO, is associated with the type of antenatal treatment is not known. The two current treatment options in TTTS are serial amnioreduction and fetoscopic laser treatment. A recently published randomized controlled trial comparing both treatments showed that perinatal mortality and neurological morbidity were significantly lower after laser surgery[10]. However, cardiovascular morbidity was not reported in this trial[10].

This study is the first controlled single center study reporting cardiovascular morbidity in TTTS after fetoscopic laser surgery. Comparison with a control group of monochorionic twins without TTTS is of crucial importance because monochorionicity is associated with increased incidence of CHD even in the absence of TTTS[19]. We found no difference

in incidence of CHD in recipient twins treated with laser compared to monochorionic twins without TTTS. A possible reason for the lack of difference between the two groups may have been that our study was underpowered. To detect a 10% difference in CHD rate (12% versus 2%) between recipient twins and monochorionic infants without TTTS we would have needed a sample size of at least 100 children in each group (with 0.05 significance and a power of 80% by two-tailed analysis). Such large cohorts in TTTS studies can only be achieved with multi-center studies or longer study-periods.

The etiology of CHD in recipients with TTTS has been linked with increased preload due to volume overload following feto-fetal transfusion, as well as increased afterload due to high levels of vasoconstrictive hormones, such as endothelin-1[249]. Endothelin-1 levels have been reported to be 2 ½ -fold higher in recipients than in donors[84]. Increased afterload and systemic hypertension during fetal life may then lead to the development of hypertrophic cardiomyopathy and eventually RVOTO. Reports of high blood pressure in recipient twins in fetal and neonatal life are consistent with this hypothesis[12;83;154]. We, as well as others, have previously shown that systemic hypertension, a clinical parameter for increased afterload, occurs more often in recipient twins than in donor twins[12;83;154]. In this study, however, we found no difference between donors and recipients in blood pressure and endothelin-1 concentrations at birth. Absence of difference in blood pressure and endolthelin-1 levels may be related to the type of antenatal treatment. Other reports showing increased afterload in recipients, have been performed in TTTS not treated with laser[12;83;84;154], whereas in our study all TTTS cases were treated with laser antenatally. Fetoscopic laser surgery occludes the placental vascular anastomoses and is therefore considered to be a causal treatment. Hypothetically, initial differences in blood pressure and endothelin-1 levels that were present before laser treatment may have gradually diminished and disappeared after laser treatment.

In conclusion, the incidence of CHD in TTTS survivors treated with fetoscopic laser occlusion of vascular anastomoses is low, but still higher than in the general population. In particular, the increased risk for RVOTO in recipient twins warrants close cardiac monitoring during fetal and neonatal life.

Part 4
Long-term outcome

Chapter 11

Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome

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Abstract

Objective: To determine the long-term neurodevelopmental outcome in children after twin-to-twin transfusion syndrome (TTTS).

Methods: Maternal and neonatal medical records of all TTTS-cases admitted to our center between 1990 and 1998 were reviewed. Neurological and mental development at school age was assessed during a home visit in all TTTS survivors.

Results: A total of 33 pregnancies with TTTS were identified. Four couples opted for termination of pregnancy. All other pregnancies were managed conservatively, 18 (62%) with serial amnioreductions and 11 (38%) without intrauterine interventions. Mean gestational age at delivery was 28.6 (range: 20-37) weeks. Perinatal mortality was 50% (29/58). Birth weight of donor twins was less than recipient twins ($p < 0.001$). Systolic blood pressure at birth was lower in donors than in recipients ($p = 0.023$) and donors required more frequently inotropic support postnatally than recipients ($p = 0.008$). The incidence of hypertension at birth was higher in recipients than in donors ($p = 0.038$). Abnormal cranial ultrasonographic findings were reported in 41% (12/29) of the neonates. All long-term survivors ($n = 29$) were assessed during a home visit. Mean gestational age at birth of the surviving twins was 31.6 (range: 25-37) weeks. Mean age at follow-up was 6.2 (range: 4-11) years. The incidence of cerebral palsy was 21% (6/29). Five out of six children with cerebral palsy had an abnormal mental development. The incidence of cerebral palsy in the group of survivors treated with serial amnioreduction was 26% (5/19). Four children were born after intrauterine fetal demise of their co-twin: two of them had cerebral palsy.

Conclusions: The incidence of adverse neurodevelopmental outcome in TTTS survivors is high, especially after intrauterine fetal demise of a co-twin.

Introduction

Cerebral palsy is estimated to occur seven times more often in twins than in singletons[1]. The higher relative risk for cerebral damage is not only attributable to the higher incidence of premature birth and low birth weight in twins compared to singletons. Monochorionic twinning predisposes to cerebral damage due to complications caused by twin-to-twin transfusions[250]. Twin-to-twin transfusion syndrome (TTTS) occurs in approximately 15% of monochorionic pregnancies and results from shunting of blood from one twin, the donor, to the other twin, the recipient. The donor becomes hypovolemic and oliguric, whereas the recipient becomes hypervolemic and polyuric[11]. The management of TTTS remains a significant challenge in perinatal medicine and the perinatal mortality rate in untreated TTTS is reported to be 75-100%[112;114]. Treatment of TTTS with serial amnioreductions or with laser coagulation of placental vascular anastomoses has decreased the perinatal mortality rate to an average rate of approximately 40%[123;124;129]. Nevertheless, the morbidity in surviving twins, which includes mainly neurological, cardiovascular and renal complications, remains high[11]. Cerebral white-matter lesions have been reported to occur antenatally in up to 35% of TTTS survivors[146]. To date, few studies have reported long-term neurodevelopmental outcome in TTTS. The incidence of cerebral palsy and global developmental delay in surviving twins varies from 4% to 23%[39;133;147;148;150;152]. However, in most studies, follow-up of the surviving twins did not extend beyond a mean age of 2 years corrected for prematurity. Assessment at school age is essential since neurological handicaps and mental retardation may only become evident several years after birth[251-253]. The main purpose of our study was to evaluate long-term neurodevelopmental outcome in school-aged twins after TTTS.

Material and Methods

We identified all cases of TTTS who were admitted at our center from January 1990 to December 1998. Written information on the aims of the study was sent to the parents of all surviving twins. Parents were asked

for consent to examine their children. Neurological outcome was assessed in all children by a single pediatrician during a home visit. Neurological outcome was defined as abnormal when evidence of cerebral palsy was found. Cerebral palsy was classified as diplegia, hemiplegia, quadriplegia, dyskinetic or mixed. We estimated the level of mental development of the children according to their school performance. School entry in the Netherlands starts at four years of age. All children with learning disabilities due to mental retardation or behavioral problems are referred to a school for special education. For the purposes of the study, children in mainstream education with or without special assistance were considered to have a normal mental development, whereas children who needed special education as well as children one or more grades below the appropriate school-level for their age were considered to have an abnormal mental development.

Diagnosis of TTTS was reached according to the following prenatal ultrasound criteria: 1.) monochorionicity established by absence of a "twin peak" sign and the presence of a thin dividing membrane, 2.) oligohydramnios (deepest vertical pocket < 1cm) in the twin sac of one fetus and 3.) polyhydramnios (deepest vertical pocket > 8cm) in the twin sac of the other fetus. During the study period, the standard treatment at our center for TTTS was serial amnioreduction. Monochorionicity was confirmed after delivery.

The following obstetrical data were extracted from the medical charts: gestational age at the time of diagnosis, number of therapeutic amnioreductions and total volume of amniotic fluid removed, intrauterine fetal death, gestational age at delivery and mode of delivery. We also recorded the stage of TTTS on admission[9]. In short, staging according to Quintero has five stages: stage I, bladder of donor twin still visible; stage II, anuria of donor twin; stage III, critically abnormal Doppler studies; stage IV, hydrops; stage V, demise of one or both twins[19]. The following neonatal data were extracted: birth weight, Apgar score at 5 minutes, arterial blood pressure on admission measured with Dinamap, hematocrit on day 1 of life. Growth discordance between recipient and donor was calculated by dividing the difference in birth weights by the birth weight of the recipient twin. Hypotension or hypertension at birth was defined as a systolic blood pressure respectively below the 3rd or above the 97th

percentile for gestational age[246]. We also recorded the use of inotropic support during the stay in our nursery. Neonatal cranial ultrasound findings were reviewed, such as periventricular leucomalacia (PVL) (grade classification according to de Vries *et al* [223]), intraventricular hemorrhage (IVH) (grade classification according to Volpe *et al*[236]), porencephalic or parenchymal cysts, subependymal pseudocysts, ventriculomegaly and lenticulostriate vasculopathy. Other significant neonatal problems were also reviewed, including transient tachypnoea of the newborn, respiratory distress syndrome, chronic neonatal lung disease, patent ductus arteriosus, necrotizing enterocolitis, renal failure, hydrops fetalis, retinopathy of prematurity and congenital malformation.
Analysis of the TTTS group according to whether the twins were donor or recipient was performed in order to detect eventual differences in perinatal mortality and morbidity as well as differences in long-term outcome. Results of categorical variables were compared using Fisher's exact test, whereas continuous normally distributed variables were examined with paired Student's *t* test. Chi-square test for trend was used in order to evaluate the relationship between stage of TTTS and outcome. A p-value < 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 10 (SPSS, Inc., Chicago, Illinois, USA).

Results

Obstetrical results

During the 8-year study period, 33 multiple pregnancies (31 twins and 2 triplets) with TTTS were admitted to our center. The mean gestational age at the time of diagnosis was 22.4 (range: 15-28) weeks. The Quintero stage at admission was I in nine cases, II in eight cases, III in ten cases, IV in three cases and V in three cases. Four couples opted for termination of pregnancy. In the remaining 29 pregnancies, intrauterine fetal demise of both twins occurred in 38% (11/29) of the pregnancies. In four pregnancies, one twin survived while the co-twin died in utero. Caesarean delivery was performed in 9 (31%) of the 29 pregnancies. The mean gestational age at delivery was 28.6 (range: 20-37) weeks. Serial amnioreduction was performed in 18 (62%) of the 29 TTTS-

pregnancies. Mean gestational age at birth of the group of twins treated with serial amnioreduction was 31.3 weeks (range: 28-35). The median number of amnioreductions per case was 1 (range: 1-7) and the mean amount of amniotic fluid removed 2 liters (range: 0.5-15) per pregnancy. Amnioreduction was not performed in the remaining 15 pregnancies either due to intrauterine death of one or both twins at presentation (n = 7), because the patient opted for termination of pregnancy (n = 4), because of mild TTTS (Quintero stage I) (n = 3), or due to imminent delivery (n = 1). We found a direct relationship between stage of TTTS and mortality rate (p = 0.042) as well as stage of TTTS and adverse outcome (cerebral palsy or death) (p = 0.015) (Table 1). Cases in which parents opted for termination of pregnancy (n = 4) and cases with stage V (n = 3) of TTTS were not included in this analysis.

TABLE 1 Mortality rate and adverse outcome (cerebral palsy or death) by stage of TTTS.

Stage	Death[a]	Cerebral palsy or Death[b]
I	31% (5/16)	37% (6/16)
II	33% (4/12)	42% (5/12)
III	61% (11/18)	83% (15/18)
IV	67% (4/6)	67% (4/6)
Total	46 % (24/52)	58 % (30/52)

Values are percentages (numbers)
Cases in which parents opted for termination of pregnancy (n = 4) and cases with stage V (n = 3) of TTTS were not included in this analysis.
[a]*Chi-square test for trend = 4.1, df = 1, p = 0.042*
[b]*Chi-square test for trend = 5.9, df = 1, p = 0.015*

Neonatal results

Thirty-six (55%) fetuses were male, 30 (45%) were female. The overall perinatal survival rate was 50% (29/58) and in the subgroup treated with serial amnioreduction, 53% (19/36). Neonatal death occurred in 3 infants, all donor twins, and was caused by terminal renal failure (n =1), *Escherichia coli* sepsis (n = 1) and severe respiratory distress syndrome (n = 1). There was no difference in overall perinatal mortality between donor and recipient twins (Table 2). The mean birth weight in donors was 1016 gr (range: 220-2740), whereas the mean birth weight in recipients

TABLE 2 Mortality and morbidity in donor and recipient twins.

	Donor (n = 29)	Recipient (n = 29)	p-value
IUFD	41% (12/29)	48% (14/29)	NS
NND	18% (3/17)	0% (0/15)	NS
Overall perinatal death	52% (15/29)	48% (14/29)	NS
Cerebral Palsy	17% (3/17)	17% (3/17)	NS

Cases in which parents opted for termination of pregnancy (n = 4) were not included in this analysis.
IUFD, intrauterine fetal demise; NND, neonatal death; NS, not significant.

was 1291 gr (range: 310-2790). The difference in birth weight between donors and recipients was significant ($p < 0.001$). Eight of the seventeen donors (47%) were also small for gestational age as compared to none of the recipients ($p = 0.003$). The mean birth weight discordance between life born recipients and donors was 24% (range: 2%-41%). The median Apgar score at 5 minutes was 8 (range: 4-10). There was no significant difference in Apgar score between donors and recipients. The mean hematocrit at birth in donors was 48.2 (range: 28-68) l/l and in recipients, 51.8 (range: 39-66) l/l. The difference in hematocrit between donors and recipients was not significant. The mean systolic blood pressure at birth in donor twins was 45.6 (range: 30-60) mmHg and in recipients 61.8 (range: 44-94) mmHg. The difference in systolic blood pressure at birth between donors and recipients was significant ($p = 0.023$). Eight of the seventeen donors (47%) also required inotropic support as compared to only one of the recipients ($p = 0.008$). Hypertension at birth was found in 27% (4/15) of the recipients, but in none of the donors ($p = 0.038$). Renal failure occurred in two neonates, both donor twins. One of them died from terminal renal failure, the other child requires hemodialysis. Fetal hydrops was found in two twins at delivery (6%). One of them was a recipient twin. The other case of fetal hydrops occurred in a donor after the co-twin died in utero and was secondary to severe fetal anemia (hemoglobin value of 4.5 g/dL) probably following acute blood loss into the dead co-twin through the vascular anastomoses. An intrauterine blood transfusion raised the hemoglobin to 13 g/dL. The donor twin was born a few days later and was still hydropic. The incidence of respiratory distress syndrome was 31% (10/29). The incidence of chronic lung disease was 10% (3/29). Patent

ductus arteriosus was found in 25% (8/32) of the neonates. Necrotizing enterocolitis was diagnosed in 9% (3/32) of the neonates. None of the neonates had retinopathy of prematurity or congenital malformations. We found no significant differences in neonatal morbidity between donors and recipients. Abnormal cranial ultrasonographic findings were found in 12 of the 29 neonates (41%) in whom a scan was performed (IVH grade I-II: n = 4, unilateral IVH grade III with intraparenchymal echodensity: n = 2, bilateral IVH grade III with intraparenchymal echodensity: n = 1, PVL grade I: n = 3, ventriculomegaly: n = 3, lenticulostriate vasculopathy: n = 1). In 3 neonates no cranial ultrasound scan was performed. We found no significant differences in abnormal ultrasonographic findings between donors and recipients.

Long-term outcome

We were able to follow-up all 29 surviving twins during a home visit. The derivation of the surviving population is shown in a flow diagram in Figure 1. The mean gestational age at birth of the surviving twins was 31.6 (range: 25-37) weeks and the mean age at follow-up was 6.2 (range: 4-11) years. The incidence of cerebral palsy was 21% (6/29) (spastic quadriplegia: n = 2, spastic diplegia: n = 3, spastic hemiplegia: n = 1). The incidence of cerebral palsy in the group treated with serial amnioreduction was 26% (5/19). Five children with cerebral palsy had an abnormal mental development, and one child with left spastic hemiplegia had a normal

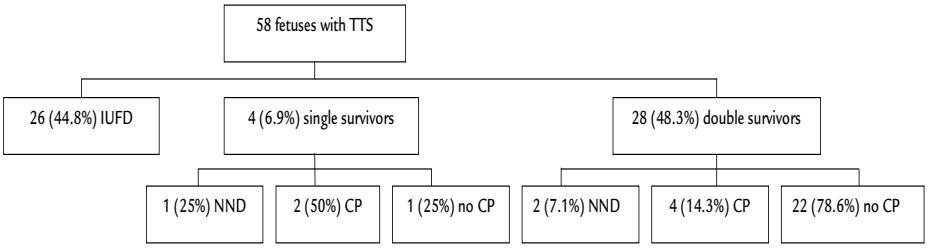

FIGURE 1 Outcome of 58 fetuses in 29 pregnancies with TTS.

Cases in which parents opted for termination of pregnancy (n = 4) were not included in this analysis.
IUFD, intrauterine fetal demise; NND, neonatal death; CP, cerebral palsy

mental development. All children with abnormal mental development needed special education. Both infants with quadriplegia were severe mentally retarded. Data regarding the 6 surviving twins with abnormal neurodevelopmental outcome are listed in Table 3. In the group of children without cerebral palsy or abnormal mental development, 22% (5/23) of the children had a mild speech delay and required speech therapy. All of these children were kept in mainstream education with special assistance from a teacher or remedial teaching.

Four survivors were born after intrauterine fetal demise of their co-twin: one of them died in the neonatal period due to sepsis caused by *Escherichia coli*, two survivors have cerebral palsy and only one survivor has a normal outcome. The incidence of adverse long-term neurodevelopmental outcome in twins whose co-twin died in utero was 67% (2/3). The incidence of adverse long-term neurodevelopmental outcome in twins who were both born alive was 15% (4/26). The difference in neurodevelopmental outcome between survivors whose co-twin died in utero compared to twins who were both alive at birth was not significant, probably because study numbers were too small.

Gestational age at birth as well as birth weight was not associated with a significantly higher incidence of adverse neurodevelopmental outcome. We found no significant difference in long-term neurodevelopmental outcome between donors and recipients. In the neonatal period, IVH grade I-II was diagnosed in 4 neonates. All of them have a normal long-term psychomotor outcome. One neonate had a bilateral IVH grade III with intraparenchymal echodensity and died in the neonatal period due to terminal renal failure. Two neonates from the same pregnancy had a unilateral IVH grade III with intraparenchymal echodensity. One of them died two days after birth due to multi-organ failure. Its co-twin had a right-sided IVH grade III with intraparenchymal echodensity and has now spastic hemiplegia on the left side. PVL grade I was diagnosed in 3 neonates. Two of them have an abnormal long-term neurodevelopmental outcome. One of them also had ventriculomegaly but has only mild symptoms (mild speech and motor delay) without further signs of cerebral palsy or abnormal mental development. Another child with ventriculomegaly died two days after birth. The neurodevelopmental outcome of the recipient twin with lenticulostriate vasculopathy was normal.

TABLE 3 Data of the 6 surviving twins with adverse neurodevelopmental outcome.

Case	Twin	No. of amnio-reductions	GA at birth (wk)	BW (gr)	Neonatal cranial ultrasound findings	Age at follow-up	Neurologic outcome	Abnormal mental outcome	Other morbidity	Outcome of co-twin
1	Recipient	3	28	780	PVL I, ventriculomegaly	10,5	quadriplegia	yes	CLD	NND
2	Recipient	3	29	1206	PVL I	10	diplegia	yes	NEC	IUFD
3	Donor	7	32	930	normal	9,5	quadriplegia	yes	renal failure	normal
4	Donor	1	35	1064	normal	6	diplegia	yes	none	normal
5	Donor	4	32	1330	normal	4,5	diplegia	yes	NEC	IUFD
6	Recipient	0	25	801	IVH III + IPE	4	hemiplegia	no	CLD	NND

GA, gestational age; BW, birth weight; IPE, intraparenchymal echodensity; NND, neonatal death; CLD, chronic lung disease; NEC, necrotizing enterocolitis

Comment

In this study we analyzed the perinatal mortality and morbidity in TTTS. We report a high perinatal mortality rate (50%) in TTTS, emphasizing the critical nature of this disease. The perinatal mortality rate in the group treated with serial amnioreduction was slightly lower (47%), and comparable to previously published mortality rates in pregnancies managed similarly[123;124;129]. We also found a direct relationship between stage of TTTS and mortality rate as well as between stage of TTTS and adverse outcome (cerebral palsy or death), confirming the prognostic significance of the Quintero staging classification. Regarding the neonatal morbidity, this study shows a significant difference in systolic blood pressure at birth between donors and recipients. Hypertension in recipients has previously been reported, and is theoretically more consistent with increased afterload rather than increased preload following volume overload[83]. Increased afterload may result from a higher endothelin-1 level in recipients[84]. Abnormal cranial ultrasonographic findings were found in 41% of the neonates who underwent cranial ultrasonography. Denbow *et al* reported an even higher incidence, 58%[146], whereas Hecher *et al* reported a lower incidence (range: 6% to 18%, depending on the type of antenatal therapy) of abnormal cranial ultrasound findings[123]. However, the definition of abnormal ultrasound findings in the study of Hecher *et al* did not include IVH grades I and II.

The main objective of our study was to evaluate the long-term neurodevelopmental outcome in TTTS. We report a high incidence (21%) of cerebral palsy and abnormal mental development in surviving twins with TTTS. This is the first study in which all TTTS survivors were at least 4 years of age at follow-up. Since the incidence of adverse neurodevelopmental outcome is positively correlated to the duration of follow-up, it is important to continue follow-up until school age[252;253]. The incidence of cerebral palsy and abnormal mental development is similar to most previous publications on long-term neurodevelopmental follow-up. Haverkamp *et al* found a 23% incidence of severe psychomotor retardation in combination with cerebral palsy in a cohort of 40 TTTS survivors who were followed until a mean age of 24 months[147]. Cincotta *et al* found a 22% incidence of cerebral palsy and global developmental

delay in 23 surviving twins who were followed to at least 2 years of age corrected for prematurity[148]. In a smaller study of 14 TTTS survivors who were followed until 2 years of age, Seng *et al* report a lower incidence (14%) of cerebral palsy with mental retardation[39]. However, the inclusion criteria for TTTS of Seng *et al* were not based on prenatal ultrasound findings, but rather on postnatal inter-twin hemoglobin and birth weight differences. Other studies, including our study, have shown that the recipient does not necessarily have a higher hemoglobin or a higher hematocrit than the donor. Reaching the correct diagnosis of TTTS is no longer guaranteed by these postnatal criteria. Therefore, some of their patients may not have been affected by TTTS, which would also explain the exceptionally high survival rate (88%) in their study. Mari *et al* also report a much lower incidence (5%) of cerebral palsy in a cohort of 42 surviving twins who were at least 2 years of age at last follow-up[150]. However, one infant in their cohort had multilocular encephalopathy but was lost to follow-up. Another infant died at 6 months of age of respiratory as well as neurological complications. Whether this child also had cerebral palsy is not clearly mentioned. Most importantly, the rate of neonatal deaths in their study was high, 16% (8/51). Half of these neonatal deaths occurred in children born at 24 and 25 weeks of gestation. The incidence of neurodevelopmental disability in children born at 24 and 25 weeks' gestation is reported to range from 12% to 45%[239]. Two other neonates who died in their study were reported to have abnormal cranial ultrasound findings (respectively brain infarction and IVH). Therefore the suspected incidence of cerebral palsy in the study of Mari *et al* could be higher. In all three neonatal deaths reported in our study, major abnormal cranial ultrasound findings were found (bilateral IVH grade III with intraparenchymal echodensity, n = 1; unilateral IVH grade III with intraparenchymal echodensity, n = 1; ventriculomegaly, n = 1). Therefore, the incidence of cerebral palsy in our study would most certainly have been higher had these three neonatal deaths not occurred. Mari *et al* also found that survivors born after 27 weeks of gestation had an excellent long-term outcome. In our study, 5 of the 6 survivors with adverse neurodevelopmental outcome were born after 27 weeks of gestation. We found that adverse neurodevelopmental outcome was associated with IUFD of a co-twin. This result supports previously published findings in

which a high incidence (27%) of serious neurological morbidity was found in survivors after death of a co-twin[11]. The major cause of cerebral white-matter damage in surviving twins whose co-twin died in utero is acute cerebral ischemia due to acute exsanguination of the surviving twin into the low-resistance vascular system of the moribund or dead twin through the vascular anastomoses[11].

In all previously reported long-term follow-up studies, TTTS pregnancies were treated with serial amnioreduction. In our study, the incidence of adverse neurodevelopmental outcome in twins with TTTS treated with serial amnioreduction was also high (26%). Recent reports suggest that laser ablation therapy of placental vascular anastomoses may be associated with a lower incidence (4-9%) of cerebral palsy in surviving twins compared to serial amnioreduction[133;152]. To assess the difference in mortality and morbidity between laser ablation therapy and serial amnioreduction, results of the first randomized control trial (www.eurofoetus.org) comparing both treatments must be awaited. Considering the high incidence of adverse neurodevelopmental outcome in TTTS, we recommend that all surviving twins be thoroughly followed up.

Chapter 12

Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery

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Submitted

Abstract

Objective: To determine the long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome (TTTS) treated with fetoscopic laser surgery.

Methods: All TTTS-cases treated at our center with laser between August 2000 and December 2003 were included in the study. Neurological, mental and psychomotor development at 2 years of age corrected for prematurity was assessed in all TTTS survivors. Neurodevelopmental impairment was defined as any of the following: cerebral palsy, deafness, blindness, mental or psychomotor development index of the Bayley Scales of Infant Development II < 2 SD.

Results: A total of 82 TTTS pregnancies were treated with fetoscopic laser surgery during the study period. Perinatal survival was 70% (115/164). The incidence of neurodevelopmental impairment was 17% (19/115) and was due to cerebral palsy (n = 8), mental developmental delay (n = 9), psychomotor developmental delay (n = 12) and deafness (n = 1).

Conclusions: The incidence of neurodevelopmental impairment in TTTS survivors treated with laser is high and warrants long-term follow-up.

Introduction

Monochorionic twinning predisposes to cerebral damage due to complications caused by twin-to-twin transfusion (TTTS). TTTS occurs in approximately 15% of monochorionic pregnancies and results from shunting of blood from one twin, the donor, to the other twin, the recipient, through placental vascular anastomoses. Untreated, TTTS is associated with high perinatal mortality and morbidity[130]. The two current treatment options in TTTS are serial amnioreduction and fetoscopic laser occlusion of vascular anastomoses[27;93;129;130]. In a recent randomized trial comparing serial amnioreduction and laser treatment, perinatal survival and neurological outcome at six months of age was significantly better in the group treated with laser[10]. Although fetoscopic laser occlusion of vascular anastomoses is increasingly being advocated as the preferred treatment for TTTS, only a few studies have been published on the long-term neurodevelopmental outcome following such treatment. The incidence of major neurological abnormalities in these reports varied from 6% to 11%[151;152;254].
The main objective of our study was to evaluate long-term neurodevelopmental outcome in a large group of TTTS survivors after treatment with fetoscopic laser surgery.

Patients and methods

All survivors of consecutive TTTS-cases treated with fetoscopic laser surgery between August 1, 2000 and December 31, 2003 at the Leiden University Medical Center were included in the study. The Leiden University Medical Center serves as the national referral center for intrauterine laser treatment in TTTS pregnancies in the Netherlands. TTTS was diagnosed using standard prenatal ultrasound criteria[105], and staged according to the criteria of Quintero[9]. The following perinatal data were recorded: gestational age at the time of laser treatment, stage of TTTS, gestational age at delivery, mode of delivery and birth weight. Inter-twin birth weight discordance was assessed and calculated as follows: ((birth weight larger twin – birth weight smaller twin)/ birth weight larger twin) x 100%. Birth

weight discordance was defined as more than 20% difference in birth weight.
The follow-up visit was assessed at 2 years of age (corrected for prematurity) and included a physical and neurological examination and an assessment of cognitive and neuromotor development using the Dutch version of the Bayley Scales of Infant Development, 2nd edition (BSID-II) (both by certified examiners). Bayley scale scores provide mental development indexes (MDI) and psychomotor development indexes (PDI). The mean score for both MDI and PDI is 100. A score below 70 is more than 2 SD below the mean score and indicates a severe delay. Infants with very low MDI or PDI scores (< 50) were assigned a score of 49 in the database. Cerebral palsy (CP) was defined according to the European CP Network and classified as diplegia, hemiplegia, quadriplegia, dyskinetic or mixed[255].
A composite outcome, termed neurodevelopmental impairment (NDI), was defined as any of the following: CP, MDI score of less than 70, PDI score of less than 70, bilateral blindness, or bilateral deafness requiring amplification. Overall adverse outcome was defined as intrauterine fetal death, neonatal death, infant death or NDI. Outcome was compared between donor and recipient twins.
The institutional review board of the Leiden University Medical Center approved the study and all parents gave written informed consent for their children.

Statistics

Results of categorical variables were compared using Fisher's exact test or Chi-square test, as appropriate. Unpaired Student's *t* test was used to compare normally distributed values between two groups. For comparisons between donors and recipients, the paired Student t test was used for normally distributed continuous variables and the Mc Nemar test for analysis of paired nominal variables. Multiple logistic regression analysis with "random twin effect" was used to measure the independent effects of potential prognostic factors on outcome. A model with "random twin effect" was applied to adjust for possible correlated effects within twins. The results of the logistic models were expressed as an odds ratio (OR) and

95% confidence intervals (CI). Chi-square test for trend was used in order to evaluate the relationship between stage of TTTS and outcome. A p-value < 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 11 (SPSS Inc., Chicago, IL, USA). Multiple logistic regression analysis was performed with EGRET version 2.0.1 for Windows (Cytel Software Corporation, Cambridge, Massachusetts, USA).

Results

During the study period, 82 TTTS pregnancies were treated with fetoscopic laser surgery at our center. Quintero stage was I in 9 cases, II in 35 cases, III in 32 cases and IV in 6 cases. Laser surgery treatment for Quintero stage I was only performed when symptomatic polyhydramnios warranted intervention. Mean gestational age at laser surgery was 20.0 weeks. Intrauterine fetal demise occurred in 41 fetuses (single intrauterine fetal demise, n = 15; double intrauterine fetal demise, n = 26). Mean gestational age at birth of the surviving infants was 33.9 ± 3.1 weeks (range: 27 to 40 weeks). Neonatal death occurred in 8 neonates. Overall perinatal survival was 70% (115/164). We were able to follow-up all 115 surviving twins. Four families refused to travel to our center for follow-up visit due to the long travel distance, but agreed to allow the complete follow-up examination (including BSID-II test) at their own home. Baseline characteristics of the TTTS survivors are presented in Table 1.

TABLE 1 Baseline characteristics in the 115 TTTS long-term survivors.

	Long-term survivors (n = 115 infants)
Gestational age at laser surgery - wk[a]	20.2 ± 3.0
Median Quintero stage (range)	2 (1-4)
Gestational age at birth - wk[a]	33.9 ± 3.1
Female - no. (%)	55 (48%)
Vaginal delivery - no. (%)	80 (70%)
Birth weight - g[a]	2015 ± 678

[a]*Value given as mean ± SD*

The incidence of NDI was 17% (19/115) and was due to cerebral palsy (n = 8), severe mental developmental delay (n = 9), severe psychomotor developmental delay (n = 12) and deafness (n = 1). Cerebral palsy was classified as quadriplegia (n=4), diplegia (n= 2) and hemiplegia (n= 2). The incidence of adverse outcome (death or NDI) was 41% (68/164). Details on long-term outcome are presented in Table 2.

TABLE 2 Long-term outcome in 115 TTTS survivors after fetoscopic laser surgery.

	Long-term survivors (n = 115 infants)
Cerebral palsy[a] - no. (%)	8 (7%)
Mental development index < 2 SD - no. (%)	9 (8%)
Psychomotor development index < 2 SD - no. (%)	12 (10%)
Bilateral deafness - no. (%)	1 (1%)
Bilateral blindness - no. (%)	0 (0%)
Neurodevelopmental impairment[b] - no. (%)	19 (17%)

[a]*Cerebral palsy included spastic quadriplegia (n = 4), spastic diplegia (n = 2) and spastic hemiplegia (n = 2)*
[b]*Neurodevelopmental impairment is defined as any of the following: cerebral palsy, mental development index < 2 SD, psychomotor development index < 2 SD, bilateral deafness or blindness.*

Intrauterine fetal demise in donors and recipients occurred in 21% (17/82) and 29% (24/82), respectively (p = 0.12). Neonatal death occurred in 5% (4/82) of donors and 5% (4/82) of recipients (p = 1.0). Characteristics and outcome in surviving donor and recipient twins at 2 years of age are presented in Table 3.
We found a direct relationship between stage of TTTS and death (Chi-square test for trend = 5.8, df = 1, p = 0.016) as well as stage of TTTS and adverse outcome (Chi-square test for trend = 9.2, df = 1, p = 0.002) (Table 4).
Multiple logistic regression was carried out to measure the independent associations between NDI and various clinical parameters (gestational age at laser, gestational age at birth, birth weight, Quintero stage and donor versus recipient status). We found a trend towards an independent association between higher Quintero stages and NDI (OR 6.6 for each stage, 95% CI 0.7 – 66.0, p = 0.079) and lower gestational age at birth and NDI (OR 1.6 for each week, 95% CI 0.8 – 3.0, p = 0.080).

TABLE 3 Characteristics and outcome in donor and recipient twins.

	Donor (n = 61)	Recipients (n = 54)	p-value
Birth weight - g[a]	1773 ± 608	2076 ± 567	< 0.001
Weight at 2 years of age - kg[a]	11.7 ± 1.3	12.3 ± 1.3	< 0.001
Length at 2 years of age - cm[a]	86.8 ± 4.1	87.6 ± 3.8	0.005
Head circumference at 2 years of age - cm[a]	48.5 ± 1.4	48.9 ± 1.4	0.006
Cerebral palsy - no. (%)	3 (5%)	5 (9%)	0.25
Mental development index[a]	96 ± 16	96 ± 18	0.90
Psychomotor development index[a]	91 ± 13	89 ± 18	0.52
Neurodevelopmental impairment[b] - no. (%)	10 (16%)	9 (17%)	1.0

[a]*Value given as mean ± SD*

[b]*Neurodevelopmental impairment is defined as any of the following: cerebral palsy, mental development index < 2 SD, psychomotor development index < 2 SD, bilateral deafness or blindness.*

TABLE 4 Mortality rate and adverse outcome (neurodevelopmental impairment or death) by stage of TTTS.

TTTS stage	Death[a]	Adverse outcome[b,c]
I	6% (1/18)	6% (1/18)
II	29% (20/70)	40% (28/70)
III	36% (23/64)	52% (33/64)
IV	42% (5/12)	50% (6/12)

[a]*Chi-square test for trend = 5.8, df = 1, p = 0.016*

[b]*Chi-square test for trend = 9.2, df = 1, p = 0.002*

[c]*Adverse outcome = Intrauterine fetal demise, neonatal death or neurodevelopmental impairment.*

Comment

The main objective of our study was to evaluate the long-term neurodevelopmental outcome in TTTS survivors treated with fetoscopic laser surgery. We were able to follow-up all (100%) TTTS survivors and report a high incidence (17%) of NDI. The long-term neurolodevelopmental outcome found in this study is in agreement with the short-term neurological outcome reported previously by our research group, in which we found a similar incidence (14%) of severe cerebral lesions in TTTS survivors after laser treatment[206].

Four studies from three different research groups have reported on long-term outcome in TTTS after laser surgery. The incidence of NDI found in this study is somewhat higher than in these other reports. However, special care must be taken when comparing results from various studies, as discrepant results may partly be due to different methodology, selection criteria and definitions of NDI. De Lia *et al* report a 6% (6/93) incidence of severe handicaps in TTTS survivors after laser surgery[131]. Mean age at follow-up was 14 months (range 1 to 34 months), which may be too soon for accurate assessment of CP or major developmental delay. Most importantly, the methods used to determine neurodevelopmental outcome were not specified, suggesting that accurate assessment of mental and psychomotor development was not performed. Sutcliffe *et al* found a 9% (6/66) incidence of CP in a cohort of TTTS survivors treated with laser[152]. Follow-up was however incomplete (81%) and in 47% (31/66) of patients neurological outcome was assessed using information from the general practitioner. In the group assessed by a pediatrician, 14% (5/36) had CP. Children assessed by pediatricians were also tested with a standardized developmental test (Griffiths' Developmental Test Scales). However, details on the number of infants with severe developmental delay (defined as a score < 2 SD) were not reported or scored as primary outcome, as opposed to the definition used in this study. The two largest follow-up cohorts have been reported by a research group from Germany. Using standardized developmental test and neurological examination, Banek *et al* and Graef *et al* report an incidence of major neurological deficiencies of 11% (10/89) and 6% (10/167), respectively[151;254]. In both studies, the definition of major neurological deficiencies did not include severe developmental delay. Therefore, as opposed to this study, infants with severe developmental delay but without CP were not included in the group with major abnormalities. Also, developmental outcome in the majority of children (112/167) in the study from Graef *et al* was only assessed by the Snijders-Oomen Non-Verbal-Intelligence Test and therefore motor abilities were not tested[254]. The incidence of CP in the studies from Banek *et al* and Graef *et al*, respectively 11% and 6%, was nevertheless similar to the 7% incidence of CP found in this study[151;254].

After treatment of TTTS with amniodrainage, most studies on long-term outcome report a high incidence of NDI, ranging from 22 to 26%[12;147;148;226].

Only one study in TTTS treated with amniodrainage reported a lower incidence of CP or multicystic encephalomalacia of 7% (3/42)[150], without assessment of neurodevelopmental delay. Overall, the reported incidence of NDI appears to be higher in TTTS survivors treated with serial amniodrainage than with laser surgery. However, different methodology may also explain the discrepancy in results between various follow-up studies. To assess the true difference in NDI in TTTS survivors treated with either laser surgery or serial amnioreduction, results of the long-term follow-up in the first randomized control trial comparing both treatments must be awaited[10].

Absence of a control group is an important limitation of this study. A case-control study comparing the long-term outcome in monochorionic twins with TTTS treated with laser and monochorionic twins without TTTS is currently being performed at our institution[153].

We found no difference in NDI between donor and recipient twins, suggesting that both are equally at risk for adverse neurodevelopmental outcome. These results are in agreement with previous studies[12;151;254]. Donor twins are significantly smaller at birth than recipient twins, and remain smaller and shorter at 2 years of age. These findings are in agreement with previous reports[12]. According to the 'fetal origins of adult disease' or 'Barker hypothesis', lower birth weight is associated with an increased risk for coronary heart disease, diabetes, hypertension and stroke in adulthood[256]. Whether reduced birth weight in donor twins in TTTS may also lead to increased incidence of adult diseases is not known yet.

We also found increasing Quintero stage to be associated with combined adverse outcome (death or NDI). Our results confirm previous findings[9;12;126;257]. Although the prognostic value of Quintero stages is subject of debate[93;258], our results suggest an important prognostic value of Quintero staging. Multivariate analysis also showed that higher Quintero stages are almost independently associated with NDI. Similarly, we found a trend towards an independent association between lower gestational age at birth and NDI. Prematurity is a well recognized risk factor for adverse neurodevelopmental outcome in twins as well as in singletons[239;258].

The objective of fetal therapy should be to reach a high percentage of intact-survival. Even though fetoscopic laser surgery appears to be the best available treatment option for TTTS, the idealistic goal of high intact-

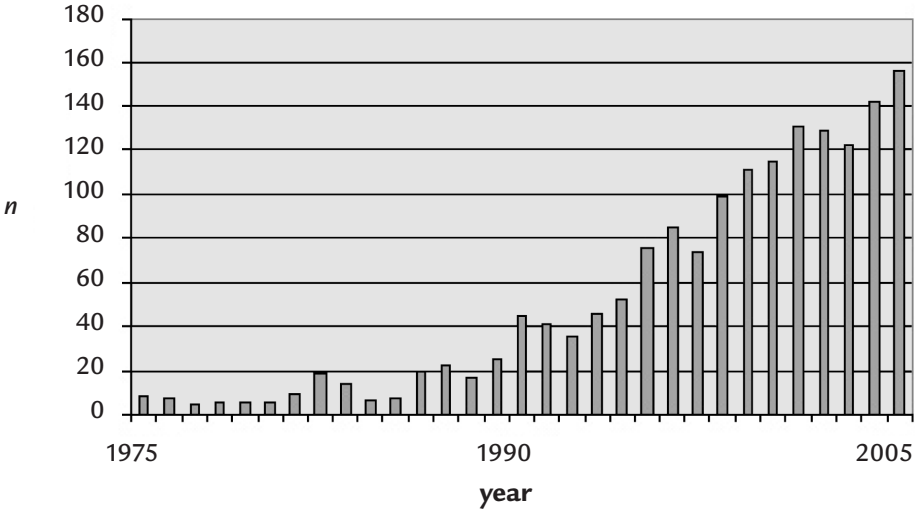
survival rate has not yet been reached. Timing of cerebral injury leading to NDI in TTTS treated with fetoscopic laser surgery is not clear. Cerebral injury may occur before, during or after laser surgery. Therefore, whether cerebral injury and subsequent NDI could be prevented by advances in laser surgery techniques such as more selective or more complete coagulation of anastomoses, or by adaptation of inclusion criteria for laser surgery is not known. Considering the high incidence of adverse neurodevelopmental outcome in TTTS, we recommend that all surviving twins be thoroughly followed up.

Chapter 13

General discussion

Twin-to-twin transfusion syndrome (TTTS) is a severe complication of monochorionic twin pregnancies resulting from unbalanced inter-twin blood flow between the donor twin and the recipient twin through placental vascular anastomoses. TTTS leads to hypovolemia and oligohydramnios in the donor and hypervolemia and polyhydramnios in the recipient. During the last decades, new insights have been gained in the pathogenesis, diagnosis and management of TTTS. An important milestone in the history of TTTS was the definition of clear and uniform diagnostic criteria[105] and a staging system[9]. Most importantly, new therapeutic modalities have led to improved outcome[10]. Finally, increased attention on TTTS from (medical) journals has resulted in improved "TTTS-awareness" in perinatologists, mid-wives and parents. A PubMed search with the term "twin-twin transfusion syndrome" between 1965 and 2005 yielded a total of 952 hits. The vast majority of these studies are of recent date. As shown in figure 1, the number of publications on TTTS is increasing every year with a current average of 3 new publications per week.

FIGURE 1 Number of TTTS publications per year.



However, despite the multitude of data currently available, various aspects of TTTS are still enigmatic and remain to be elucidated.

The *pathogenesis* of TTTS is nowadays regarded as a multifactorial process. Various studies have shown that unbalanced inter-twin blood flow occurs principally in the absence of equilibrating superficial arterio-arterial anastomoses[194]. However, the view that TTTS may develop only from simple blood transfer from donor to recipient has been discarded. Inter-twin transplacental transfer of various hormones, in particular hormones related to the renin angiotensin system, play an important role in the development of TTTS[259]. Understanding of the pathophysiology of TTTS is hampered by the absence of a suitable animal model. Computer models for TTTS have proved to be a valid alternative for animal models. However, computer models require input from clinical data. Determination of anastomotic blood flow is an example of the type of data which can be used to validate these computer models. In the TTTS case presented in Chapter 3, we calculated that the blood flow across the anastomoses was approximately 28 ml/24h at 29 weeks' gestation. Meanwhile, an almost identical TTTS case was treated at our institution, in which we also measured the percentage of transfused adult red blood cells (or adult hemoglobin) in the "new" recipient after birth. Blood flow through the only patent residual arterio-venous anastomosis in this second TTTS case was determined to be 18ml/24h at 29 weeks' gestation, which is in excellent agreement with the arterio-venous flow measured in the previous case. These results confirm the accuracy of the two different calculation methods. These mathematical experiments which enabled us to calculate arterio-venous anastomotic flow, can also be repeated in similar TTTS cases found by other research groups. The main pre-requisites required for these tests are: (1) hemoglobin levels (fetal and adult hemoglobin values) measured before and after the various intrauterine transfusions and at birth and (2) a placental injection study showing patent arterio-venous anastomoses in only one direction. These measurements and their correlations with different anastomotic patterns may further enhance computer modeling for TTTS and improve our understanding of the pathogenesis of TTTS.

It is now generally accepted that TTTS is not a homogeneous disease, and should be subdivided into two major forms: chronic TTTS and acute TTTS. *Diagnosis* of chronic TTTS is based on strict antenatal ultrasound criteria.

The main criterium required to reach the diagnosis of chronic TTTS is the twin oligo-polyhydramnios sequence (TOPS). However, as shown in the two reported cases in Chapter 6, significant chronic inter-twin blood transfusion can also occur in the absence of TOPS. We named this atypical form of chronic TTTS, twin anemia-polycythemia sequence (TAPS) and established new diagnostic criteria required to reach the diagnosis of TAPS. We have recently detected another case of TAPS at our center with similar hematological and placental findings as in the two previously reported cases. In a consecutive series of 81 monochorionic twins/triplets without chronic TTTS delivered at our center since 2002, TAPS was found in only 3 cases, which yields an incidence of TAPS of 4% (3/81). This would suggest that TAPS is not a very frequent phenomenon in monochorionic twinning. However, recent studies report an unexpected and unexplained high risk (5%) of late fetal death in monochorionic twins without TTTS[260], which may, hypothetically, be caused by TAPS. In addition to the "natural" form of TAPS, recent studies also report the existence of an iatrogenic form of TAPS[214]. In a large study of 151 TTTS cases treated with laser, Robyr *et al* found that 13% of the cases developed isolated anemia-polycythemia sequence without oligo-polyhydramnios sequence[214], so-called iatrogenic TAPS. Interestingly, it was usually the former recipient who became anemic, whereas the former donor became polycythemic[213;214]. In a recent study with mathematical modeling, we were able to explain this clinical presentation of feto-fetal transfusion reversal after incomplete laser therapy and predict significant discordant hematocrit values in both twins in the absence of amniotic fluid abnormalities due to residual anastomoses[261]. This phenomenon results from, first, the increased colloid oncotic pressure present in the former recipient prior to and shortly after laser, and second, continued feto-fetal transfusion[261].

The acute form of TTTS can be divided in acute perimortem TTTS and acute perinatal TTTS. The occurrence of acute perimortem TTTS has been undoubtedly proven and is known to be associated with a high risk of perinatal mortality and morbidity in the surviving twin[13-16;30-33]. In contrast, acute perinatal TTTS is only based on a few, often incomplete case reports[34-39]. Most case reports of acute perinatal TTTS failed to determine the inter-twin difference in reticulocyte count (or erythropoietin levels) and lacked accurate placental injection study. Therefore some of the cases

may well have been TAPS cases instead of acute perinatal TTTS. In our series of 81 consecutive uncomplicated monochorionic twins, no cases of acute perinatal TTTS have been observed. However, we noticed another important perinatal hematological phenomenon which may often occur in monochorionic twinning and is related to birth order and placento-fetal transfusion. As shown in Chapter 7, second-born twins have higher hemoglobin levels than first-born twins. Hypothetically, after clamping of the first-born's umbilical cord, uterine contractions may allow placental blood from the first twin's low-pressure placental bed to be transfused through the low-resistance superficial vascular anastomoses into the higher pressure circulation of the second twin. Large inter-twin hemoglobin differences in monochorionic twins without chronic TTTS may thus be due to acute placento-fetal transfusion rather than feto-fetal transfusion.

The optimal *management* of TTTS is limited by a paucity of well-designed studies. Most studies are observational and few studies have focused on the long-term outcome. Only two randomized controlled trials on the management of TTTS have been published. Moise *et al* compared serial amnioreduction and septostomy and reported similar short-term outcomes between both treatments[140]. Senat *et al* compared serial amnioreduction and fetoscopic laser surgery and found that perinatal mortality and short-term neurologic morbidity were significantly lower in the group treated with laser surgery[10]. Despite the significant improvement due to treatment with fetoscopic laser surgery, perinatal mortality and morbidity in TTTS remain strikingly high, especially when considering that both fetuses were probably initially structurally normal. Several studies presented in this thesis, report in detail the significant short and long-term perinatal morbidity in TTTS survivors treated with laser. The most deleterious type of morbidity encountered in TTTS survivors is severe cerebral injury. We found that 14% of TTTS survivors after laser surgery have severe cerebral injury on cranial ultrasound. Nevertheless, the incidence of severe cerebral injury in TTTS treated with laser is still significantly lower than in TTTS treated with amnioreduction. In the randomized trial from Senat *et al*, extensive cystic periventricular leucomalacia grade III or IV occurred less often in the laser group than in the amnioreduction group, 6% versus 14%, respectively (RR 0.39, 95% CI 0.18 - 0.86, p = 0.02)[10]. The timing of

cerebral injury in TTTS treated with laser is not known, and could occur before, during or after laser surgery. To answer this question, a sequential fetal MRI study performed before and after laser surgery is required.
The origin of cerebral damage in TTTS is not clear and may be related to hemodynamic imbalance in cerebral flow causing hypoxic-ischemic or hemorrhagic injury. Recently, Rodeck *et al* presented a new hypothesis (the placental 'steal' phenomenon) which can explain the severe neurological outcome seen in TTTS cases treated with amnioreduction[262]. They suggest that amnioreduction may lead to a radical shift of blood from the fetus into the placenta causing hypoxic-ischemic brain damage. Since single amnioreduction is also performed routinely in patients during fetoscopic laser surgery procedures, the placental 'steal' phenomenon may also occur in TTTS treated with laser.
The 14% incidence of severe cerebral injury in TTTS survivors after laser treatment found in our study is in accordance with the 17% incidence of neurodevelopmental impairment detected in long-term survivors (see Chapter 12). Nevertheless, the reported incidence of neurodevelopmental impairment in TTTS treated with laser appears to be lower than in TTTS treated with serial amniodrainage (see also Chapter 11)[12;147;148;151;152;226;254]. Obviously, caution is advised when comparing the various results in view of the observational nature of these follow-up studies and the methodological differences between the studies. To assess the true difference in neurodevelopmental impairment in TTTS survivors treated with either laser surgery or serial amnioreduction, results of the long-term follow-up in the ongoing randomized trial must be awaited[10].

In conclusion, despite significant progression during the last decades, the management of TTTS remains one of the most challenging medical problems in fetal medicine. More research is necessary to further improve the prognosis of TTTS.

Chapter 14

Future perspectives

Fetal medicine is a new discipline, gradually evolving from a field with mainly diagnostic function to a broader field with also life-saving therapeutic possibilities. However, few interventions in fetal medicine are evidence-based. Most fetal medicine studies are observational and often limited by small numbers of included patients. To date, only few randomized controlled trials in fetal medicine have been performed. Interestingly, two of these trials involved the treatment of TTTS[10;140]. Hopefully, TTTS can pave the way for more randomized controlled studies in fetal medicine.

There are still several aspects in TTTS requiring further investigation:

1. The exact *pathogenesis* of TTTS needs to be further unraveled. Due to the absence of animal models, computer modeling of TTTS may prove to be a valuable alternative. However, for computer models to be reliable, they require realistic input from *in vivo* cases. Intensive collaboration between perinatologists and computer modeling scientists may lead to new important discoveries. New research should also keep focusing on the role of various hormonal factors. These data may then in return be used to improve computer modeling of TTTS.
   Routine placental injection study of all monochorionic placentas, with and without TTTS, has already yielded interesting discoveries (e.g. in our studies) and can still lead to new insights. Injection studies of TTTS placentas treated with laser may also help in understanding the underlying cause of the high incidence of residual anastomoses. This information may be crucial for the individual fetal surgeon to reduce the frequency of residual anastomoses. Furthermore, determination of the type and number of placental vascular anastomoses can be used to study the possible relation between angioarchitecture and timing of onset of TTTS. As suggested at the last Eurofoetus meeting (Paris, May 2006) late onset TTTS (i.e. after 26 weeks) may occur when a large amount of anastomoses is present. Finally, more placental casting studies are needed to determine the incidence and clinical implications of the recently reported deep anastomoses which are not visible on the placental surface[263;263;264]. We recently finished a casting study of monochorionic placentas with TTTS (after laser treatment) and without TTTS and are currently in the process of analyzing the data.

2. The *diagnosis* of TTTS and its related complications may, in the near future, gain more accuracy with the use of improved imaging techniques. Several studies have investigated the value of (Doppler) ultrasound examination in predicting the development of TTTS or the outcome in TTTS, including a study performed at our institution by Sueters *et al* (TULIPS study)[68;109]. Other studies have reported that detection of an arterio-arterial anastomosis decreases the risk of developing TTTS by 9-fold[66;68]. Fetal magnetic resonance imaging (MRI) is a new promising technique which is nowadays increasingly being used[17;265-267]. Fetal brain MRI is particularly useful in TTTS as it appears to detect brain lesions earlier and with better definition than prenatal ultrasound[177]. However, more research is required to evaluate the optimal timing of fetal MRI in TTTS and determine the exact prognosis of each type of detected lesion. A study entitled "Cerebral Imaging in Monochorionic Twins: CIMT study" will soon start at the Leiden University Medical Center. The objective of this study is to evaluate the incidence, character and origin of cerebral lesions in monochorionic twins with TTTS treated with laser using sequential fetal and neonatal MRI and 4D ultrasound. A group of monochorionic twins without TTTS will serve as control group. The most important research question is whether cerebral injury occurs before or after laser surgery. If severe cerebral lesions occur before laser and can be detected accurately, then this could have major implications for future management strategies in TTTS.

3. The optimal *management* of TTTS, and especially the occurrence of early and late complications in TTTS treated with laser, requires further investigation. Future studies should focus on the optimization of various treatments to improve the outcome in TTTS. New techniques, such as combined laparoscopy and fetoscopy in complete anterior placenta developed at our center, may further improve outcome (Eurofoetus meeting, Paris 2006). Recently, we also proposed the use of laser surgery in TTTS up to 28 weeks of gestation, as neonatal morbidity was lower compared to TTTS cases treated with amnioreduction (Eurofoetus meeting, Paris 2006). Other major issues, such as reduction of preterm delivery and rupture of membranes after fetoscopy, may further increase survival rates in TTTS.

4. There is paucity of *long-term follow-up* studies in monochorionic twins. More data on long-term studies in monochorionic twinning are strongly required. We are currently investigating the long-term neurodevelopmental outcome at 2 years of age in all monochorionic twins with and without TTTS delivered at our center between June 2002 and September 2005. We also intend to examine this cohort of monochorionic twins at 5 years of age to assess the neurodevelopmental outcome at school age. Well documented cohorts of monozygotic twins with single intrauterine growth restriction form also an ideal group to test the 'Barker hypothesis'[256]. According to this hypothesis, the risk of adult diseases such as coronary heart disease, diabetes, hypertension and stroke is increased in adults who were growth restricted during fetal life[256]. Monozygotic twins are genetically identical but may be subjected to a different intrauterine environment. Whether monozygotic twins with discordant fetal growth also have a discordant risk of adult diseases requires further investigation.

5. *In general*, there are several important aspects to be taken into consideration for future research in TTTS. One of the main limitations of most TTTS studies is the small size of included patients, which is inherent to the rarity of the disorder itself. To overcome this major problem, future study designs should preferentially be multicentered. A second general problem concerns the definition of adverse outcome. To date, most TTTS studies focus on "survival" instead of "disease-free survival". Given the high percentage of cerebral injury in TTTS, future studies should aim at evaluating long-term neurodevelopmental outcome, or at least short-term neurological outcome.
   To reach a consensus on the optimal treatment in monochorionic twin pregnancies and TTTS in particular, more randomized trials are required. Various trials have already been proposed. Moise *et al* suggested that a randomized trial of combination amnioreduction/septostomy versus laser surgery in early stage TTTS should be performed[140]. Quintero *et al* suggested that a trial of laser surgery versus expectant management is indicated in monochorionic twins with highly discordant intrauterine growth restriction[135]. Lastly, given the high rate of unexpected late fetal deaths in uncomplicated monochorionic

twins, Barigye *et al* suggested that a trial should be designed to test the potentially beneficial role of early delivery in monochorionic twins[260].

Although these trials may certainly be indicated, studies in TTTS should not only focus on perinatal "survival" but especially on "intact-survival". If the fetus is truly to become a unique patient[268], then primary outcome measures in fetal medicine, and TTTS in particular, should be "survival without severe morbidity".

Chapter 15

Summary

Twin-to-twin transfusion syndrome (TTTS) is a severe complication of monochorionic twin pregnancies associated with high perinatal mortality and morbidity rates. Placental vascular anastomoses, almost invariably present in monochorionic placentas, are the essential anatomical substrate for the development of TTTS. TTTS is thought to result from unbalanced inter-twin blood flow between the donor twin and the recipient twin through the vascular anastomoses, leading to hypovolemia and oligohydramnios in the donor and hypervolemia and polyhydramnios in the recipient. Despite significant developments in the diagnosis, staging and management of TTTS, the pathogenesis of TTTS is still poorly understood and, most importantly, perinatal mortality and morbidity in TTTS remain strikingly high.

In this thesis, several studies on TTTS are presented regarding various aspects of this disease, including studies on monochorionic placentas to investigate the pathogenesis of TTTS, description of a new form of chronic TTTS and the short and long-term outcome in TTTS treated with fetoscopic laser surgery.

In *Chapter 2*, an overview of the literature is presented. This review analyzes the possible pathophysiologic mechanisms involved, discusses the latest findings in diagnosis, therapy and prognosis, and focuses on neonatal and pediatric morbidity associated with TTTS.

In *Chapter 3* we describe a novel technique to calculate the net feto-fetal blood flow through placental arterio-venous anastomoses in a case of TTTS treated with laser surgery and subsequent intrauterine transfusion. In this study we determined that the net blood flow through the five residual unidirectional arterio-venous anastomoses was approximately 28 ml/24h, much lower than previously measured with Doppler ultrasound. This finding may also explain the inaccuracy of Doppler flow measurements, as such low flow velocities cannot possibly be detected with current Doppler techniques. Measurements of anastomotic blood flow are of major importance for the validation and development of accurate computer modeling in TTTS.

In *Chapter 4* we studied the role of velamentous cord insertion and discordant placental territories in the pathogenesis of TTTS by comparing monochorionic placentas with and without TTTS. Previously, several studies reported an increased incidence of velamentous cord insertions in TTTS placentas and suggested a direct relation between velamentous cord insertion, unequal placental territories and the development of TTTS. In this study we examined 76 monochorionic placentas with TTTS and 63 monochorionic placentas without TTTS. The incidence of velamentous cord insertion (per fetus) in the TTTS group and the no-TTTS group was 13% and 14% ($p = 0.79$), respectively. Placental territory discordancy in the TTTS group and the no-TTTS group was 20% in both groups ($p = 0.83$). In the TTTS group, donor twins had more often a velamentous cord insertion than recipient twins (24% and 3%, respectively, $P < 0.001$) and smaller placental territories (44% and 56% respectively, $p < 0.001$). Our findings suggest that velamentous cord insertion and placental territory discordancy are not critical factors for the development of TTTS.

In *Chapter 5* the frequency of residual placental vascular anastomoses after fetoscopic laser surgery for TTTS was studied. Presence of residual anastomoses was investigated in relation to adverse outcome and to inter-twin hemoglobin difference at birth. Residual anastomoses were detected in 33% (17/52) of placentas. Adverse outcome (fetal demise, neonatal death or severe cerebral injury) was similar in the groups with and without residual anastomoses, 18% (6/34) and 29% (20/70), respectively ($p = 0.23$). Large inter-twin hemoglobin differences (> 5 g/dL) were found in 65% (11/17) of cases with residual anastomoses and 20% (7/35) of cases without residual anastomoses ($p < 0.01$). The first conclusion of this study is that laser treatment needs to be improved as only 2/3 of monochorionic placentas are functionally "dichorionized". The second conclusion is that residual anastomoses in this study are not associated with adverse outcome. Lack of association between residual anastomoses and adverse outcome may partly be due to the small size of most residual anastomoses (< 1mm diameter in 64% of the cases) and the presence of "protective" residual superficial anastomoses in 35% of the cases. Finally, we concluded that residual anastomoses are often associated with neonatal hematological complications.

In *Chapter 6* we describe two pairs of monochorionic twins without TTTS but with marked discordant hemoglobin levels. We named this new form of TTTS, the twin anemia-polycythemia sequence (TAPS). In the two reported TAPS cases, both donor twins were severely anemic requiring blood transfusion and both recipients were polycythemic, one requiring partial volume exchange transfusions. Inter-twin difference in reticulocyte counts was extremely high, suggesting a chronic form of inter-twin blood transfusion. Placental injection studies revealed a preponderance of very small (< 1 mm) arterio-venous anastomoses in one direction. Nowadays, routine prenatal measurements of middle cerebral artery peak systolic velocity using Doppler ultrasound are recommended after laser surgery to rule out fetal anemia or (iatrogenic) TAPS. We suggest that routine Doppler studies also be performed in uncomplicated monochorionic twin pregnancies without TOPS. Signs of fetal anemia in a monochorionic twin should then alert the perinatologist of the possibility of TAPS. TAPS should be diagnosed when a large inter-twin discordance in fetal or neonatal hemoglobin levels and reticulocyte counts is found, in the absence of TOPS. Placental injection studies may then reveal a preponderance of very small arterio-venous anastomoses.

Discordant hemoglobin levels occur not only in chronic TTTS and in TAPS, but are also reported to occur in uncomplicated monochorionic pregnancies due to acute peripartum TTTS. In *Chapter 7* we studied the inter-twin difference in hemoglobin levels at birth in monochorionic twins without TTTS compared to a control group of dichorionic twins, in relation to birth order and placental vascular anatomy. We found that hemoglobin differences occur more frequently in monochorionic twins without chronic TTTS than in dichorionic twins, but only when measured on the second day of life. Furthermore, hemoglobin differences in monochorionic twins are associated with birth order and with the presence of superficial vascular anastomoses. We found that second-born monochorionic twins have significantly higher hemoglobin values than first-born twins. Our findings support the hypothesis that second-born monochorionic twins are more likely to receive a large placental blood transfusion rather than lose blood into the placenta. We also report that hemoglobin differences in monochorionic twins are greater in the presence of superficial vascular

anastomoses. In analogy to acute perimortem TTTS, it is conceivable that superficial vascular anastomoses may also be responsible for rapid placento-fetal blood transfusion during delivery.

Chapter 8, 9 and *10* focus on the short-term outcome in TTTS treated with fetoscopic laser surgery. The neonatal outcome in TTTS survivors treated with laser at our center is presented in *Chapter 8*. We compared the outcome in a TTTS group after laser treament with a control group of monochorionic twins without TTTS delivered at our center. We found that neonatal mortality in the TTTS and no-TTTS group was 8% (6/76) and 3% (3/90), respectively (p = 0.03). Overall, the incidence of adverse neonatal outcome (neonatal mortality, major neonatal morbidity or severe cerebral lesions) in the TTTS and no-TTTS group was 26% (20/76) and 13% (12/90), respectively (RR = 1.97, 95% CI = 1.03 to 3.77). We concluded that the risk for adverse neonatal outcome is two-fold increased in TTTS treated with laser than in monochorionic twins without TTTS.

Details on the short-term neurological outcome in TTTS survivors treated with fetoscopic laser surgery are presented in *Chapter 9*. Again we compared the results with a control group of monochorionic twins without TTTS. Incidence of antenatally acquired severe cerebral lesions in the TTTS group was 10% (8/84) and 2% (2/108) in the no-TTTS group (p = 0.02). Incidence of severe cerebral lesions at discharge was 14% (12/84) in the TTTS group and 6% (6/108) in the no-TTTS group (p = 0.04). Antenatal injury was responsible for severe cerebral lesions in 67% (8/12) of the TTTS group. We conclude that the incidence of severe cerebral lesions in TTTS treated with fetoscopic laser surgery is high and results mainly from antenatal injury.

Details on the short-term cardiac outcome in TTTS survivors treated with fetoscopic laser surgery are presented in *Chapter 10* and compared to a control group of monochorionic twins without TTTS. Echocardiography was performed within one week after delivery. At birth, blood pressure was measured in all survivors and endothelin-1 was determined in umbilical cord blood. Data on right ventricular outflow tract obstruction in TTTS treated with laser surgery at our center but delivered elsewhere

were reviewed retrospectively from medical records. We found that the incidence of right ventricular outflow tract obstruction in recipients was 4% (3/75). We found no difference in afterload parameters between donors and recipients after laser treatment. We concluded that the incidence of congenital heart disease in TTTS survivors treated with fetoscopic laser occlusion of vascular anastomoses is around 5%, which is higher than in the general population (0.5%). In particular, the increased risk of right ventricular outflow tract obstruction in recipient twins warrants close cardiac monitoring during fetal and neonatal life.

The long-term neurodevelopmental outcome in TTTS is presented in *Chapter 11* and *12*. *Chapter 11* describes the long-term neurodevelopmental outcome in TTTS treated conservatively. All TTTS-cases admitted at our center between January 1990 and December 1998 were included in the study. Perinatal mortality was 50% (29/58). Neurological and mental development at school age was assessed during a home visit in all TTTS survivors (n = 29). The incidence of adverse neurodevelopmental outcome in TTTS survivors was 21% (6/29) and was due to cerebral palsy (n = 6) and developmental delay (n = 5). The incidence of adverse neurodevelopmental outcome in the group of survivors who were treated with amnioreduction was 26% (5/19). Two of the four children born after intrauterine fetal demise of their co-twin had cerebral palsy.

Chapter 12 describes the long-term neurodevelopmental outcome in TTTS treated with fetoscopic laser surgery. All TTTS-cases treated consecutively at our center between August 2000 and December 2003 were included in the study. Perinatal mortality was 30% (49/164). Neurological, mental and psychomotor development at 2 years of age was assessed in all TTTS survivors (n = 115). Overall, the incidence of neurodevelopmental impairment was 17% (19/115) and was due to cerebral palsy (n = 8), mental developmental delay (n = 9), psychomotor developmental delay (n = 12) and deafness (n = 1). In both long-term follow-up studies, we concluded that neurodevelopmental delay in TTTS, regardless of type of antenatal treatment, is high and warrants long-term follow-up.

In conclusion, although laser surgery appears to be the best available treatment option for TTTS, perinatal mortality and morbidity rates in TTTS treated with laser are still significant. More research and new developments are required to further improve the short and long-term outcome in TTTS.

Samenvattting

Er zijn 2 soorten tweelingen: een-eiig en twee-eiig. Twee-eiige tweelingen komen vaker voor dan een-eiige tweelingen (65% versus 35%). Alle twee-eiige tweelingen hebben ieder een eigen gescheiden placenta (dichoriaal). Dichoriale placenta's bevatten vrijwel nooit vaatverbindingen, ook wel vaatanastomoses genoemd, die de bloedcirculatie van beide foetussen met elkaar verbindt. De meeste een-eiige tweelingen hebben daarentegen een zogenaamde gezamenlijke placenta (monochoriaal). Monochoriale placenta's bevatten juist wel vrijwel altijd vaatanastomoses, waardoor de circulatie van beide foetussen met elkaar verbonden is. Ten gevolge van deze vaatanastomoses, kan in 15% van de monochoriale een-eiige tweelingen het tweeling-transfusie syndroom (TTS) ontstaan. Voor de Nederlandse situatie betekent dit dat er naar schatting jaarlijks ongeveer 60 à 90 TTS zwangerschappen voorkomen. TTS ontstaat doorgaans in het 2e trimester van de zwangerschap. Het eerste symptoom is meestal het plotseling ontstaan van te veel vruchtwater (polyhydramnion) bij één van de foetussen. Het polyhydramnion ontstaat doordat de ene foetus (de recipiënt) via de vaatanastomoses teveel bloed ontvangt van de andere foetus (de donor). De donor is dientengevolge ondervuld en heeft zeer weinig vruchtwater (oligohydramnion). Het polyhydramnion bij de recipiënt kan leiden tot een acute toename van de buikomvang van de zwangere en vervolgens tot vroegtijdige weeën en vroeggeboorte. Indien er dan geen actie ondernomen wordt, kan TTS leiden tot een schrikbarend hoge mortaliteit en morbiditeit (> 80%).
Tot enkele jaren geleden was er in Nederland maar één behandeling mogelijk voor TTS, namelijk het herhaaldelijk aftappen van overtollig vruchtwater (amniondrainage), hetgeen primair bedoeld was om de klachten van de zwangere te reduceren en tegelijktertijd de kans op vroeggeboorte te verminderen. Deze behandeling heeft echter geen direct effect op TTS zelf, waardoor het ziekteproces onverminderd door kan gaan. Sinds Augustus 2000 wordt in het Leids Universitair Medisch Centrum een nieuwe therapie, foetoscopische laser coagulatie van de vaatanastomoses, toegepast. Deze techniek werd in de jaren '90 in de Verenigde Staten uitgevonden. Hierbij worden door middel van een foetoscoop en laserlicht de vaatverbindingen op de placenta dicht gecoaguleerd. (De foetoscoop is een soort "kijkbuis" die via een kleine incisie in de buikwand in de baarmoeder wordt ingebracht.) Het effect van deze laser behandeling bij

kinderen met TTS was nog niet goed onderzocht.

Het primaire doel van dit proefschrift was het beschrijven van de korte en lange termijn gevolgen van deze laser behandeling voor de kinderen met TTS. Dit onderzoek , genaamd Leidse TTS study (LETTS' study), ging in juni 2002 van start. Tijdens de loop van deze studie, werden ook andere studies naar TTS opgestart. Onder ander werd er een aantal studies verricht naar de oorzaak van TTS. Hiervoor werden de monochoriale placenta's uitvoerig bestudeerd, hetgeen o.a. leidde tot de identificatie van een nieuwe, atypische vorm van TTS.

Hoofdstuk 2 bevat een samenvatting van de literatuur en een opsomming van de vele nieuwe inzichten ten aanzien van de pathogenese, diagnostiek en behandeling van TTS.

In *Hoofdstuk 3* beschrijven wij een nieuwe techniek om de netto bloedstroom (flow) door placentaire vaatanastomoses te berekenen. Deze berekening is uitgevoerd aan de hand van een bijzondere met laser behandelde TTS casus. De ex-recipiënt in deze casus vertoonde na de laserbehandeling tekenen van bloedarmoede (anaemie), hetgeen een éénmalige intra-uteriene bloedtransfusie noodzakelijk maakte. Het placenta onderzoek na de geboorte liet zien dat er nog 5 arterio-veneuze rest-anastomoses aanwezig waren die van de ex-recipiënt naar de ex-donor liepen. Gebruikmakend van de daling van het hemoglobine bij de ex-donor tussen het moment van de intrauteriene transfusie en het moment van geboorte, hebben we kunnen uitrekenen dat de flow door de rest-anastomoses ongeveer 28 ml/dag was. Deze berekeningen kunnen gebruikt worden om computer modellen te maken om TTS te simuleren en zodoende het inzicht te vergroten. Door de afwezigheid van adequate diermodellen, zijn deze computer modellen van groot belang voor verder onderzoek naar TTS.

In *Hoofdstuk 4* hebben wij het verband onderzocht tussen de aanwezigheid van velamenteuze navelstreng inserties, een onevenredige (discordante) placenta verdeling en het ontstaan van TTS beschreven. Voorheen werd gesuggereerd dat er een directe relatie zou zijn tussen velamenteuze

navelstreng inserties, discordante placenta verdeling en TTS. In ons onderzoek hebben we een grote serie monochoriale placenta's met en zonder TTS vergeleken. De incidentie van velamenteuze navelstreng inserties in de groep met en zonder TTS was respectievelijk 13% (20/152) en 14% (18/126) ($p = 0.79$). Het gemiddelde verschil in placenta oppervlak in de groep met en zonder TTS was identiek (20%). In de TTS groep hadden donor tweelingen vaker een velamenteuze navelstreng insertie dan de recipiënten (respectievelijk 24% en 3%, $p < 0.001$) en een kleiner placenta deel (respectievelijk 44% en 56%, $p < 0.001$). Onze bevindingen suggereren dat velamenteuze navelstreng inserties en discordante placenta verdeling geen directe oorzaak van TTS zijn.

In *Hoofdstuk 5* hebben wij onderzocht hoe vaak er in TTS placenta's die met laser behandeld waren, nog sprake was van rest-anastomoses. Tevens bekeken wij de klinische gevolgen van deze rest-anastomoses. Wij vonden in 33% (17/52) van de met laser behandelde placenta's rest-anastomoses. De meeste rest-anastomoses (64%) waren extreem klein (diameter < 1 mm). De aanwezigheid van rest-anastomoses was echter niet geassocieerd met een slechtere uitkomst (gedefiniëerd als intrauteriene of neonatale sterfte, of ernstige hersen (cerebrale) schade). Rest-anastomoses waren daarentegen wel duidelijk geassociëerd met grotere hemoglobine-verschillen (> 5 g/dL) tussen de beide kinderen. Grote hemoglobine-verschillen werden gedetecteerd in 65% (11/17) van de gevallen met rest-anastomoses vergeleken met 20% (7/35) van de gevallen zonder rest-anastomoses ($p < 0.01$). Aldus hebben wij aangetoond dat 1/3 van de door ons met laser behandelde TTS placenta's nog rest-anastomoses bevatten en dat deze rest-anastomoses vooral kunnen leiden tot hematologische complicaties.

In *Hoofdstuk 6* beschreven wij twee monochoriale tweeling-paren met een nieuwe, atypische vorm van chronische TTS, die wij tweeling anaemie-polycythemie sequentie (TAPS) hebben genoemd. TAPS onderscheidt zich van de klassieke chronische TTS doordat er bij antenataal echografisch onderzoek geen typische tweeling oligo-polyhydramnion sequentie (TOPS) aanwezig is. Beide donoren waren bij de geboorte anaemisch en werden behandeld met een bloedtransfusie. De beide recipiënten

daarentegen waren polycythemisch, waarbij een van de recipiënten partiële wisseltransfusies nodig had. Het reticulocyten aantal bij de donoren was extreem hoog, hetgeen wees op een chronische vorm van TTS. Onderzoek van de placenta liet slechts enkele minuscule arterioveneuze vaatanastomoses zien. Recentelijk zijn er meer gevallen van TAPS (zonder TOPS) na laserbehandeling beschreven. Er wordt derhalve geadviseerd om na laserbehandeling vanwege TTS routinematig door middel van echo Doppler naar de aanwezigheid van foetale anaemie te kijken. Wij adviseren ook om Doppler onderzoek routinematig te gebruiken om TAPS uit te sluiten in ongecompliceerde monochoriale tweelingzwangerschappen. Daarnaast lijkt het raadzaam dat perinatologen in geval van onverwacht grote hemoglobine-verschillen post partum bij monochoriale tweelingen zonder TTS, ook het aantal reticulocyten bepalen en de placenta laten onderzoeken.

Hemoglobine-verschillen bij monochoriale tweelingen hoeven niet alleen door chronische TTS of TAPS veroorzaakt te worden, maar zouden ook kunnen ontstaan door een acute vorm van TTS die optreedt rondom de geboorte (peri-partum TTS). In *Hoofdstuk 7* hebben wij de hemoglobine verschillen bij monochoriale tweelingen zonder TTS vergeleken met die van dichoriale tweelingen. Wij vonden vaak grote hemoglobine verschillen bij monochochoriale tweeling-paren, met name wanneer de bepaling op de 2^{e} dag na de geboorte werd herhaald. Hemoglobine-verschillen waren vooral geassociëerd met de volgorde van geboorte en de aanwezigheid van oppervlakkige vaatanastomoses. Het kind dat als tweede was geboren, had vaker hogere hemoglobine waardes. Onze bevindingen passen bij de hypothese dat, na de geboorte van het eerste kind, er bij het tweede kind, via de oppervlakkige vaatanastomoses, een grotere placento-foetale bloed transfusie zou kunnen optreden.

Hoofdstuk 8, 9 en *10* bevatten studies naar de korte termijn uitkomsten na laser behandeling vanwege TTS.

In *Hoofdstuk 8* hebben we gekeken naar de neonatale uitkomsten bij TTS tweelingen behandeld met laser, vergeleken met monochoriale tweelingen zonder TTS. Neonatale mortaliteit in de groep met TTS en zonder TTS

was 8% (6/76) en 3% (3/90). De incidentie van een slechte uitkomst (gedefiniëerd als neonatale sterfte of ernstige neonatale morbiditeit) was 2 keer hoger in de groep met TTS vergeleken met de groep zonder TTS, namelijk respectievelijk 26% (20/76) en 13% (12/90) (p = 0.03). In het licht van deze bevindingen adviseren wij om TTS tweelingen na laser behandeling te beschouwen als een hoog-risico groep met een verhoogde kans op neonatale complicaties.

In *Hoofdstuk 9* wordt de incidentie, het karakter en de origine van cerebrale schade in TTS tweelingen na laser behandeling beschreven. Het in dit hoofdstuk beschreven onderzoek, werd verricht door middel van cerebrale echografie, waarbij het eerste echografische onderzoek binnen 24 uur na de geboorte plaats vond. Hierbij betrof de controle groep wederom monochoriale tweelingen zonder TTS. De incidentie van antenataal opgelopen ernstige cerebrale schade in de groep met en zonder TTS was respectievelijk 10% (8/84) en 2% (2/108) (p = 0.02). De incidentie van ernstige cerebrale schade bij ontslag in de groep met en zonder TTS was respectievelijk 14% (12/84) en 6% (6/108) (p = 0.04). Antenatale schade was derhalve verantwoordelijk voor 67% (8/12) van de ernstige cerebrale schade bij TTS tweelingen. Gezien de hoge incidentie van cerebrale schade adviseren wij om bij alle TTS tweelingen (zowel na laser behandeling, als zonder laser behandeling) post-partum echografisch onderzoek van de hersenen te laten verrichten.

In *Hoofdstuk 10* zochten wij naar hart (cardiale) afwijkingen bij TTS tweelingen die behandeld waren met laser en vergeleken deze groep met een controle groep van monochoriale tweelingen zonder TTS. Dit onderzoek vond plaats door middel van cardiale echografie binnen 1 week na de geboorte. De incidentie van obstructie van de bloed flow door de rechter hartkamer was 4% (3/75). Over het geheel genomen was de incidentie van congenitale hart aandoeningen bij TTS tweelingen behandeld met laser veel hoger (rond de 5%) dan in de gewone bevolking (0.5%). Derhalve is alertheid geboden ten aanzien van de verhoogde kans op foetale en neonatale cardiale afwijkingen.

Hoofdstuk 11 en *12* bevatten studies over de lange termijn uitkomst bij TTS.

In *Hoofdstuk 11* beschrijven wij een studie naar de lange termijn uitkomsten bij TTS tweelingen na conservatieve behandeling. Alle kinderen met TTS geboren in het Leids Universitair Medisch Centrum tussen 1990 en 1998 werden geïncludeerd in deze studie. De perinatale mortaliteit betrof 50% (29/58). Alle kinderen hadden inmiddels de school-leeftijd bereikt. De primaire uitkomstmaat was een abnormaal neurologisch onderzoek en/of achterstand in de geestelijke (mentale) ontwikkeling. De incidentie van een abnormale geestelijke en/of lichamelijke (psychomotore) ontwikkeling was 21% (6/29). De incidentie van abnormale psychomotore ontwikkeling in de TTS groep die behandeld was met amniodrainage was 26% (5/19).

In *Hoofdstuk 12* beschrijven wij de lange termijn uitkomst bij TTS na laser behandeling. Alle kinderen met TTS die tussen 2000 en 2003 in het Leids Universitair Medisch Centrum met laser behandeld zijn, werden geïncludeerd in deze studie. De perinatale mortaliteit betrof 70% (49/164). De neurologische status en de psychomotorische ontwikkeling werden bepaald op de leeftijd van 2 jaar, gecorrigeerd voor de prematuriteit. Een abnormale psychomotore ontwikkeling werd gedefiniëerd als één of meerdere van de volgende factoren: spasticiteit, doofheid, blindheid of een mentale of motorische ontwikkelingsindex van onder de 70 (dat is meer dan 2 standdaarddeviaties verlaagd). De incidentie van een abnormale psychomotore ontwikkeling was 17% (19/115) en was gebaseerd op spasticiteit (n = 8), doofheid (n = 1), achterstand in mentale ontwikkeling (n = 9) en achterstand in motorische ontwikkeling (n = 12). In beide hoofdstukken concluderen wij dat kinderen met TTS langdurige follow-up behoeven, gezien de hoge kans op spasticiteit en ontwikkelingsachterstand.

Samenvattend kunnen we stellen dat hoewel laser behandeling de beste beschikbare therapeutische optie voor TTS is, de perinatale morbiditeit en mortaliteit nog steeds aanzienlijk blijft. Om de korte en lange termijn prognose voor TTS zwangerschappen verder te verbeteren, is voortgaand onderzoek derhalve noodzakelijk.

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List of abbreviations

AA	Arterio-arterial
AV	Arterio-venous
ASD	Atrial septal defect
BSID	Bayley Scales of Infant Development
CHD	Congenital heart disease
CP	Cerebral palsy
DC	Dichorionic
Hb	Hemoglobin
IUFD	Intrauterine fetal demise
IVH	Intraventricular hemorrhage
LUMC	Leiden University Medical Center
MC	Monochorionic
MCA-PSV	Middle cerebral artery peak systolic velocity
MDI	Mental development index
NDI	Neurodevelopmental impairment
NND	Neonatal death
PDI	Psychomotor developmental index
PVL	Periventricular leucomalacia
RA	Residual anastomoses
RVOTO	Right ventricular outflow tract obstruction
TAPS	Twin anemia-polycythemia sequence
TOPS	Twin oligo-polyhydramnios sequence
TTTS	Twin-to-twin transfusion syndrome
VSD	Ventricular septal defect
VV	Veno-venous

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